HYDROTHERMAL SYNTHESIS OF NEAR-INFRA-RED EMITTING QUANTUM DOTS FOR FLUORESCENT AND MAGNETIC BIMODAL IMAGING

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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Dedicated to my parents, who taught me the importance of perseverance and patience, and encouraged me to pursue my dreams.
ACKNOWLEDGEMENTS

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<td>Cross linked iron oxide</td>
</tr>
<tr>
<td>DAPI</td>
<td>4’, 6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>DI</td>
<td>Distilled</td>
</tr>
<tr>
<td>DMSA</td>
<td>Dimercaptosuccinic acid</td>
</tr>
<tr>
<td>EDC</td>
<td>N-(3-dimethylaminopropyl)-N-ethylcarbodiimde hydrochloride</td>
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<tr>
<td>EDS</td>
<td>Energy dispersive x-ray spectroscopy</td>
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<td>EPR</td>
<td>Electron paramagnetic resonance</td>
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<tr>
<td>EXAFS</td>
<td>Extended x-ray absorption fine structure</td>
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<td>FC</td>
<td>Field cooled</td>
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<tr>
<td>HRTEM</td>
<td>High resolution transmission electron microscopy</td>
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<tr>
<td>ICP</td>
<td>Inductively coupled plasma mass spectrometry</td>
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<tr>
<td>IR</td>
<td>Infra Red</td>
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<tr>
<td>LED</td>
<td>Light emitting device</td>
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<tr>
<td>MAA</td>
<td>Mercaptoacetic acid</td>
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<tr>
<td>MEIO</td>
<td>Metal doped iron oxide</td>
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<td>MNP</td>
<td>Magnetic nanoparticle</td>
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<tr>
<td>MPA</td>
<td>Mercapto-propionic acid</td>
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<td>MR</td>
<td>Magnetic Resonance</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>MUA</td>
<td>Mercaptoundecanoic acid</td>
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<td>MWIR</td>
<td>Mid wavelength infrared</td>
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<tr>
<td>NAC</td>
<td>N-Acetyl-Cysteine</td>
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<td>NHS</td>
<td>N-hydroxysuccinimide</td>
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<tr>
<td>Acronym</td>
<td>Definition</td>
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<td>------------------------------------------------</td>
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<tr>
<td>NIR</td>
<td>Near-Infra-Red</td>
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<td>NPs</td>
<td>Nanoparticles</td>
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<td>PET</td>
<td>Position emission tomography</td>
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<td>PL</td>
<td>Photo luminescence</td>
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<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
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<td>QD</td>
<td>Quantum dot</td>
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<td>QD750</td>
<td>Quantum dot emitting at 750 nm</td>
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<td>QY</td>
<td>Quantum Yield</td>
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<tr>
<td>ROI</td>
<td>Reactive oxygen intermediates</td>
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<td>SAD</td>
<td>Selected area diffraction</td>
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<td>SEM</td>
<td>Scanning Electron Microscopy</td>
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<td>SPECT</td>
<td>Single photon emission computed tomography</td>
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<td>SQUID</td>
<td>Superconducting quantum interference device</td>
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<td>SWIR</td>
<td>Short wavelength infrared</td>
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<tr>
<td>TAT</td>
<td>Trans-activator of transcription</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
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<tr>
<td>TGA</td>
<td>Thioglycolic acid</td>
</tr>
<tr>
<td>TSPETE</td>
<td>Triacetic acid trisodium salt</td>
</tr>
<tr>
<td>Vis</td>
<td>Visible</td>
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<tr>
<td>WSIO</td>
<td>Water soluble iron oxide</td>
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<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
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<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
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<tr>
<td>ZFC</td>
<td>Zero-field cooled</td>
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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

HYDROThERMAL SYNTHESIS OF NEAR-INFRA-RED EMITTING QUANTUM DOTS FOR FLUORESCENT AND MAGNETIC BIMODAL IMAGING

By

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August 2011

Chair: Brij M. Moudgil
Major: Materials Science and Engineering

Synthesis and characterization of water dispersible, near-infra-red (NIR) emitting and magnetic QDs of sizes between 3-6 nm for magnetic and fluorescent bimodal imaging are reported in this dissertation. QDs are semiconducting materials that exhibit quantum confinement with sizes below the excitonic Bohr radius of the material. NIR emitting QDs have potential to act as excellent probes for non-invasive monitoring of biological processes because NIR photons permit deep tissue penetration due to low absorption by water and other tissue components and also due to minimum tissue autofluorescence in the NIR wavelength regime of 700 – 900 nm. QDs synthesized by the conventional organometallic route require surface modifications with hydrophilic ligands to enable dispersion in aqueous biological conditions. However, these procedures result in significant reduction in their optical properties such as quantum yields (QYs). In this research 3-6 nm alloyed QDs were synthesized by heating the precursor solutions at 180 °C for various time intervals (30 – 100 min) under hydrothermal conditions. No separate ligand exchange steps for the QDs were necessary for water dispersibility. NIR emission tunability was achieved by modifying
the sizes of the QDs and also by developing a CdS rich shell on core CdTeS QDs. The alloy core and core/shell structure of the QDs were characterized using TEM, XRD, Energy dispersive X-ray spectroscopy (EDS) and XPS. The functionalization of the QDs with a non-toxic N-Acetyl-Cysteine (NAC) creates surface carboxylic acid groups which also allow subsequent bio-conjugation for targeted delivery. The QDs exhibited fluorescence in the visible-NIR 530-820 nm range and yielded high photoluminescence QYs with the maximum being about 60%. The functionality of the QDs was evaluated using *in vitro* mouse phantom experiments. The 800 nm emitting core/shell QDs exhibited bright photoluminescence inside the mouse phantom when excited with NIR light (710 – 745 nm) in the Xenogen IVIS® Spectrum Biophotonic Imager indicating their viability as NIR contrast agents.

In order to produce QDs that could also be traced by magnetic resonance imaging (MRI) the synthesis route was modified to develop novel magnetic QDs. This was achieved by controlled doping of the CdTeS QDs with Fe. Bimodal contrast agents with optical and magnetic properties integrated into one single nanoparticle (NP) resulted in a group of new materials that can be utilized in two highly complementary imaging techniques viz. fluorescence imaging and MRI. The fluorescent and magnetic Fe doped CdTeS QDs were characterized by superconducting Quantum interference device (SQUID) and MRI measurements. Fe doped QDs emitting between 530-740 nm exhibited QY within 40-60%. The saturation magnetization (M_s) values for QDs emitting at 740 nm and 730 nm were measured to be 2.8 emu/gm and 1.7 emu/gm, respectively, at room temperature. The relaxivity coefficient of the Fe doped QD emitting at 740 nm (732.4 mM⁻¹s⁻¹) was determined to be 88% higher than that for Feridex® I.V. (389.2 mM⁻¹
1 s⁻¹), a commercial magnetic contrast agent (now withdrawn). The performance of the magnetic QDs was determined by *in vitro* labeling with J774 macrophages. *In vivo* experiments were also performed by injecting QD labeled macrophages into the leg muscle of mouse followed by whole animal fluorescence imaging and MRI. Significant fluorescence and magnetic contrasts were generated by the QDs with respect to the neighboring tissues inside the animal. These 3-6 nm QDs because of their small size can be cleared from the blood circulation in a short time span. The magnetic QDs have significant potential as biological contrast agents because of their dual fluorescent and magnetic properties in addition to their small size.
CHAPTER 1
INTRODUCTION

1.1 Quantum Dots

1.1.1 What are Quantum Dots?

Quantum dots (QDs) are semiconducting materials in which the excitons (electron-hole pairs) are confined in all three dimensions¹. These nanoparticles (NPs) are smaller in size relative to the spatial extension of the holes and electrons in the bulk semiconductors and show quantum size effects. The size regime in which these effects are typically observed is 1-10 nm. The material properties of QDs lie in between molecules and bulk semiconducting materials.

1.1.2 Optical Properties and Quantum Confinement in QDs

QDs can vary in size from less than 1 nm to greater than 20 nm in diameter. Those with sizes less than 1 nm are nearly molecular and contain less than 100 atoms while those with diameter greater than 20 nm can contain more than 100,000 atoms¹. The emission wavelengths of QDs depend on their size due to quantum confinement, which is the change of the electronic states from semiconducting bulk materials to QDs (Figure 1-1)². In a bulk semiconducting material an exciton is typically bound within a certain length called the exciton Bohr radius. The properties of the semiconducting material change when the excitons are constrained further by changing the size of the semiconductor.

1.1.3 Visible-NIR QDs

Among the different properties that QDs exhibit the most striking is the optical property variation with size. With reduction of the size of a nanocrystal the electronic excitations transitions into higher energy. Thus tunability of emission wavelength is
attained with nanocrystal size variation. For example, the band gap of CdSe can be tuned from 1.7 eV to 2.4 eV with reduction of the cluster size from 200 Å to 20 Å and changes in light emissions in the visible wavelength regime of the electromagnetic spectrum from deep red to green\(^2\). The band gap of CdTe is tunable from 1.51 eV to 2.34 eV with change in emission wavelengths from NIR emission to visible green emission with change in particle diameter from 200 Å to 20 Å.

1.1.4 Why is the Study of QDs Important?

Interest in QDs is growing over the years because of their unique properties that include size tunable emission, good stability of the QDs against photobleaching relative to organic dyes and continuous size dependent absorption profiles from the ultraviolet wavelengths to the visible. Due to these properties QDs have numerous potential applications in biology and optoelectronics\(^3\)-\(^5\). They have attracted considerable research interest recently than conventional dyes due to their excellent photostability along with narrow and symmetric emission peaks and broad absorption spectra\(^3\)-\(^5\). Size tunable QDs can emit in different wavelength ranges. Visible QDs emit in the 400 – 700 nm while NIR QDs emit in the 700 – 900 nm wavelength regimes. The near-infrared (NIR) QDs emitting within the wavelength region 700 and 900 nm are of particular interest because in this range the tissue absorbance and autofluorescence are minimum leading to low background noise\(^7\).

In the field of life sciences investigation and understanding of various fundamental processes is dependent on reliable, fast and sensitive detection of the interactions of biomolecules among themselves and also with other ionic/molecular species. Fluorescence spectroscopy is one among the techniques available that is well suited to realize these goals. The different properties that are required in suitable fluorescent
label are\(^8\) (a) convenient excitability of the fluorophore alone without the biological matrix being excited simultaneously and it should be detectable using conventional techniques and instrumentation, (b) brightness i.e. high molar extinction co-efficient at the wavelength of excitation with high fluorescence QY, (c) solubility in cell culture media, body fluids and relevant buffers, (d) stability in relevant experimental conditions, (e) presence of functional groups for site specific labeling, (f) availability of reported data on its photophysics and (g) availability in reproducible quality. There are other requirements depending on the application of the fluorescent label. They are (h) size, steric effects, (i) toxicity, (j) possibility of the label to be delivered to cells, (k) suitability for multiplexing and (l) compatibility for signal amplification techniques.

These properties were compared for two different types of fluorescent labels viz. QDs of type II-VI (CdTe, CdSe, CdS etc.) III-V (GaAs, InP etc.) and organic dyes, which are widely used for diagnosis in medicine and biological analysis.

**1.1.5 Advantages of QDs over Dyes as Fluorescent Labels**

QDs in comparison to organic dyes have the desirable property of increased absorption at shorter wavelengths below the first excitonic peak. The emission bands of QDs are also narrower and symmetrical compared to the organic dyes. The quantum size effect phenomenon allows emission wavelength tunability in QDs by variation of size. The emission peak width is mainly a function of the particle size distribution. The broad absorption in QDs makes free selection of excitation wavelength possible, which allows for straightforward separation of emission from that of the excitation. The molar absorption co-efficient of QDs at the first absorption band are usually larger compared to the organic dyes. For QDs the typical values of the molar absorption coefficients are within the range of 100,000 – 1,000,000 M\(^{-1}\)cm\(^{-1}\) whereas for the dyes the typical values
lies within \(25,000 \text{–} 250,000 \text{M}^{-1}\text{cm}^{-1}\) at the main absorption band. In the visible wavelength regime (400 – 700 nm) the QY of QDs with proper surface-passivation are high in most cases. The QY reported for CdSe was within 65 – 85%, for CdS the value was less than 60% and for CdTe, CdHgTe in the NIR range of >700 nm was within 30 – 75%. For PbS and PbSe emitting above 800 nm the QY value ranges were 30 – 70% and 10 – 80% respectively. In comparison to QDs organic dyes have high fluorescence QYs in the visible range but moderate in the NIR wavelength regime. In NIR imaging applications QDs are favored over organic dyes because of the combined limitations of low QY along with low photostability of the NIR-wavelength dyes. The fluorescence lifetimes for QDs are typically large in the range of five to hundreds of nanoseconds compared to that of organic dyes which are about 5 ns and 1 ns (few exceptions such as acridone dyes) in visible and NIR wavelengths respectively. The extended lifetime of QD fluorescence also offers some advantages for using QDs over organic dyes in imaging applications that involve lifetime measurements.

1.1.6 Magnetic and Fluorescent Bimodal QDs

Bimodal QDs designed with two functionalities that of fluorescence and magnetic property, integrated in one single nanoparticle are an exciting class of new bioimaging materials. Size controllable multimodal nanoparticles/QDs ranging from a few nanometers to tens of nanometers are of particular interest because they are smaller than cells but comparable to viruses, genes and proteins. Because of the small size these particles can interact with biomolecules by crossing the biological membranes. These magnetic and fluorescent QDs can be used to perform two important but complementary diagnostic imaging of tissues and cells viz. fluorescent and magnetic
resonance imaging (MRI). Like their fluorescent counterparts the magnetic properties of NPs are different from bulk magnetic materials\textsuperscript{10}.

**Unique properties of magnetic NPs.** Magnetic nanoparticles (MNPs) exhibit variety of phenomena which are unique and drastically different from their bulk counterparts. These properties could be utilized for various applications which include their use as storage media in magnetic memory devices, as probes and vectors in biomedical research etc.\textsuperscript{10} The magnetic properties of the nanoparticles vary from bulk magnetic materials mainly in two aspects. The magnetic coupling/interaction of the surface atoms in MNPs with neighboring atoms will be different from those in bulk materials resulting in mixed surface and volume magnetic properties\textsuperscript{10}. This is due to the different local environment experienced by the surface atoms in MNPs which have large surface-to-volume ratio relative to the bulk magnetic materials.

Superparamagnetism is one of the size-dependent phenomena exhibited by magnetic NPs. The magnetic anisotropic energy (U) of bulk materials is much larger than the thermal energy (kT). However, for the small NPs the magnetic anisotropy energy is lower than the thermal energy (Figure 1-2). The thermal energy of the small NPs is high enough to readily invert the magnetic spin direction but it is not sufficient to overcome the spin-spin exchange coupling energy. This kind of magnetic behavior in NPs leads to a net magnetization of zero and is called superparamagnetism. The temperature at which the transition occurs from ferromagnetism to superparamagnetism is called blocking temperature ($T_B$). The property of superparamagnetism occurs for a single particle which is also a single domain. The magnetization behavior of ferromagnetic and superparamagnetic NPs in the presence and absence of an external
applied field is illustrated in Figure 1-3 (a). In the presence of external magnetic field
domains in the ferroelectric NPs aligned in the direction of the field. The single domain
of superparamagnetic NP also aligned with the external field. In the absence of the
external magnetic field the ferromagnetic NPs maintained a net magnetization whereas
there was no net magnetization for superparamagnetic NPs. In Figure 1-3 (b) the
relationship between the size of magnetic NPs and their magnetic domain structures is
indicated. MNPs with sizes below $D_s$ exhibit superparamagnetism and each particle is a
single domain. With NP sizes above $D_c$ the MNPs acquire multidomain structure.$^{11}$

For ferromagnetic materials the intensity of the applied magnetic field required to
bring the magnetization of the material to zero after it was subjected to saturation
magnetization is called coercivity ($H_c$) or coercive field/force of the ferromagnetic
material. The unit of coercivity is oersted or amp/meter. Magnetic coercivity of
ferromagnetic NPs is different from those of bulk ferromagnetic materials. NPs exist as
single magnetic domains below a critical size, $D_c$ where there is unidirectional alignment
of all magnetic spins. Coercivity of MNPs increase with size according to the
relationship$^{10}$,

$$H_c = \frac{2K_u}{m_s} [1 - 5 \left(\frac{kT}{K_u V}\right)^{\frac{1}{2}}]$$  \hspace{1cm} (1-1)

where, $m_s$ is the saturation magnetization, $K_u$ is the magnetic anisotropic constant and $V$
is the volume of NP. Multidomain magnetism exists for NP sizes greater than $D_c$ and a
small amount of magnetic field ($H_c$) is required to bring down the net magnetization to
zero.

**MRI using MNPs.** By using MRI 3 dimensional images of soft and opaque tissue
can be generated with relatively high tissue contrast and spatial resolution. Hence it is
the most versatile diagnostic imaging tool available in the clinic. The magnetic property of the MNPs enhances the sensitivity of MRI. Very high magnetic moment of the MNPs/QDs along with high transverse relaxivity values makes high detection sensitivity possible for MRI\textsuperscript{12}.

Contrast agents generate contrast in MRI by shortening the $^1$H relaxation times of the surrounding water\textsuperscript{12}. By the process of relaxation the protons in a magnetic field that were initially excited by a radio frequency magnetic pulse in the MRI scanner return to thermal equilibrium. The process of relaxation is divided into two principle relaxation types: longitudinal relaxation or spin-lattice (characteristic time $T_1$[s] or relaxation rate $R_1$[s$^{-1}$]) and transverse relaxation ($T_2$, $R_2$). Magnetic field inhomogeneities can accelerate the rate of relaxation in the transverse process. In such a case the relaxation process is referred as $T_2^*$ . For the $T_1$ weighted MR sequences, bright images are produced in areas with short $T_1$ (positive contrast). While for $T_2$ weighted MR sequences, dark images are produced in areas of short $T_2$ (negative contrast).

The strength of a contrast agent to produce contrast by accelerating the relaxation rate is defined by the change in the relaxation rate per unit concentration of the contrast agent. For longitudinal relaxation the proportionality constant is denoted by $r_1$ and for transverse relaxation the proportionality constant is denoted as $r_2$. Their unit is mM$^{-1}$s$^{-1}$, where $r_2 \geq r_1$. A contrast agent can never exclusively be a positive or a negative contrast agent because the $T_1$ and $T_2$ processes are not completely independent of each other. The ratio $r_2/r_1$ can be used to determine the suitability of a contrast agent either as $T_1$-weighted positive contrast agent or as $T_2$-weighted negative contrast agent. Contrast
agents with \( r_2/r_1 \) between 1 and 2 are usually suitable as \( T_1 \) contrast agent while those with ratio larger than \( r_2/r_1 \) are suitable as \( T_2 \) contrast agent\(^{12}\).

1.1.7 QD Toxicity

Toxicity of QDs has become an important area of research in recent years because of the emergence of studies involving biological imaging using QDs. As discussed earlier QDs are attractive compared to dyes because of their high photoluminescence QY and broad absorption spectrum which permits excitation with multiple wavelengths simultaneously. However, most of the QDs that are optically suitable for biological imaging are cadmium based materials. The most popular among QDs that possess high fluorescence QY for light emissions across the visible and near infrared wavelength regime of the electromagnetic spectrum are the CdSe and CdTe QDs. The elements of Cd, Se and Te using which the QDs were synthesized are considered to be highly toxic to cells and organisms\(^{13}\). These elements were thought to be released from the QDs in the form of ions when the surfaces of the QDs were oxidized. QD toxicity was attributed to leaching of Cd, Te, Se ions from the QDs and also due to the formation of reactive oxygen intermediates (ROI)\(^{14}\). Synthesis of DNA, RNA and proteins gets inhibited by Cadmium which is also responsible for damaging DNA strands and mutating chromosomes\(^{15-16}\). One way to minimize toxicity by QDs is to enclose the core QD with a shell and thus prevent the oxidation and leaching of ions from the QDs. But, core encapsulation is not full proof and there were reports\(^{13}\) on ions leaching from the cores and subsequent toxicity associated from the leached ions for even well protected cores. Reactive oxygen intermediates (ROI) were also reported to be responsible for some amount of toxicity. Encapsulation of QD cores can minimize leaching of toxic ions and mitigate toxic effects to some degree. However, toxicity due to
the produced ROIs is less controllable as the ROIs are produced due to the transfer of energy from QDs to molecular oxygen and can happen without any barrier\textsuperscript{17-19}.

1.2 Literature Review

1.2.1 Quantum Dot Applications, Synthesis and Characterization

QDs can be tailor made to fluoresce over a wide range of wavelengths which includes visible (400 – 700 nm) and NIR (700 – 1000 nm) wavelength regimes of light. Visible light emitting QDs have applications in light-emitting devices (LEDs)\textsuperscript{20-22}, photonic\textsuperscript{23-24} structures, solar cells\textsuperscript{25} etc. whereas NIR QDs have applications in biomedical and solar cells. Visible light emitting QDs have been widely researched during the last few decades and a large volume of literature on such QDs are available. The interest in NIR emitting QDs is newer in comparison to visible wavelength emitting QDs and the amount of literature available on their application, synthesis and characterization are relatively fewer than those available for the visible QDs. Here in this dissertation we will focus on the application, synthesis and characterization of NIR emitting QDs.

1.2.1.1 Applications and types of NIR QDs

While visible applications of QDs are popular, the need for QDs emitting in the NIR is also apparent. For example, solar cells can benefit from QDs that absorb across\textsuperscript{25} the visible and into the NIR. The wavelengths of light energy emitted by the sun span the visible (400 – 700 nm) along with NIR (700 – 1000 nm), short-wavelength infrared (SWIR) (1000 – 2000 nm) and mid-wavelength infrared (MWIR) (2000 – 8000 nm). While visible light accounts for only half of the sun’s energy, the remaining half lies beyond 700 nm into the IR region\textsuperscript{25}. Solar cells made of silicon can transform only 25% of the sun’s energy into electrical power; however, 41% of the sun’s energy can be
converted if solar cells are used containing layers of visible and infrared photovoltaic devices. This construct requires the integration of various semiconductor crystals of various compositions, band gaps, and lattice structures onto a single substrate. Lattice mismatch causes crystal strain and defects, resulting in less efficient solar energy conversion; thus, it becomes necessary to find materials which can minimize the lattice mismatch between the semiconductors. Varying the QD composition can allow for continuous light absorption through the visible and NIR because of broad QD absorption spectra. With a single semiconductor system composed of QDs, only their size needs to be changed to alter their optical properties; thus, the ability to precisely control size in a robust system using process control is vital to the scaled-up manufacturing of QDs.

In analytical research fluorescent probes are very popular however applying these labels for bioimaging of multicellular organisms poses a lot of challenges. These probes emit light mostly in the visible wavelength region which has poor transmission through animal tissues. Thus most of the emitted light from the probes will be attenuated. Also the scattering properties of tissues can decrease the signal intensity. In addition endogenous fluorophores like collagen emit in the visible wavelength spectrum (400 -700 nm) which is also the wavelength spectrum for visible fluorophores. This can produce an overlap of the signals making the detection of visible fluophores difficult. Fortunately, there exists a clear wavelength region between 650 – 900 nm in most biological tissue where tissue absorption is the lowest with low Rayleigh scattering which make this region suitable for fluorescence imaging (Figure 1-4). This allows NIR emission wavelengths to penetrate tissues deeply leading to excellent non-invasive imaging applications. QDs emitting in the near-infra red (NIR) region of 700-900 nm is
useful for biomedical applications because of minimal autofluorescence and maximum
tissue penetration in this wavelength range\textsuperscript{27}. NIR QDs can also be used in light-
emitting devices (LEDs), which have potential applications in the medical field.
Photobiomodulation is a therapy that uses low intensity light in the far red to NIR (630-
1000 nm) to modulate various cellular functions\textsuperscript{28}. These NIR LEDs can accelerate
wound healing and attenuate degeneration of damaged optic nerves. They can also
improve healing of ischemic injury of the heart. All of these effects happen due to the
improvement in the mitochondrial energy metabolism and production when subjected to
NIR light\textsuperscript{28}. QDs can even potentially be used as photosensitizers\textsuperscript{29-30}.

1.2.1.2 Types of NIR QDs

NIR QDs can be of different types depending on their composition. Typically NIR
QDs are made from II-VI, III-V and IV-VI binary alloys. However, research on core/shell
and ternary alloy (AB\textsubscript{x}C\textsubscript{1-x}) QDs are increasingly being reported\textsuperscript{31}.

**Binary alloy NIR QDs.** The QDs of type II-VI forms the largest group among QDs.
The Cd based QDs belong to this group. The sulfide, telluride and selenide QDs of Cd,
Zn and Hg are uniform in shape and size compared to other QDs. At room temperature
they also display sharp absorption profiles with good emission features\textsuperscript{7} relative to other
QDs. But the number of NIR wavelength emitting II-VI type QDs are less compared to
visible wavelength emitting QDs. The QDs containing Hg are highly toxic for the
environment which limits their application. CdTe QDs can emit NIR light around the 800
nm wavelength region. From Table 1-1 it is evident that NIR emitting CdTe QDs
synthesized by the organometallic technique has a QY less than 20% whereas some of
the QDs synthesized using aqueous techniques has higher QY > 50%. Thus by
aqueous synthesis it is possible to obtain good quality QDs with good emission properties.

The III-V type QDs can have NIR emissions. InAs and GaAs QDs fall under this category. These QDs are mainly used in optoelectronic devices. Alivisatos\textsuperscript{32} group reported the fabrication of InAs QDs having sizes within 2.5 nm and 6 nm by the organometallic synthesis technique. The synthesis of III-V QDs which emit in the NIR wavelength region is complicated and difficult\textsuperscript{7} compared to II-VI type QDs. The QDs synthesized have wide size distribution, poor stability and low fluorescence efficiency with QY of 2.5%. Hence the literature available on the synthesis of III-V type QDs is much less than that available for II-VI type QDs.

The IV-VI type NIR emitting QDs are Pb based materials viz. PbTe, PbSe and PbS. These Pb based QDs are environmentally hazardous and are thus less attractive for applications. However, these QDs can be easily size tuned in the NIR or infra-red (IR) wavelength regions.

**Core/shell NIR QDs.** Core/shell QDs are especially desirable for bio-imaging because thick shell QDs exhibit less blinking and greater photostability\textsuperscript{33}. Recently some researchers\textsuperscript{34-36} reported the synthesis of thick shelled NCs with suppressed blinking in the single particle level. The reported core/shell QDs were all prepared using multistep organometallic techniques. Deng et al.\textsuperscript{33} reported the fabrication of core/shell QDs in the aqueous phase.

The core/shell QDs have a structure that resembles an onion with layers. More than two semiconducting materials are used in core/shell QDs. In these QDs the basic fluorescence properties are controlled by the core. Core/shell QDs containing the
different semiconducting compound materials viz. II-VI, III-V and IV-VI can be classified as type I, reverse type I and type II depending on the band alignment in core/shell QDs. The bandgap of the shell material along with the relative electronic energy level positions of the core and the shell are responsible for the different optical properties of the core/shell material. In case of type I core/shell QDs the band gap of the shell is larger than that of the core and in this case all electrons and holes remain confined in the core. In reverse type I alignment the shell has smaller band gap than that of the core. For the reverse type I core/shell QDs the thickness of the shell determines whether the confinement of the electrons and holes inside the shell will be complete or partial. In case of type II core/shell QDs the band alignment is staggered with the result that the effective band gap of the material is smaller than either core or shell\textsuperscript{37}. The staggered alignment of the band gaps results in the spatial separation of the holes and electrons in different regions inside the core/shell QDs (Figure 1-5)\textsuperscript{38}.

The shell in type I QDs is used to minimize the surface defects in order to improve their fluorescence efficiency. The dangling bonds at the surface are reduced by the growth of the shell. These dangling bonds when present can minimize the QY of the QDs by acting as surface trap states for charge carriers. Another function of the shell in type I core/shell QDs is that it separates the optically active core physically from the surrounding environment. Thus the effect of surrounding water or oxygen molecules on the surface of the QDs is minimized\textsuperscript{37}. One example of this system is CdSe/ZnS. In this case the ZnS shell improves the fluorescence QY significantly and also contributes toward stability against photobleaching. The shell growth gives rise to small red shift in emission wavelength by 5-10 nm which is due to partial leakage of electrons from the
core into the shell\textsuperscript{37}. In reverse type I core/shell QDs, the shell material has a narrower band gap than the core. The charge carriers in this system are delocalized at least partially into the shell material and the wavelength of emission can be fine tuned by varying the thickness of the shell. The change is shell thickness is usually accompanied by a red-shift in the emission wavelength. ZnSe/CdSe\textsuperscript{39}, CdS/HgS\textsuperscript{40} and CdS/CdSe\textsuperscript{41} are the most extensively studied systems of this type. Photobleaching properties and QY for these QDs can be improved by coating these particles with another shell over their core/shell structure.

In the core/shell type II system, the growth of the shell leads to significant redshift of the wavelength of emission. The effective band gap of the material due to the staggered alignment is reduced to a value that is smaller than either of the core or the shell material. Interest in having this kind of system lies in the tunability of the emission wavelength to values which will be difficult to achieve in other systems. These type of QDs can have NIR emissions. Some common examples are CdTe/CdSe and CdSe/ZnTe. Relative to type I QDs, type II systems have long PL decay times due to smaller overlap of excitonic wavefunctions. In this case either the hole or electrons will be located in the shell and a second shell will be required to improve their fluorescence efficiency and photostability.

**Ternary alloy NIR QDs.** Alloyed quantum dot semiconductors \((AB_xC_{1-x})\textsuperscript{31}\) have potential in research areas related to nanoscale engineering due to their physical and optical properties which can be continuously tuned by varying their chemical composition variable \(x\). This allows for an added degree of freedom by which the exciton energy of the QDs can be fine-tuned keeping size unchanged to obtain emission
wavelength ranges that may be difficult to achieve using binary QDs. Different other properties can be achieved in these QDs by fine tuning the composition either homogeneously or by producing a gradient throughout the nanocrystal. The fluorescence properties of these QDs are good and on par with binary QDs\textsuperscript{31}. The different areas where these QDs can find applications include LEDs\textsuperscript{42}, bio-imaging\textsuperscript{43-46} and solar cells\textsuperscript{47-48}. In case of LEDs the QDs should emit at a specific wavelength, for bio-imaging the QDs are required to be of small size with emission wavelength in the NIR region while for the solar cells wide absorption wavelength range with small size are preferred. All of these properties are achievable with alloy core QDs.

Synthesis of various II-VI alloyed nanocrystals have been reported viz. CdS\textsubscript{x}Te\textsubscript{1-x}, Cd\textsubscript{x}Zn\textsubscript{1-x}Se\textsuperscript{49-50}, CdS\textsubscript{x}Se\textsubscript{1-x}\textsuperscript{51}, Cd\textsubscript{x}Zn\textsubscript{1-x}S\textsuperscript{52}, CdSe\textsubscript{x}Te\textsubscript{1-x}\textsuperscript{43} and HgSe\textsubscript{x}S\textsubscript{1-x}\textsuperscript{53}. These materials can be categorized into two groups according to the elements they contain.

a. The compounds in this group contain one transition metal element along with two chalcogen atoms – CdS\textsubscript{x}Te\textsubscript{1-x}, CdS\textsubscript{x}Se\textsubscript{1-x}, CdSe\textsubscript{x}Te\textsubscript{1-x} and HgSe\textsubscript{x}S\textsubscript{1-x}

b. This group will contain compounds having two transition elements and one chalcogen atom - Cd\textsubscript{y}Zn\textsubscript{1-x}Se and Cd\textsubscript{y}Zn\textsubscript{1-x}S

It has been reported in recent articles that the ratio of the chalcogen atoms in the first group of alloys has a significant non-linear influence on the properties of these materials. The properties of the second group of alloys change quasi-linearly with their compositions. The non-linear effects, called "optical bowing" have been reported previously for bulk semiconductors and explained by Bernard and Zunger\textsuperscript{54}.

1.2.1.3 Synthesis and characterization of NIR QDs

The conventional organometallic route developed by Murray\textsuperscript{55} is currently the most widely used technique for synthesizing QDs. However, despite numerous efforts by various researchers, preparation of high quality NIR QDs by the organometallic process
that will be useful for bioimaging applications remained elusive\textsuperscript{33}. There are reports on the synthesis of various NIR QDs viz. CdTeS/ZnS, InAs etc. by the organometallic synthesis techniques. However for using them in biological applications the surface of these QDs need to be modified using hydrophilic ligands in aqueous solution. This is a cumbersome process which gives rise to several drawbacks like low QY and limited stability.

Typical NIR QDs that have been synthesized by various researchers are listed in Table 1-1. This table is adapted from the review paper by Ma et al.\textsuperscript{7} (with some modifications) and contains information on emission wavelength, size, capping agent, applications, synthesis technique and QY of the QDs. Thiol coated II-VI quantum dots synthesized by aqueous synthesis processes provide an alternative route to the conventional synthesis processes that use high boiling organic solvents. Thiol usage in QDs is useful not only for surface functionality leading to high dispersibility in water but also for controlling the kinetics of particle formation, stability in solution and passivation of surface dangling bonds\textsuperscript{81}. Various applications of thiol coated QDs have been reported viz. in optoelectronics like light emitting diodes (LEDs), photosensitive films. They are useful for bioimaging applications. In the following section some of the published research on NIR emitting QDs synthesized using different thiol ligands viz. mercaptopropionic acid (MPA)\textsuperscript{78}, N-Acetyl cysteine (NAC)\textsuperscript{79} and thioglycolic acid (TGA)\textsuperscript{80} etc. are discussed.

CdTeS alloyed QDs emitting in the NIR wavelength range and capped by MPA was reported by Mao et al.\textsuperscript{78} Hydrothermal synthesis technique was used for the preparation of the QDs. The synthesis process was carried out at a temperature of 180
°C. The QDs synthesized emitted wavelengths ranging from 530 – 800 nm. The maximum QY of 68% was achieved for QDs having emission wavelength of 726 nm. The authors discussed the effects of precursor concentrations on the emission wavelength of the synthesized QDs. The 800 nm emitting QDs were obtained for CdCl₂ concentration of 30 mM. With lower CdCl₂ precursor concentration of 2, 10 and 20 mM only smaller QDs with emission wavelengths much less than 800 nm were obtained.

Zhao et al.\(^ {79}\) first reported the synthesis of water dispersible, NIR emitting CdTe/CdS QDs using NAC as the capping agent by the hydrothermal process at 200 °C. The QDs synthesized have emission wavelengths varying from 652 nm and 795 nm. The QY of the QDs varied within 45-62%. HK-1 cells were labeled using QDs emitting at 685 nm and subjected to fluorescence imaging to demonstrate the potential of these QDs for bio-imaging applications.

Zhang et al.\(^ {80}\) synthesized CdTe nanocrystals using hydrothermal technique from CdCl₂ and NaHTe solution with TGA as the capping agent. The as prepared nanocrystals emitted wavelengths varying from green to red. The highest QY achieved for the nanoparticles was 30%. The nanoparticles were then utilized for labeling L929 mouse cells.

Rogach et al.\(^ {81}\) reported the synthesis of thiol capped CdTe nanocrystals by aqueous method. In this process they used Cd(ClO₄)₂ as the source of Cd and H₂Te gas as the source of Te with MPA as the stabilizer. The emission wavelengths of the nanoparticles synthesized varied within 500 – 800 nm with 40 – 60% QY (QY). The authors compared the effects of using two different stabilizers, TGA and MPA on the optical properties of the synthesized nanoparticles. They observed that by using MPA
nanoparticles with sizes more than 4.5 nm emitting upto 800 nm can be prepared which is not possible for TGA capped nanoparticles. By using TGA maximum emission wavelength of 750 nm was achievable.

There are only a few reports on the aqueous synthesis of core/shell NIR emitting QDs. Deng et al.\textsuperscript{33} published a paper on the multistep synthesis of MPA capped core/shell CdTe/CdS QDs using aqueous techniques. The core/shell QDs were tetrahedral in shape and emitted NIR wavelengths upto 820 nm. The QDs were synthesized using various temperatures within 20 - 90 °C for different time intervals. The maximum QY achieved was 70% for the 715 nm emitting QDs.

Qian and co-workers\textsuperscript{74} reported the synthesis and characterization of core/shell CdHgTe/CdS QDs by a multistep process. The precursors used for the synthesis were CdCl\textsubscript{2}, Hg(ClO\textsubscript{4})\textsubscript{2} and NaHTe in the presence of MPA as stabilizer. The photoluminescence (PL) exhibited by the QDs ranged from 600 – 830 nm while the QY varied within 20-50%.

Type II NIR emitting core/shell CdTe/CdSe QDs were synthesized in the aqueous system by Zhang et al.\textsuperscript{80}. The QDs can emit within the wavelength range of 613-813 nm. The maximum QY achieved for these particles was 12%.

He et al.\textsuperscript{82} developed aqueous NIR emitting CdTe QDs using a microwave synthesis process. The QDs exhibited PL in the 700-800 nm wavelength regimes with QY variation within 15-20%. These QDs were conjugated to antibodies and then used subjected to in-vivo imaging inside mouse. The authors demonstrated that these QDs have potential as NIR emitting optical contrast agents for biological applications.
1.2.2 Magnetic NPs

During the last two decades scientists and researchers investigated numerous NPs as contrast agents for MRI. Magnetic iron oxide NPs have been extensively studied by the researchers as MRI contrast agents as they have the ability to reduce the T$_2^*$ relaxation time in animal tissues. Iron oxide NPs can be classified into micrometer sized paramagnetic iron oxide (MPIO; several micrometers), superparamagnetic iron oxide (SPIO; hundreds of nanometers) and ultrasmall superparamagnetic iron oxide (USPIO; less than 50 nm) depending on their particle sizes. SPIOs coated with dextran have been clinically used for the diagnosis of liver diseases because of the selective uptake of SPIOs in the Kupffer cells of liver, bone marrow and spleen. USPIOs were used for imaging lymph nodes. However, the T$_2$ contrast agents have several disadvantages for clinical applications. The T$_2$ contrast agents being negative imaging agents produce a signal decreasing effect. There are possibilities of confusing the dark signal produced by the contrast agents with other pathogenic conditions. Thus T$_2$ contrast agents produce images of lower contrast than those produced by T$_1$ contrast agents. T$_2$ contrast agents also possess susceptibilities high enough to distort the magnetic field on the surrounding normal tissues. This produces obscure images and demolishes the background of the region of interest.

To overcome the limitations of the iron-oxide based T$_2$ contrast agents researchers recently conducted extensive research to develop T$_1$ contrast agents based on NP technology. The T$_1$ contrast agents are mostly Gadolinium complexes conjugated to nanostructured materials viz. dendrimers, silicas, perfluorocarbon NPs and nanotubes. Researchers also have investigated Gadolinium compounds such as Gd$_2$O$_3$, GdF$_3$ and GdPO$_4$ as T$_1$ MRI contrast agents. Besides Gadolinium compounds
other T₁ contrast agents like MnO NPs have also been investigated. The various properties of the NP based T₂ and T₁ contrast agents, viz. magnetization and relaxivity coefficients (r₁, r₂) are listed in Tables 1-2 and 1-3 respectively.

1.2.3 Bimodal – Fluorescent and Magnetic NPs

Bimodal nanoparticles, with magnetic and fluorescent functionalities broaden the applicability of nanocrystals in different applications like bioseparation, pathogenic detections, immunoassay along with multimodal imaging. These particles can be classified into four different categories depending on their structure and composition.

Type A – Core/shell and heterostructures. In this type the QD and the MNPs form a fused heterostructure or core/shell. Magnetic and semiconductor nanocrystals usually have a large lattice mismatch. However, inspite of this mismatch, researchers demonstrated that combination of the two materials into a single nanocrystal is possible. Gao et al. demonstrated the synthesis of ~3 nm core FePt nanoparticles with a ~3-5 nm thick shell of CdSe or CdS. The NPs were synthesized by the organometallic technique. These nanoparticles emitted at wavelengths around 465 nm and had a QY of 7-10% which was much lower relative to the CdSe QDs alone. The synthesized particles were paramagnetic and had a blocking temperature of 14K. The quenching of fluorescence was due to the interaction of the magnetic core with that of the fluorescent shell. Gu et al. reported FePt-CdS heterodimers with magnetofluorescent properties and synthesized using the organometallic process. According to the authors the FePt/CdS core/shell initial structure transformed on heating into a heterodimer structure. The final product emitted at 438 nm and had a QY of 3%. They were superparamagnetic with a coercivity of 0.85 kOe at 5K and had a blocking temperature of 11K.
**Type B – Doped QDs.** Fluorescent and magnetic NPs can be synthesized by doping fluorescent QDs with paramagnetic ions. This is a direct synthetic procedure by which dual functionalities like fluorescence and magnetic properties can be achieved in a single nanoparticle. Doping paramagnetic ions into semiconductors have been tried by various researchers over several decades\textsuperscript{102}. Although some successes have been reported however for unknown reasons most efforts in doping crystals have failed\textsuperscript{103}. Bhargava et al.\textsuperscript{104} published one of the first reports on doped semiconductor nanocrystals after which research in this field increased rapidly. In this publication the authors reported the optical properties of ZnS nanocrystals of size 3.5 to 7.5 nm doped with Mn\textsuperscript{2+}. The nanocrystals emitted in the 590 nm region and exhibited QY of 18%. Doped nanocrystals can be of two types:

**Type B (i) – Paramagnetic ion doped in QDs.** During the last few years synthesis and characterization of various doped magnetic QDs of this type have been reported\textsuperscript{105-109} viz. ZnS:Mn\textsuperscript{2+}, ZnO:Co\textsuperscript{2+}, ZnSe:Mn\textsuperscript{2+}, CdSe:Mn\textsuperscript{2+}, CdS:Mn/ZnS. These nanoparticles emit light in the visible wavelength range. One important aspect in this research is the doping of the paramagnetic ion into the core of the semiconducting nanoparticle. Proving the location of the dopant ion inside the core or on the outside surface of the particle is difficult. Various characterizing techniques viz. extended X-ray absorption fine structure (EXAFS) and electron paramagnetic resonance (EPR) have been used to verify the location of the dopant\textsuperscript{110}. The potential of doped nanocrystals for bioimaging has been realized. Santra et al.\textsuperscript{109} demonstrated the use of CdS:Mn/ZnS QDs for multimodal imaging. These NPs were synthesized using the microemulsion technique and were 3.1 nm in size. The QDs emitted at wavelength ~ 575 nm and
exhibited a hysteresis loop when characterized using SQUID. These QDs were imaged in-vivo using MRI after they were conjugated with HIV-1 trans-activator of transcription (TAT) peptide and injected into the brain of a rat. Details of the MRI measurements were not reported.

Yong reported the preparation of fluorescent MNPs that emit in the NIR wavelength region\(^1\). To the best of our knowledge this is the only published report on magnetic NIR emitting QDs. The authors reported the synthesis and characterization of Mn-doped CdS-capped CdTe\(_{0.25}\)Se\(_{0.75}\) QDs of size 4-5 nm by the organometallic synthesis route. The amount of Mn doping in the QDs was 3 atom\%. The QDs emitted at 822 nm with a QY of 15\%. The Mn doped QDs exhibited hysteresis and had coercivity of 125 G at room temperature.

**Type B (ii) – Paramagnetic ion doped in the shell of core/shell QDs.** In this type the paramagnetic ions were doped inside the shell of the core/shell QDs. Wang et al.\(^1\) reported the synthesis of CdSe/Zn\(_{1-x}\)Mn\(_x\)S QDs which have both magnetic and fluorescent properties. The ~ 5 nm QDs were synthesized using the organometallic technique and emitted within 570 – 650 nm wavelength range. The QDs had a QY of more than 20\%. The Mn\(^{2+}\) ions were located inside the CdS shell and exhibited \(r_1\) relaxivity values of 10 – 18 mM\(^{-1}\)s\(^{-1}\). The suitability of these QDs for bimodal imaging was assessed by attaching these QDs with macrophages imaging these macrophages using fluorescence spectroscopy and MRI.

**Type C – Integrated composite particles containing semiconducting and magnetic nanoparticles.** In this type a carrier material contains two different nanoparticles, one magnetic and the other fluorescent integrated into a single entity.
This type of nanoparticles can be further classified into two types depending on their structure.

**Type C (i) – Incorporation of the fluorescent and magnetic nanoparticles inside the carrier material.** In the type C (i) case the carrier material either contains all the nanoparticles inside it or attached to its outside. The carrier material can be silica or polymer based particle. The final particles containing the fluorescent and MNPs entities are usually larger than the type A or type B particles.

Yi et al.\textsuperscript{113} demonstrated the synthesis of silica particles of size ~ 50 nm that are doped with CdSe/ZnS (size 3.5 nm) QDs and iron oxide NPs (size 12 nm) by a microemulsion synthesis technique. The composite particle exhibited PL at 554 nm with a QY of 5%. The QY of CdSe/ZnS QDs decreased from 15% to 5% on being incorporated into the silica matrix. The reason for the decrease of QY was probably the interaction of the QDs with iron oxide or due to the incorporation of the QDs in the silica shell by the microemulsion process. Magnetization values reported were 60-80 emu/g\(\gamma\)Fe\(_2\)O\(_3\) at 5K and and 40-60 emu/g\(\gamma\)Fe\(_2\)O\(_3\) at 300K. Kim et al.\textsuperscript{114} described a similar process of incorporating CdSe/ZnS QDs and iron oxide NPs inside 150 nm silica matrix. The composite particle showed both fluorescent and magnetic properties.

**Type C (ii) – Incorporation of QDs inside the carrier material with MNPs attached to the outside of the carrier material or vice versa.** The interaction of the QDs with MNPs results in the decrease of the fluorescence QY of the QDs which makes the composite particles less bright. One way to overcome this limitation is to increase the distance between these particles inside the composite material. Maceira et al.\textsuperscript{115} synthesized a composite particle where the MNPs were incorporated into silica particles.
70 nm in size while the QDs were attached to the surface of the silica particles using charged polymers. The resulting silica particles carrying the MNPs and QDs were further coated with a 20 nm silica shell. The final particle was 220 nm in diameter. The authors maintained that quenching of the QDs were prevented using this technique. Two similar particles were synthesized which emitted at 567 nm and 623 nm. Magnetic measurements were carried out on the particles. Saturation magnetization of 1.34 emu/gm and a coercivity of 175 Oe (at 5K) along with a blocking temperature of 150 K were recorded.

**Type D – Paramagnetic Gd coated on QDs using chelates.** Gd$^{3+}$ is used as a paramagnetic $T_1$ contrast agent for MRI. It has a high magnetic moment and its ground state is symmetrical. There is toxicity issues associated with Gd$^{3+}$. Hence, to decrease the effect of toxicity it is often used along with organic chelates in the form of a Gd-complex. Chelated Gd-DTPA (diethylenetriamine pentaacidic acid) is a widely used contrast agent. However, there are many other compounds which are being studied for better relaxivity properties and varied biomedical applications$^{116}$. The paramagnetic chelated complexes are attached to dyes and QDs to impart fluorescent property to the complex. The attachments of the Gd-complexes to the fluorescent materials are done by covalent/non-covalent bonding. Gd-complexes can be directly attached to the QDs or they can be attached on the shell material surrounding the QDs. Depending on the how the Gd-chelates are attached to the QDs the Gd-chelate-QD-composite particles can be divided into two types.

**Type D (i) – Gd-chelates attached to QDs.** Mulder et al.$^{117}$ first reported Gd-chelate attachments to QDs. The QDs were surrounded by a lipidic micelle to facilitate
non-covalent attachment of the Gd-chelates to the QDs. The composite particle synthesized emitted at around 560 nm and exhibited $r_1$ relaxivity of 12.4 mM$^{-1}$s$^{-1}$ and $r_2$ relaxivity of 18 mM$^{-1}$s$^{-1}$. The $r_2/r_1$ ratio was 1.5 which is good enough for these particles to be suitable for imaging in the T$_1$ mode.

**Type D (ii) – Gd-chelates attached to the shell surrounding the QDs.** The number of paramagnetic ions present on the surface of the QDs will determine the overall relaxivity of the composite particles. Hence it will be useful to increase the surface area of the particles by coating them with a shell and then attaching the paramagnetic ions on the shell. Yang et al.$^{118}$ reported the synthesis of 3 nm CdS:Mn/ZnS QDs coated with 7 nm thick silica shells. Gd ions were chelated with Triacetic acid trisodium salt (TSPETE) and the Gd-TSPETE complex was attached to the silica shell coating on the surface of the QDs. The QY of the composite particles emitting yellow light was 28% and the $r_1$, $r_2$ relaxivity values reported were 20.5 and 151 mM$^{-1}$s$^{-1}$ respectively. The $r_2/r_1$ ratio is 7.4 indicating that the composite particles are most suitable as T$_2$ contrast agents.

### 1.2.4 QD Toxicity

The nature of cadmium, selenium and tellurium whether bounded covalently as CdTe/CdSe in the QDs or released as free ions will determine the toxicity of the QDs.$^{119}$ Cadmium toxicity studies on animals were carried out by Manca et al.$^{120}$ Rats were administered 25-1250 µg cadmium/kg in the form of CdCl$_2$. The Cd dosage was found to be toxic to the animals which were sacrificed 24 hours after treatment. Lin et al.$^{119}$ carried out experiments to investigate the chemical fate of Cd/Se/Te based QDs emitting at 705 nm on mice. The authors reported that 100% of the QDs were retained in the body after 16 weeks of exposure and free Cd was released from the QDs. Su et
investigated short and long term toxicity of CdTe QDs synthesized by the aqueous method. CdTe QDs of concentration 0.2 nmol and volume 0.1 ml was intravenously injected into the tail vain of mice. Post injection the QDs accumulated in the liver in a short time span of (0.5 - 4h) and then into the kidneys in increasing amounts after (15 - 80 days). However there was no overt toxicity from the QDs even after 80 days of exposure.

The first in-depth research on the toxicity effects of CdSe QDs coated with various coatings on hepatocytes was reported in 2004 by Derfus and coworkers. CdSe QDs coated with mercaptoacetic acid (MAA) and prepared by organometallic synthesis technique and kept in an inert atmosphere showed no toxicity. However, when these QDs were exposed to air prior to MAA coating, the viability of the cells decreased with increasing dosage of QDs. The reason for cell viability decrease was attributed to leaching of Cd ions due to the surface degradation following surface oxidation. Photoluminescence data indicating blue shifting of emission wavelength due to the decrease of particle size and lower peak amplitude reinforced the finding by the authors that the surface was degraded by oxidation. Release of Cd and associated cytotoxicity was also increased with increasing exposure of QDs to UV light. MAA coated CdSe/ZnS core/shell QDs were found to be less cytotoxic than bare QDs when they were subjected to oxidation solely by air. Some degradation of the ZnS coating was observed while the core CdSe remained unaffected. The stability of the same QD decreased when exposed to UV light and was expected to be more cytotoxic. The authors concluded that the QDs can be safely used in the cells without much damage if used in low concentrations and subjected to limited UV exposure.
The effect of QD capping agent on cytotoxicity was also investigated by the authors\textsuperscript{13}. The capping ligands used for the study were Mercaptoundecanoic acid (MUA), thioglycerol and cysteamine. Cytotoxicity of QDs coated with a mixture of these capping ligands was also evaluated. Cell deaths were reported for QDs coated with MUA. QDs coated with other ligands did not show significant cell death and DNA damage. The authors concluded that cytotoxicity of QDs were due to coatings and not due to the material composition.
<table>
<thead>
<tr>
<th>QDs (core/shell)</th>
<th>Emission peak/nm</th>
<th>Size/ nm</th>
<th>Modification strategy or coupling reagent</th>
<th>Area of application</th>
<th>Synthesis Approach (Reference)</th>
<th>Quantum yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>CdTe/CdS</td>
<td>715</td>
<td>6-7</td>
<td>3-Mercaptopropionic acid-capped</td>
<td>— (^a)</td>
<td>AS(^c) (33)</td>
<td>70%</td>
</tr>
<tr>
<td>InAs/ZnS</td>
<td>750</td>
<td>4–16</td>
<td>Coated with different lengths of short chain PEGs</td>
<td>Organ-and tissue-selective biodistribution</td>
<td>OR(^b) (56)</td>
<td>20%</td>
</tr>
<tr>
<td>InAs</td>
<td>820–1240</td>
<td>2–6</td>
<td>TOP-capped</td>
<td>— (^a)</td>
<td>Colloidal chemical synthesis (57)</td>
<td>2.50%</td>
</tr>
<tr>
<td>InAs/CdSe</td>
<td>700–1400</td>
<td>3–5</td>
<td>TOP-capped</td>
<td>— (^a)</td>
<td>OR(^b) (58)</td>
<td>Up to 90%</td>
</tr>
<tr>
<td>InAs/ZnCdS</td>
<td>700–900</td>
<td>10</td>
<td>(1) Coupled with poly(ethylene glycol) (2) Covalent conjugation of streptavidin</td>
<td>Imaging of HeLa cells in vivo</td>
<td>OR(^b) (59)</td>
<td>35–50%</td>
</tr>
<tr>
<td>InAs/InP/ZnSe</td>
<td>800</td>
<td>15.9</td>
<td>(1) Encapsulated with poly(ethylene glycol) (2) Coupled with arginine-glycine-aspartic acid or arginine-alanine-aspartic acid peptides</td>
<td>Imaging of subcutaneous U87MG tumor</td>
<td>OR(^b) (60)</td>
<td>19%</td>
</tr>
<tr>
<td>PbS</td>
<td>900–1600</td>
<td>5.3</td>
<td>Mercaptoundecanoic acid-capped</td>
<td>Imaging of HT29 cells</td>
<td>AS(^c) (61)</td>
<td>10%</td>
</tr>
<tr>
<td>PbS</td>
<td>700–1600</td>
<td>10 ± 0.5</td>
<td>Straightforward mercaptan-PEGylation ligand exchange</td>
<td>— (^a)</td>
<td>OR(^b) (62)</td>
<td>26%</td>
</tr>
<tr>
<td>PbSe</td>
<td>1200–1500</td>
<td>7–12</td>
<td>Coated with a silica shell</td>
<td>Imaging of NIH-3T3 cells and HepG2 cells</td>
<td>OR(^b) (63)</td>
<td>— (^a)</td>
</tr>
<tr>
<td>CdTe/CdSe</td>
<td>750</td>
<td>7</td>
<td>Loaded in PLGA nanosphere</td>
<td>— (^a)</td>
<td>OR(^b) (64)</td>
<td>— (^a)</td>
</tr>
<tr>
<td>QDs</td>
<td>Emission peak/nm</td>
<td>Size/nm</td>
<td>Modification strategy or coupling reagent</td>
<td>Area of application</td>
<td>Synthesis Approach (Reference)</td>
<td>Quantum yield</td>
</tr>
<tr>
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<td>-------------------------------------------</td>
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</tr>
<tr>
<td>CdTe/CdSe</td>
<td>743</td>
<td>9.5</td>
<td>L-Cysteine-capped</td>
<td>Fixed HeLa cell staining</td>
<td>Layer-by-layer (65) epitaxy approach</td>
<td>8%</td>
</tr>
<tr>
<td>CdTe/CdSe</td>
<td>752.2</td>
<td>4.5</td>
<td>Thiol-capped</td>
<td>Sensing of copper</td>
<td>ASc (66)</td>
<td>11.40%</td>
</tr>
<tr>
<td>CdTe/CdSe</td>
<td>840–860</td>
<td>10</td>
<td>Oligomeric phosphine organic coatings</td>
<td>Sentinel lymph node mapping</td>
<td>ORb (67)</td>
<td>— a</td>
</tr>
<tr>
<td>CdTe/CdSe</td>
<td>750</td>
<td>5</td>
<td>Cysteine-capped</td>
<td>Detecting cysteine, homocysteine and glutathione</td>
<td>Layer-by-layer (68) epitaxy approach</td>
<td>— a</td>
</tr>
<tr>
<td>CdSe/CdTe/ZnSe</td>
<td>850–1100</td>
<td>6</td>
<td>3-Mercaptopropionic acid-capped</td>
<td>— a</td>
<td>ORb (69)</td>
<td>60%</td>
</tr>
<tr>
<td>CdTeSe</td>
<td>750</td>
<td>5–6</td>
<td>Aminoethanethiol-capped</td>
<td>— a</td>
<td>Chemical aerosol flow synthesis (70)</td>
<td>— a</td>
</tr>
<tr>
<td>CdSe0.25Te0.75/CdS</td>
<td>859</td>
<td>7</td>
<td>Coated with lysine</td>
<td>Long-term targeted imaging in vivo</td>
<td>Hot colloidal synthesis approach (71)</td>
<td>10–15%</td>
</tr>
<tr>
<td>CdTe1–xSexCdS</td>
<td>650–850</td>
<td>— a</td>
<td>Exchanged with mercaptoundecanoic acid</td>
<td>Multiplexed imaging</td>
<td>ORa (72)</td>
<td>— a</td>
</tr>
<tr>
<td>CdSeTe/CdS</td>
<td>785</td>
<td>10 ± 0.2a</td>
<td>Binding of Gd3+ -DOTA complexes</td>
<td>Fluorescence/magnetic resonance imaging</td>
<td>ORa (73)</td>
<td>— a</td>
</tr>
<tr>
<td>CdHgTe</td>
<td>800</td>
<td>5.68</td>
<td>Capped with a CdS shell</td>
<td>In vivo imaging of a mouse</td>
<td>ASc (74)</td>
<td>&lt;20%</td>
</tr>
<tr>
<td>CdHgTe</td>
<td>775</td>
<td>40d</td>
<td>Loaded in gelatin nanospheres</td>
<td>Imaging of cells and mouse</td>
<td>ASc (75)</td>
<td>— a</td>
</tr>
<tr>
<td>Mn:CdTe</td>
<td>720</td>
<td>— a</td>
<td>Conjugated with EDC and NHS</td>
<td>FRET</td>
<td>ASc (76)</td>
<td>15–20%</td>
</tr>
<tr>
<td>Cu:InP</td>
<td>630–1100</td>
<td>4</td>
<td>TOP-capped</td>
<td>— a</td>
<td>ORb (77)</td>
<td>20%</td>
</tr>
<tr>
<td>CdTe/CdS</td>
<td>820</td>
<td>11</td>
<td>3-Mercaptopropionic acid-capped</td>
<td>— a</td>
<td>AS (78)</td>
<td>70%</td>
</tr>
<tr>
<td>CdTe/CdS</td>
<td>735</td>
<td>4.3</td>
<td>N-Acetyl-L-cysteine-capped</td>
<td>Imaging of HK-1 cells</td>
<td>Hydrothermal route(79)</td>
<td>60.02%</td>
</tr>
<tr>
<td>QDs</td>
<td>Emission peak/nm</td>
<td>Size/nm</td>
<td>Modification strategy or coupling reagent</td>
<td>Area of application</td>
<td>Synthesis Approach</td>
<td>Quantum yield</td>
</tr>
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<td>-----------------------------------------------</td>
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</tr>
<tr>
<td>QD705</td>
<td>705</td>
<td>15–20(^a)</td>
<td>Coated with arginine-glycine-aspartic acid peptide</td>
<td>Detecting U87MG glioblastoma tumors</td>
<td>Purchased</td>
<td>— (^a)</td>
</tr>
<tr>
<td>QD800</td>
<td>800</td>
<td>— (^a)</td>
<td>Coated with 4-(maleimidomethyl)-1-cyclohexanecarboxylic acid N-hydroxysuccinimide ester</td>
<td>Imaging of Human prostate cancer C4-2B</td>
<td>Purchased</td>
<td>— (^a)</td>
</tr>
<tr>
<td>QD800</td>
<td>800</td>
<td>17.6(^d)</td>
<td>Formed PEG-PE-QDs based micelle</td>
<td>Quantification of tumors</td>
<td>Purchased</td>
<td>— (^a)</td>
</tr>
<tr>
<td>QD705/800</td>
<td>705/800</td>
<td>— (^a)</td>
<td>Conjugated with long-chain (2000 Da) amino-PEG, (5000 Da) methoxy-PEG</td>
<td>Analysis of chick CAM vasculature</td>
<td>Purchased</td>
<td>— (^a)</td>
</tr>
</tbody>
</table>

where,
\(^a\) — indicates no record.
\(^b\) ‘OR’ represents the traditional organometallic synthesis route.
\(^c\) ‘AS’ represents the aqueous synthesis route.
\(^d\) Indicates the diameter after modification.
Table 1-2. Properties of T<sub>2</sub> contrast agents. (Adapted from Ref. 83)

<table>
<thead>
<tr>
<th>Name</th>
<th>Core Material</th>
<th>Surface</th>
<th>Diameter of Core (nm)</th>
<th>HDD&lt;sup&gt;c&lt;/sup&gt; (nm)</th>
<th>Magnetization (emu/gm)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>r&lt;sub&gt;2&lt;/sub&gt; (mM&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>B&lt;sub&gt;0&lt;/sub&gt;(T)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferumoxides (Feridex)</td>
<td>Fe&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;, γ-Fe&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Dextran</td>
<td>4.96</td>
<td>160</td>
<td>45</td>
<td>120</td>
<td>1.5</td>
<td>84</td>
</tr>
<tr>
<td>Ferucarbotran (Combidex)</td>
<td>Fe&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Carboxyldextran</td>
<td>4</td>
<td>60</td>
<td>186</td>
<td>1.5</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Ferumoxtran (Combidex)</td>
<td>Fe&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Dextran</td>
<td>5.85</td>
<td>35</td>
<td>61</td>
<td>65</td>
<td>1.5</td>
<td>85</td>
</tr>
<tr>
<td>CLIO-Tat</td>
<td>Fe&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Dextran</td>
<td>5</td>
<td>30</td>
<td>60</td>
<td>62</td>
<td>1.5</td>
<td>86</td>
</tr>
<tr>
<td>WSIO (MEIO)</td>
<td>Fe&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>DMSA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>25</td>
<td>78</td>
<td>1.5</td>
<td>10, 87</td>
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<td></td>
<td></td>
<td></td>
<td>6</td>
<td>43</td>
<td>106</td>
<td>10</td>
<td>87</td>
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<td></td>
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<td></td>
<td>9</td>
<td>80</td>
<td>130</td>
<td>10</td>
<td>87</td>
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<td>12</td>
<td>101</td>
<td>218</td>
<td>10</td>
<td>87</td>
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<tr>
<td>FeNP</td>
<td>α-Fe</td>
<td>PEG</td>
<td>10</td>
<td>10</td>
<td>129</td>
<td>1.5</td>
<td>88</td>
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<tr>
<td>MnMEIO</td>
<td>MnFe&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>DMSA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6</td>
<td>68</td>
<td>208</td>
<td>1.5</td>
<td>10, 89</td>
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<td>9</td>
<td>98</td>
<td>265</td>
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<td>12</td>
<td>110</td>
<td>358</td>
<td>10</td>
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<tr>
<td>CoMEIO</td>
<td>CoFe&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>DMSA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12</td>
<td>99</td>
<td>172</td>
<td>1.5</td>
<td>10, 89</td>
<td></td>
</tr>
<tr>
<td>NiMEIO</td>
<td>NiFe&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>DMSA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12</td>
<td>85</td>
<td>152</td>
<td>1.5</td>
<td>10, 89</td>
<td></td>
</tr>
<tr>
<td>Au-Fe&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Fe&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>PEG</td>
<td>20</td>
<td>114</td>
<td>3.0</td>
<td>90</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Au-FePt</td>
<td>FePt{fcc}</td>
<td>PEG</td>
<td>6</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Magnetic properties were measured at 1.5 T external field.

<sup>b</sup>2, 3-Dimercaptosuccinic acid.

<sup>c</sup>Hydrodynamic diameter.

*B<sub>0</sub> - magnetic field

CLIO – Cross linked iron oxide

MEIO - Metal doped iron oxide

WSIO – Water soluble iron oxide
Table 1-3. Properties of $T_1$ contrast agents. (Adapted from Ref. 83)

<table>
<thead>
<tr>
<th>Name</th>
<th>Core Material</th>
<th>Diameter of Core (nm)</th>
<th>Relaxivity based on concentration of whole atoms</th>
<th>Relaxivity based on number of particles</th>
<th>Relaxivity based on surface $B_0(T)*$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_1$(mM$^{-1}$s$^{-1}$) $r_2$(mM$^{-1}$s$^{-1}$)</td>
<td>$r_1$(mM$^{-1}$s$^{-1}$) $r_2$(mM$^{-1}$s$^{-1}$)</td>
<td>$r_1$ $r_2$</td>
<td></td>
</tr>
<tr>
<td>Gd-DTPA</td>
<td>Gd</td>
<td>ion</td>
<td>4.1 4.9</td>
<td>4.1 4.9</td>
<td>7 92</td>
<td></td>
</tr>
<tr>
<td>Dextran-SPGO</td>
<td>Gd$_2$O$_3$</td>
<td>4.8 16.9</td>
<td></td>
<td></td>
<td>7 93</td>
<td></td>
</tr>
<tr>
<td>PEG-Gd$_2$O$_3$</td>
<td>Gd$_2$O$_3$</td>
<td>3 9.4 13.4</td>
<td></td>
<td></td>
<td>1.5 94</td>
<td></td>
</tr>
<tr>
<td>GadoSiPEG</td>
<td>Gd$_2$O$_3$</td>
<td>2.2 8.8 11.4</td>
<td>3700 4800</td>
<td></td>
<td>7 92</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.8 8.8 28.8</td>
<td>18600 60700</td>
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<tr>
<td></td>
<td></td>
<td>4.6 4.4 28.9</td>
<td>38800 65000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGP/dextran-K01</td>
<td>GdPO$_4$</td>
<td>13.9 15</td>
<td></td>
<td></td>
<td>0.47 95</td>
<td></td>
</tr>
<tr>
<td>GdF$_3$:cit</td>
<td>GdF$_3$</td>
<td>3.17 2.0 $\times 10^7$</td>
<td></td>
<td></td>
<td>227.3$^a$ 14.2 96</td>
<td></td>
</tr>
<tr>
<td>GdF$_3$/LaF$_3$:AEP</td>
<td>GdF$_3$/LaF$_3$</td>
<td>2.71 8.8 $\times 10^5$</td>
<td></td>
<td></td>
<td>77.2$^a$ 14.2 96</td>
<td></td>
</tr>
<tr>
<td>PGP/dextran-K01</td>
<td>GdPO$_4$</td>
<td>13.9 15</td>
<td></td>
<td></td>
<td>0.47 95</td>
<td></td>
</tr>
<tr>
<td>MnO</td>
<td>MnO</td>
<td>7 0.37 1.74</td>
<td>3000 14000</td>
<td>33$^b$ 154$^b$</td>
<td>3 97</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 0.18 0.57</td>
<td>15000 46000</td>
<td>34$^b$ 121$^b$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 0.13 0.52</td>
<td>25000 99000</td>
<td>33$^b$ 102$^b$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 0.12 0.44</td>
<td>46000 165000</td>
<td>39$^b$ 139$^b$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FeCo/GC</td>
<td>FeCo</td>
<td>4 31 185</td>
<td></td>
<td></td>
<td>1.5 98</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 70 644</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Relaxivities based on the Gd$^{3+}$ on the shell (unit: mM$^{-1}$s$^{-1}$)

$^b$Relaxivities based on the surface area of the nanoparticles (unit: m s$^{-1}$)

*B$_0$ - magnetic field
Figure 1-1. Density of states in one band of a semiconductor as a function of dimension. Adapted from Ref. 2.
Figure 1-2. Energy diagram of large and small NPs with different magnetic spin alignment. Ferromagnetism and superparamagnetism exhibited by the large and small NPs respectively. Adapted from Ref. 10.
Figure 1-3. (a) Magnetization properties of ferromagnetic and superparamagnetic NPs with and without the influence of external magnetic field (b) Dependence of magnetic domain structures on NP sizes, \( D_s \) and \( D_c \) are the thresholds for ‘superparamagnetism’ and ‘critical’ size. Adapted from Ref. 11.
Figure 1-4. Absorption coefficient versus wavelength plot. Adapted from Ref. 26. (Hb is hemoglobin, HbO₂ is oxy-hemoglobin)
Figure 1-5. Schematic representation of Type I and Type II QDs. The positions of conduction and valence bands of the core (center) and the shell materials are represented using rectangles. Adapted from Ref. 38.
CHAPTER 2
MATERIALS AND METHODS

2.1 Core/Shell Design Considerations

Efficient NIR QDs have attracted a lot of attention for various applications. As discussed earlier bio-imaging using these QDs holds a lot of potential. The properties that are desirable in a good NIR emitting material used for bio-imaging are: good fluorescence property with QY > 10%, small in size with hydrodynamic diameters < 10 nm to facilitate transportation and circulation inside living organisms, good stability when exposed to physiological environment and against photobleaching. QDs synthesized by aqueous techniques like hydrothermal can have the above mentioned properties. The criteria used for choosing the different materials used in this research are provided below.

2.1.1 CdTe as QD Core Material

As explained in section 1.2.1.2, II-VI systems are the largest group among QDs. The synthesis of CdX, ZnX and HgX (X=Te, Se, S) are widely reported because these QDs when synthesized have uniform shape and size compared to other QDs. At room temperature they also display sharp absorption profiles with good emission features relative to other QDs. Among these particles Zn based QDs emit in the visible range (Figure 2-1). While the Hg based QDs emit in the mid NIR range, they are highly toxic to the environment. Thus the Cd based QDs appears to be the material of choice. However, from Figure 2-1 it is evident that CdSe and CdS emit in the visible region while CdTe in the NIR region. CdTe has a bulk bandgap 1.5 eV which corresponds to 828 nm emission. Thus CdTe was chosen as the core QD material because it can emit around 800 nm which is desirable for NIR applications.
2.1.2 CdS as QD Shell Material

In addition to band alignment the other requirement for core/shell nanocrystals with good optical properties is epitaxial shell growth. Epitaxial growth of shells on core nanoparticles signifies that the shell material will crystallize on the core with very little lattice mismatch.\(^{123}\) Large lattice mismatch between core and shell leads to strain which generates defect states at the interface of core and shell or inside the shell. These defects can in turn act as trap states for charge carriers resulting in the reduction of fluorescence QY.\(^{123}\) The difference in lattice parameter of CdTe (a=6.482Å) and CdS (a=5.818Å) is small. Thus CdTe/CdS form good core/shell materials because they have a small lattice mismatch (11.4\%) as illustrated in Figure 2-2. CdTe core with CdS shell forms a pseudo type II QD\(^{124}\). By varying the thickness of the CdS shell the emission wavelength of the QD can be fine-tuned. In the type II QD the staggered band alignment produces smaller band gap which gives rise to significant red shift of the emission wavelength.

2.1.3 N-Acetyl-Cysteine (NAC) as the Dispersing Ligand

The common thiols used for nanoparticle synthesis are thioglycolic acid (TGA)\(^{126}\), L-cysteine/mercaptoethylamine\(^{127}\), 1-thyoglicerol\(^{127-128}\), mercaptopropionic acid (MPA)\(^{129}\). However, MPA and NAC are the only thiol reported that have been utilized for synthesizing NIR emitting QDs\(^ {79}\). CdCl\(_2\) forms a white precipitate with MPA during the initial mixing stages with pH lower than 7.3. Above pH > 7.3 the precipitate dissolves to form a clear solution. MPA also has toxic properties with awful odor\(^ {79}\). With NAC CdCl\(_2\) do not form any precipitate in the wide pH range of 2.4 – 12 and NaOH solution can be used to vary the pH. This provides flexibility of QD synthesis and allows production of QDs at any pH lying in the above mentioned range. Also, NAC is used as an antioxidant
and there are reports which indicate that NAC protects cells from oxidative stress and cyto-toxicity arising from toxic QDs\textsuperscript{79}. All of these factors led to the choice of NAC as the desirable ligand for synthesizing NIR QDs reported in this work. The chemical structure of the ligand is shown in Figure 2-3.

2.2 Synthesis of the QDs

2.2.1 Synthesis of the Core CdTeS QDs

Hydrothermal synthesis of core CdTeS QDs was carried out in Teflon lined autoclaves. The flowchart for the synthesis of core and core/shell QDs is outlined in Figure 2-4. The synthesis process is similar to the method used by Zhao et al.\textsuperscript{79} In a typical process 0.23 gm CdCl\textsubscript{2} (1.26 mmol) was dissolved in 100 ml Argon saturated DI water in a 250 ml round bottom flask to produce a 12.5 mM solution. N-Acetyl-Cysteine (NAC) was added to the solution so that Cd:NAC molar ratio was 1:2.5. In a separate 20 ml air tight glass bottle 0.05 gm (1.32 mmol) NaBH\textsubscript{4} and 0.08 gm (0.63 mmol) Tellurium powder were mixed in 2 ml Argon saturated DI water to produce sodium hydrogen telluride (NaHTe). The molar ratio of NaBH\textsubscript{4} to Te used was 2:1. The glass vial containing the solution was sealed with paraffin film to make it air tight. The as prepared NaBH\textsubscript{4}/Te solution was kept in the refrigerator at 4 °C for the reaction to be completed. This reaction produces a deep red solution of NaHTe. The time period allowed for reaction was more than 8 hrs. The equation for the reaction is as follows:

\[
2\text{NaBH}_4 + \text{Te} + 2\text{H}_2\text{O} = \text{NaHTe} + \text{NaBO}_2 + 11/2\text{H}_2
\]  \hspace{1cm} (2-1)

It is very important to use DI water that is oxygen free or with negligible oxygen content. Te is susceptible to oxidation and its oxidation number can vary from -2(H\textsubscript{2}Te) to +6(TeO\textsubscript{4}\textsuperscript{2-})\textsuperscript{130}. In CdTe the oxidation number of Te is -2. In case of oxidation of Te atoms, the CdTeS QD properties prepared from partially oxidized tellurium will be poor.
Hence it is important to use Argon saturated DI water for making solutions. The color of the NaHTe solution turned wine red. The pH of the precursor solution was adjusted to (8.0 – 8.4) by using 2M NaOH solution. Several polytetrafluoroethylene (PTFE) lined autoclaves (Parr acid digestion bombs) were then used for heating the solution. 10 ml of solution was filled in each of these containers with maximum capacity of 23 ml. Subsequent heating of the solutions were carried out for different time intervals (30 – 100 min) at 180 °C. The autoclaves were cooled by a hydro-cooling process after the completion of heating. The QDs thus prepared were taken out from the autoclaves and characterized after washing. Washing of the QDs were done using Amicon Ultra-15 Centrifugal Filter Unit with membranes suitable for 30 kDa protein filtration and centrifuging them at 2600 rpm for 4-5 minutes. The QDs were washed 8-9 times with DI water to get rid of unreacted reactants.

2.2.2 Coating of the Core CdTeS QDs with CdS Shells

10 ml of the crude CdTeS QDs were taken in a 100 ml two necked round bottom flask and 0.5 gm (2.73 mmol) of CdCl₂ was added to the solution. In another two necked 100 ml round bottom flask 0.03 gm (0.39 mmol) of anhydrous Na₂S and 0.125 gm (0.77 mmol) of NAC were added to 20 ml of DI water (Figure 2-4). This solution was bubbled with Argon gas for 30 minutes to get rid of any dissolved oxygen. The Argon saturated solution was then added dropwise to the crude CdTeS QD solution very slowly using a syringe pump at the rate of 300 µl per minute. The solution thus obtained was heated in batches of 10 ml in 23 ml teflon lined autoclaves at 130 °C for various time intervals (60 – 150 min). The QDs thus obtained were centrifuged at 2600 rpm and washed with DI water using 30 kDa Amicon Ultra-15 Centrifugal Filter Units. The washing steps were
repeated 8 to 9 times to get rid of all the unreacted chemicals. The washed QDs were then characterized by different techniques.

### 2.2.3 Synthesis of Magnetic Fe Doped CdTeS QDs

The synthesis of Fe doped CdTeS QDs is schematically represented in Figure 2-5. The basic synthesis technique was the same as used for core CdTeS QDs. The Cd:Te molar ratio was maintained as 2:1. In a typical process a 30 mM solution of CdCl₂ in DIW was prepared by adding 0.55 gm (3 mmol) of CdCl₂ to 100 ml DI water. Sodium hydrogen telluride (NaHTe) was prepared from NaBH₄ and Tellurium powder in Argon saturated DI water. The molar ratio of NaBH₄ to Te used was 2:1. The as prepared NaBH₄/Te solution was kept in the refrigerator at 4 °C for the reaction to be completed. The time period allowed for reaction was more than 8 hrs. CdCl₂ (30 mM) solution in 100 ml DI water was prepared in a 250 ml round bottom flask. NAC was added to the solution so that Cd:NAC molar ratio was 1:2.5. FeCl₂.4H₂O was added to the resulting solution so that Cd:Fe molar ratio was 8.5:1. The solution was Argon bubbled for more than 30 min and then the NaHTe solution was added to this solution. The color of the solution turned wine red. The pH of the precursor solution was adjusted to (8.0 – 8.4) by using 2M NaOH solution with subsequent heating in PTFE lined autoclaves for different time intervals (30 – 100 min) at 180 °C. The autoclaves were cooled by a hydro-cooling process after the heating was completed. The QDs thus prepared were taken out and characterized after washing.

### 2.3 Characterization of the QDs

The QDs thus prepared were characterized using fluorescence spectroscopy, TEM, EDS, XRD and XPS to determine their particle size, chemical and phase composition. SpectraMax M5 was used for ultraviolet-visible absorption emission
spectra at room temperature. The PL QYs of the core and core/shell particles were
determined at room temperature by comparing with Rhodamine 6g dissolved in ethanol
which was taken as 95%\textsuperscript{131}. The absorbance or optical density (OD) of Rhodamine 6g,
the core and the core/shell QDs were adjusted to less than 0.1 for QY determination.
JEOL 2010F was used for high resolution transmission electron microscopy (HRTEM)
of the QDs. The transmission electron microscopy (TEM) sample preparations were
done by dropping the nanoparticles suspended in water on carbon coated Cu grids and
dried overnight at room temperature. The powder diffraction patterns of the
nanoparticles were obtained using a Philips APD 3720 instrument using Cu Kα
radiation. XPS studies were done using Perking Elmer 5100 XPS system. The x-ray
photoelectron spectroscopy (XPS) sample preparations were done by drying drops of
core and core/shell suspensions on silicon wafers at room temperature.

Performance assessment of the QDs was carried out by incubating the QDs with
macrophages and subsequent imaging of the QD doped cells with NIR detecting
camera. J774 mouse macrophage/monocytic cells were grown using standard
Dulbecco’s modified eagle’s medium (DMEM), supplemented with 10% fetal bovine
serum, glutamax and penicillin/streptomycin. Prior to labeling, cells were seeded into 4-
well chamber slides and allowed to attach before subsequent incubation with 200 µg/ml
core/shell CdTeS/CdS QDs, emitting at 800 nm, for 6 hours. The cells were imaged at
40X magnification. Prior to acquiring images, cells were counterstained with 4’, 6-
diamidino-2-phenylindole (DAPI) for visualization of cell nuclei. Near infrared images
was acquired using a custom made infrared camera setup with a 15 second exposure
time and subsequently pseudo colored red using ImageJ software (NIH website). The
time resolved Photo luminescence (PL) spectra measurement were done using time-correlated-single-photon-counting (PicoQuant – PicoHarp 300). The samples were excited by 375 nm light.

The hysteresis curves for the commercial Feridex® I.V. sample and the magnetic Fe doped CdTeS QDs were obtained using a superconducting quantum interference device (SQUID) magnetometer at different temperatures (10K and 300K). The applied field was varied from -1500 Oe to 1500 Oe. Magnetic measurements were performed on a series of QDs emitting between 660 - 740 nm. The diamagnetic contribution from the substrate was subtracted from the crude data to obtain the QD magnetization values. Non-magnetic filter-paper was soaked with 15 µL QD sample and placed inside non-magnetic empty gelatin capsule. The gelatin capsule was placed within the measuring straw inside the SQUID. Magnetization loops were obtained by varying the magnetic field over the range (±5 T) and during the measurement the temperature was kept constant.
Figure 2-1. Energy gap (eV) versus lattice parameter (Å) for bulk semiconducting materials. Adapted from Ref. 122.
Figure 2-2. Band gap (eV) versus lattice spacing (Å) for compound semiconductors. Adapted from Ref. 125.
Figure 2-3. Structure of the N-Acetyl-Cysteine (NAC) ligand.
Figure 2-4. Schematic representation of the core and core/shell QD synthesis by hydrothermal technique.
Figure 2-5. Flowchart for the synthesis of magnetic Fe doped CdTeS QDs.
CHAPTER 3
RESULTS AND DISCUSSIONS

3.1 Synthesis of Core CdTeS QDs Emitting up to 750 nm

Aqueous synthesis technique using short chain thiols as stabilizing agents is a useful alternative to the oil based organometallic synthesis of CdSe, CdS, CdTe, CdHgTe, HgTe and ZnSe quantum dots. Thiols not only passivate the dangling bonds at the surface of the QDs but also help in controlling the kinetics of the nanoparticle synthesis. Thus chemical stability of the QDs along with surface functionality and good water dispersibility of the particles were obtained. The different aqueous methods of nanoparticle synthesis reported using thiols include hydrothermal, ultrasonic, microwave irradiation and illumination.

In this research aqueous synthesis of QDs using NAC thiol as the dispersing ligand is reported. Highly luminescent CdTeS QDs were prepared by reacting CdCl₂ with NaHTe and NAC in teflon-lined autoclaves by a process similar to that reported by Zhao et al. The quality of the QDs synthesized by this process is highly influenced by molar ratio of reactants, reaction temperature, pH, reaction time, and Cd²⁺ concentration. Therefore to obtain QDs that are of high quality all these parameters were strictly controlled.

3.1.1 Effect of Reactant Concentration

The Cd:Te:NAC molar ratio used for QD synthesis plays an important role in the quality of the resultant QDs. The optimal reactant ratios were determined after experimentation. In the core CdTeS QD synthesis the optimum molar ratio was found to be Cd:Te:NAC 1:0.5:2.4.
3.1.2 Effect of Precursor pH

The pH of the precursor solution is an important parameter that influences the QY as well as the nanocrystal growth rate\textsuperscript{79}. The pH of the precursor solution for the core CdTeS QDs was kept within 8-9 because for CdTe/CdS QDs pH higher or lower than this range causes particle aggregation resulting in the decrease of their fluorescence property\textsuperscript{79}.

3.1.3 Effect of Heating Temperature

The structure of the QD surface is dependent on the reaction temperature. Faster growth rate due to higher temperatures minimizes the formation of surface defects and thus improve the fluorescence properties of the QDs\textsuperscript{79}. One important advantage of hydrothermal synthesis is that it permits high temperature synthesis of QDs. The heating temperature was kept constant at 180 °C. Lower temperature produced QDs with poor quality while temperature greater than 240 °C caused precipitation in the autoclave\textsuperscript{79}.

3.1.4 Effect of Heating Time

Heating time is directly proportional to the size of the QDs. Longer heating time produced larger QDs that emitted at higher wavelengths. The solutions were heated in different time intervals within 30 – 100 minutes. During heating the emitted wavelengths evolved from green to NIR as larger particles were produced when heated for longer time periods (Figure 3-1). The evolution of emitted wavelength with heating time for the core QDs is presented in Figure 3-2. This experiment was repeated several times to ascertain the variation of emission wavelength with heating time. The emission wavelength variation obtained for QDs heated for a particular time interval that lies within the 30 – 100 min period at a constant temperature of 180 °C was within 30 nm.
Above 96 minutes heating time which produced QDs that emitted at 750 nm, particles started to agglomerate in the autoclave. The agglomerated QDs with emission wavelengths longer than 750 nm exhibited significantly lower QYs than the non-agglomerated QDs emitting below 750 nm. The probable reason for the agglomeration of the QDs emitting above 750 nm is the decomposition of high amount of NAC ligand leading to the decrease in the amount of ligand available for dispersion of the QDs.

### 3.2 Characterization of the Core CdTeS QDs

#### 3.2.1 Particle Size of the Core CdTeS QDs

The core CdTeS QDs were characterized using TEM to determine their particle sizes. Figure 3-3 shows the TEM image of QD700. Size of the CdTeS QDs were measured from TEM and compared with values calculated using the equation reported by Yu et al.\textsuperscript{141} The core QDs emitting within 530 – 750 nm have particle size within 3 - 6 nm. The calculated and measured sizes of the QDs are tabulated in Table 3-1.

\[
D = (9.8127 \times 10^{-7})\lambda^3 - (1.7147 \times 10^{-3})\lambda^2 + (1.0064)\lambda - 19484
\]  

(3-1)

where,

- \(D\) (nm) is the diameter of the quantum dot
- \(\lambda\) (nm) is the wavelength of the first excitonic absorption peak of the corresponding sample

There is some variation observed in the measured and calculated particle sizes. The particle sizes reported by Yu et al.\textsuperscript{141} are for CdTe QDs. However, the QDs reported in this dissertation have CdTe\(_{1-x}\)S\(_x\) alloy composition (discussed later in section 3.2.2). The change in lattice parameter and density due to the change in composition might be the reason behind the difference in values observed between the measured
and the calculated particle sizes. The particle sizes of the QDs were also measured using Dynamic Light Scattering (DLS). However, because of the small size of the QDs there were large variations in the obtained results. The data were not reproducible and hence not reported in this dissertation.

3.2.2 Chemical Composition Variation in the Core CdTeS QDs

The selected area diffraction (SAD) pattern of the core CdTeS QDs emitting at 700 nm is shown in the inset of Figure 3-3. The 3 rings correspond to the peaks observed in the XRD pattern of the core CdTeS as depicted in Figure 3-11. The d value for [111] in core CdTeS 700 nm is 3.59 Å. This value is intermediate between CdS, cubic [111] (3.36 Å) and CdTe, cubic [111] (3.74 Å) which indicate that the QDs have an alloy composition of Cd, Te and S. The S in the QDs comes from the decomposition of the NAC ligand at high temperature.

The chemical compositions of the QDs were also determined by Energy-dispersive X-ray spectroscopy (EDS) analysis and X-ray photoelectron spectroscopy (XPS). The EDS spectra for core QDs emitting at 560 nm and 700 nm with Cd, Te and S peaks are shown in Figure 3-4. The variation of chemical composition with increasing emission wavelength and corresponding increase in particle size is illustrated in Figure 3-5. From the figure it is evident that the amount of Te decreases while that of S increases with increasing emission wavelength. The amount of Cd remains almost constant around 50 atomic%.

The bigger QDs with higher emission wavelengths have more S and less Te than the QDs having smaller size and lower emission wavelength. No significant change in their optical properties that are desirable for bioimaging applications seems to be affected by the size dependent variation in QD chemical composition. Ohata et al.142
reported the change in phase, density and lattice parameter with S addition to bulk powders of CdTe. With increasing value of \( x \) in CdTe\(_{1-x}\)S\(_x\) the density of the bulk powder particles and the lattice parameter ‘\( a \)’ decreased with cubic CdTe changing to wurtzite CdS. The QDs might follow similar change in phase, lattice parameter and density with change in chemical composition as happens with bulk CdTe\(_{1-x}\)S\(_x\) powders. More investigations are required to figure out the change of phase and lattice parameter of the CdTeS QDs with increasing size and sulfur content.

3.2.3 Fluorescence Quantum Yield of the Core CdTeS QDs

The QY of the QDs was determined relative to the standard Rhodamine 6g dye which has a QY of 95% in ethanol\(^{131}\). The optical densities for both QDs and Rhodamine 6g were determined using SpectraMax M5. Value of absorbance in either case was kept below 0.08 at the wavelength of excitation. Same excitation wavelength was used for both the QD samples and the standard dye. The integrated emission intensity from the QDs was compared to that of the dye using the following equation\(^{131}\).

\[
QY = QY_{St} \frac{1-10^{-A_{St}}}{1-10^{-A}} \times \frac{\eta^2}{\eta_{St}^2} \times \frac{I}{I_{St}} \quad (3-2)
\]

where,

- \( QY \) and \( QY_{St} \) are quantum yields for sample and standard.
- \( A \) and \( A_{St} \) are absorbance values of sample and standard at the excitation.
- \( \eta \) and \( \eta_{St} \) are refractive indices of the sample and standard solvents, and
- \( I \) and \( I_{St} \) are the integrated emission areas for the QD samples and the standard, respectively.
The QY thus measured for the core CdTeS emitting in the 530-700 nm region varied within 40-60%. The QDs emitting in the wavelength range 700-740 nm exhibited QY between 20-40%.

3.2.4 Extinction Co-Efficient Calculations for the Core QDs.

The extinction co-efficients of two core QDs viz. QD630 and QD710 per mole of particles (ε) were calculated. The calculations were based on Beer-Lambert’s law\textsuperscript{141}

\[ A = \varepsilon CL \]  

where,

A is the absorbance for a sample at the first exciton absorbance peak.

C is the molar concentration (mol/L) of the QDs of the same sample.

L is the path length (cm) of the exciting light used for obtaining the absorbance spectrum.

The value of L was fixed at 1 cm. The extinction co-efficient was calculated per mole of the QDs and its unit is L (mol)\textsuperscript{-1} (cm)\textsuperscript{-1} or M\textsuperscript{-1} cm\textsuperscript{-1}. The sizes of QD630 and QD710 calculated from Equation 3-1 were 3.7 nm and 4.4 nm respectively. Molecular weights (MWs) of the QDs were determined (calculations available in Appendix) and their absorbance versus concentration plots obtained from the UV/Vis spectra (Figure 3-6). From the slopes of the linear plots extinction co-efficients were estimated. The extinction co-efficient values for QD630 and QD710 were found to be $5.8 \times 10^{-5}$ M\textsuperscript{-1} cm\textsuperscript{-1} and $3.7 \times 10^{-5}$ M\textsuperscript{-1} cm\textsuperscript{-1} respectively. The extinction co-efficient values for CdTe reported by Yu et al.\textsuperscript{141} are also in the $10^{-5}$ M\textsuperscript{-1} cm\textsuperscript{-1} range. However, extinction co-efficients for more QDs need to be determined and a trend line plotted with increasing particle size to figure out the variation of extinction co-efficient with changing QD sizes.
3.3 Synthesis of Core/Shell CdTeS QDs Emitting up to 820 nm

As discussed earlier the objective of this research was to obtain QDs emitting close to 800 nm. However, using the one pot synthesis technique core QDs with good fluorescence property above 750 nm could not be prepared. This necessitated the search for alternative techniques that can overcome the above mentioned limitation. Core/shell QDs provided a good alternative and by fabricating a CdS shell over the CdTeS cores emission wavelength tunability near the 800 nm region was achieved. By changing the thickness of the shell emission wavelength tunability over a wide range is possible\textsuperscript{33}. In principle CdS can be considered as a good shell material for CdTe core QDs because of three factors viz. CdS has a wider band gap (2.5 eV) than CdTe (1.5 eV), relatively small lattice parameter mismatch of CdTe with CdS (11.4%) when compared to other candidates like ZnS (16.5%) and ZnSe (12.5%)\textsuperscript{124}. Core/shell CdTeS/CdS will probably be Type II QDs (Figure 3-7) as their electrons are considered to be confined in the CdS shells while the holes are mostly in the CdTeS cores as depicted by their band offsets\textsuperscript{124}. According to the hypothesis by Zeng et al.\textsuperscript{124} the CdTe/CdS core/shell system slowly evolves from Type I to Type II system with increasing shell thickness as represented in Figure 3-7(b).

**CdS Coatings on the Core CdTeS QDs:** The CdS coatings of the core CdTeS QDs were done using the experimental protocol presented in Figure 2-4. The core QDs used for CdS coatings have emission wavelengths of 575 nm, 720 nm and 735 nm respectively. The emission wavelengths for these QDs when coated with the CdS shell increased with increasing thickness of the shell produced by prolonged heating of the core QDs in the precursor solution containing Cd and S.
With an increase in CdS shell thickness first exciton peak in absorption along with the fluorescence peak undergo a significant redshift. This redshift caused emission wavelength to increase for all three core CdTeS QDs (Figure 3-8). With the CdS shell formation on the CdTeS core QDs maximum tunability upto 820 nm was achieved.

For overcoating QDs with an inorganic shell there are a few criteria that need to be followed\textsuperscript{123}, (a) the conditions for shell deposition should be such that the core QDs will be able to withstand those conditions (b) both the core and shell materials should have similar surface energies so that the barrier for heterogeneous nucleation of the shell will be lower than homogeneous nucleation. (c) under the conditions of shell deposition the core and shell material must not readily interdiffuse. In a typical core/shell synthesis the core QDs synthesized by one of the standard techniques are redispersed into a solution of stabilizers and solvent. The inorganic shell precursors are then gradually added to the solution which is heated and held at a particular temperature. During this process the shell materials are heterogeneously nucleated on the core QDs. The rate of precursor addition is controlled so that it never exceeds the rate of deposition of shell material on the core QDs. In such a case the precursor concentration does not cross the threshold for homogeneous nucleation of the shell material. In the case of core/shell CdTeS/CdS QD synthesis the above criteria were followed. Temperature for shell formation was different than that was used for the synthesis of core QDs. The CdS shell was formed at a temperature of 130 °C which was much lower than the temperature of core QD formation, i.e. 180 °C. The core CdTeS QDs were mixed with excess cadmium in a two necked 100 ml flask. In another two necked 100 ml flask Na\textsubscript{2}S was dissolved in DI water containing NAC. The Na\textsubscript{2}S solution was then slowly added to the solution.
containing the CdTeS core QDs in a dropwise manner using a syringe pump so that the precursor concentration never reaches the homogeneous nucleation threshold. The pH of the final solution was adjusted to 12.0 – 12.2. The resultant solution was then heated in autoclaves at 130 °C for different time intervals to achieve different thickness of the CdS shell. After the heating was completed the autoclaves were taken out of the oven and cooled by running tap water. The QDs were then washed with DI water several times and characterized using TEM, XRD, fluorescence spectrophotometer etc. Rogach et al. reported that high pH of the precursor solution in the range of 11-12 facilitate high growth rate of CdTe QDs coated with thioglycolic acid (TGA). In the research reported here the optimum pH range for coating the NAC stabilized core QDs with the CdS shell was also found to be 12.0-12.2, which is similar to the pH used by Rogach. The emission wavelength tunability achieved at pH lower than 12 was smaller than 180 nm. The zeta potential (by the Huckel method) of the precursor solution containing core CdTeS QDs emitting at 690 nm decreased from -70 mV to -20 mV with pH change from 7 to 12 (Figure 3-9). Thus with high pH the core QDs probably had smaller distance of separation among them leading to high growth rate. At pH 12 the maximum wavelength tunability achieved for a core QD emitting at 620 nm was 180 nm when heated at 180 °C for 3 hours thus producing core/shell CdTeS/CdS QDs emitting at 800 nm. From these results we can conclude that high pH of the precursor solution favors high growth rate of QDs.

3.4 Characterization of the Core/Shell CdTeS/CdS QDs

The core/shell QDs synthesized were characterized using TEM, XRD, EDS and XPS techniques. TEM was used to determine the size and shape of the QDs whereas XRD, EDS and XPS gave the phase and chemical composition of these particles. The
TEM image of core/shell QDs emitting at 720 nm is shown in Figure 3-10. That the QDs are crystalline is evident from the presence of lattice fringes in the TEM image. From the TEM images it was determined that all core/shell QDs emitting between 530 nm – 820 nm have sizes within 3-6 nm. The core/shell CdTeS/CdS QDs have the same size range of 3-6 nm as that of the core CdTeS QDs which implies that the CdS shell on the core CdTeS QDs is less than 1 nm. The core/shell structure of the QDs was not distinct in the TEM image. Chemical composition studies using EDS and XPS were performed to determine the core/shell characteristics of these QDs.

The XRD of the core CdTeS QDs and the core/shell CdTeS/CdS QDs are presented in Figure 3-11. From the figure 3 distinct peaks can be identified that correspond to planes [111], [220] and [311] respectively. The 3 diffraction peaks for both core and core/shell QDs lies within the 2θ values for corresponding cubic CdTe and cubic CdS peaks. For cubic CdTe the 2θ values assigned for [111], [220] and [311] planes are 24.2°, 40.3° and 46.8° respectively while that for cubic zinc-blende CdS the corresponding 2θ values are 27.1°, 44.8° and 53.4° respectively. That the diffraction peaks of the core and core/shell QDs lie within the corresponding peaks for CdTe and CdS illustrate the fact that these QDs are alloys of CdTe and CdS. According to Ohata et al.\textsuperscript{142} CdTe\textsubscript{1-x}S\textsubscript{x} type bulk mixed crystals possess cubic zincblende structure for x < 0.2 and hexagonal wurtzite structure for x > 0.2. More investigations are required to confirm the phase composition of the core and core/shell QDs reported in this dissertation.
3.4.1 Determining Core/Shell Structure of the QDs Using Chemical Composition Studies

The core/shell structure of the QDs was not distinct in the (Figure 3-10) TEM picture. EDS and XPS studies of the QDs were carried out to investigate the presence of CdS shell. The chemical composition of 3 particles were analyzed viz. core 670 nm, core 750 nm and core/shell 750 nm emitting QDs. The core/shell 750 nm emitting QDs were obtained by coating the core 670 nm emitting QDs with CdS shells.

3.4.1.1 Characterization of the QDs using EDS

The EDS spectra for the core QD750 and core/shell QD750 are shown in Figures 3-12(a) and (b) respectively. The Cd, Te and S peaks depicted in these figures confirm the presence of the elements in these samples. The O peak in the samples comes from the NAC ligand attached to the QDs while the Cu peak is from the Cu-TEM grids used as sample holders. The chemical composition values are for all of the three QDs are shown in Table 3-2. The amount of sulfur in the core/shell QD750 (47.3 atomic %) is significantly higher than the sulfur present in the core QD670 (34.7 atomic %) from which they were developed using a CdS coating. These results indicate the presence of CdS rich shell on the surface of CdTeS core QDs. Also the sulfur amount is higher in the core/shell 750 nm emitting particle than just the core 750 nm (35.5 atomic%) emitting QDs. Hence the core/shell 750 nm particle has a different chemical composition than that of a core 750 nm emitting particle without a shell which is expected.

3.4.1.2 Characterization of the QDs using XPS

X-ray photoelectron spectroscopy (XPS) is regarded as a sensitive tool for determination of the surface chemical composition of materials. Hence, this technique
was utilized for the surface analysis of the QDs. The surface composition of 3 different QDs viz. core QD670, core QD750 and core/shell QD750 were analyzed using XPS. Peaks due to Cd, Te, O, C and S appear in the XPS spectra of the QDs shown in Figure 3-13(a). C and O came either from the NAC ligand or from the environment. The appearance of Cd, Te and S peaks were due to the fact that these elements were present in the core and shell of the QDs. The high resolution spectra showing the plot of binding energy vs. intensity for the elements Cd, Te and S species are depicted in Figures 3-13 (b), 3-14 (a) and (b) respectively. Peaks of Cd 3d$_{5/2}$ at 405 eV, Cd 3d$_{3/2}$ at 411 eV, Te 3d$_{5/2}$ at 571 eV, Te 3d$_{3/2}$ at 582 eV, S 2p at 161 eV and S 2p at 225 eV appeared in all the XPS spectra. Thus the presence of these elements in the QDs was confirmed. The elemental composition of the QD surfaces is presented in Table 3-3. The amount of S for the core/shell QD750 (37.9 atomic %) is higher than that in the core QD750 (34.0 atomic %). The amount of S in core QD670 (32.5 atomic %) is the lowest among the 3 QDs. The reverse trend in observed for Te. Among the three QDs the amount of Te in the core QD670 (12.5 atomic%) is the highest followed by the amount of Te in core QD750 (8.8 atomic%) and core/shell QD750 (3.0 atomic%). The trend observed with the S and Te amounts present in the QDs by XPS is the same as was observed from the chemical analysis of the QDs using EDS. The results indicate that the amount of S on the surface increases with increasing QD size i.e. as the emission wavelength increases for the core QDs. Also, the core/shell QDs have even higher S on the surface relative to the core QDs due to the presence of CdS rich shell on the core CdTeS QDs. The QDs were further etched using Argon and the chemical composition of the surfaces determined using XPS. The chemical compositions of the etched
surfaces of the same three particles viz. core 670 nm, core 750 nm and core/shell 750 nm emitting QDs were determined after subjecting them to Ar etching for 0, 5 and 10 minutes. Tables 3-4, 3-5 and 3-6 show all the chemical composition values obtained after etching the samples with Argon for 0, 5 and 10 minutes. For all the QD samples the amount of Te increases with increasing etching time which is expected because as discussed earlier the QDs are Te rich inside and are S rich on the outside. These values agree with the previous finding that the QDs are of gradient alloy composition with a CdS rich shell. The presence of Te in all the XPS results confirms that the shell is an alloy of Cd, Te and S.

3.4.2 Time Resolved Luminescence Properties of Core and Core/Shell QDs

The fluorescence decay curves for the 620 nm emitting core QDs and that of the core/shell 800 nm are shown in Figure 3-15. The core/shell 800 nm emitting QDs were obtained by coating the 620 nm emitting QDs with CdS rich shells. The PL decay measurements were taken after exciting the QDs with 375 nm laser. The 620 nm and 800 nm emitting QDs have lifetimes of 33 ns and 121 ns respectively. Figure 3-16 shows the comparisons of lifetimes among CdTeS cores 670 nm and 750 emitting QDs along with core/shell 750 nm emitting QDs. The core/shell 750 nm QDs were obtained by coating the 670 nm emitting QDs with CdS shells. The lifetimes for the core 670 nm, 750 nm and core/shell 750 nm emitting QDs are 51 ns, 75 ns and 82 ns respectively. For the core QDs an increase in the average PL decay times with increasing size was observed. This phenomenon is typical of II-VI QDs and reported by other researchers\textsuperscript{143}. The PL decay times can be higher for the larger QDs with longer wavelength of emission for two reasons:
a. The number of discrete energy levels increases in larger QDs which can trap electron-hole pairs and thus delay the recombination process resulting in larger PL decay times.

b. The number of excitons increases with larger QDs. Thus it takes longer for the decay to be complete.

PL decay times for core/shell QDs are larger than the core QDs. There are two probable reasons behind this phenomenon:

a. Type I nanocrystals transform into type II nanocrystals during the growth of CdS shells on the core CdTe QDs\textsuperscript{33}. This causes the electrons to be accommodated the shell and holes in the cores. As a result the overlap in the electron-hole integral decreases producing significant increase in the PL lifetimes\textsuperscript{144}.

b. The shell reduces the surface states on the surface of the core QDs. Thus the high amount of non-radiative relaxations originating from the surface states gets minimized. When the percentage of radiative relaxations rises due to the passivation of the surface states the average PL decay time for the core/shell QDs increases.

Deng et al.\textsuperscript{33} reported the PL decay times for CdTe/CdS core/shell QDs made by classic aqueous reflux method. The lifetimes measured for QDs emitting at 623, 660, 740, 760, 800, 820 nm were 44, 58, 110, 138, 183, 245 ns respectively. The PL decay values for core 620, 670 and 750 nm emitting QDs obtained from our experiments were 33 ns, 51 ns and 75 ns which are close to the values reported by the above researchers. However, our value for 800 nm emitting QDs was 121 ns which is much lower than 183 ns reported by Deng et al.\textsuperscript{33}. The possible reason for this difference in
lifetime might be the higher shell thickness for the QDs prepared by the authors. They prepared the core/shell 800 nm emitting QDs from 465 nm cores whereas in our system the 800 nm emitting core/shell QDs were prepared from 620 nm cores.

3.5 Performance Assessment of the NIR Emitting Core/Shell QDs

The usefulness of the QDs for applications in bioimaging was investigated by performing a staining test of J774 macrophages/monocytes cells using core/shell CdTeS/CdS 800 nm wavelength emitting QDs. These cells were incubated with 200 μg/ml core/shell QDs for 6 hrs. Unattached QDs were discarded by washing the sample. For nuclei visualization the cells were stained with DAPI before imaging. As depicted in Figure 3-17 (a) the CdTeS/CdS core/shell QDs emitting at 800 nm were up taken by the J774 macrophages/monocytes. That the QDs have been absorbed by the cells was confirmed by comparing the images given in Figure 3-17 for cells incubated (a) with the QDs and (b) without the QDs. However, from the images it was difficult to ascertain whether the QDs were on the surface of the cells or inside the cells.

The QDs were also imaged with mouse phantoms to determine their usefulness for animal imaging. Figure 3-18 shows the mouse phantom imaged with core/shell CdTeS/CdS QD emitting at 800 nm embedded in it and using different excitation wavelengths and emission filters. The picture represented in Figure 3-18 (a) is that of the mouse phantom containing the 800 nm QDs when it is excited with 640 nm light and imaged using 720 nm emission filters. The auto fluorescence from the phantom when viewed with the excitation wavelength/emission filter combination of 640 nm/720 nm overshadows the emission from the QDs at 720 nm. The QDs are distinctly visible only when the emission wavelengths of the QDs as well as the emission filters are in the NIR range. By just changing the excitation wavelength/filter combination to the NIR region
and keeping everything else exactly the same as before the QDs can be easily detected in the phantom as shown in the remaining figures. In Figures 3-18 (b) and (c) the excitation wavelength/emission filter combinations used are 710 nm/780 nm and 710 nm/840 nm respectively. In both these images the fluorescence from the QDs from inside the mouse phantom could be detected without any difficulty. The imaging results from experiments with both the J774 cells and the mouse phantom demonstrate that the QDs emitting at 800 nm can be useful for bioimaging of animal cells and tissues.

3.6 Bimodal Imaging Using Magnetic QDs

Visualization and understanding of tissues and specific molecules in vivo can be achieved using molecular imaging. The molecular imaging techniques that are currently used for various disease diagnoses are position emission tomography (PET), single photon emission computed tomography (SPECT), MRI, optical and ultrasound\textsuperscript{145}. Table 3-7 illustrates the different characteristics of these imaging techniques. None of these techniques are perfect and each one has its own advantages and disadvantages. The information required for comprehensive imaging can be obtained only by synergistic combination of the different imaging modalities.

Nanoparticles, QDs which are 100-10,000 times smaller in size than the cells have the ability to pass through the cell membrane easily and reach the target site. Thus nanoparticles, QDs can potentially enhance not only the imaging sensitivity and resolution but also specificity. Multimodal imaging probes based on nanoparticles, QDs which are detectable by the above mentioned imaging modalities can be used for multitargeting and monitoring, as well as in improving diagnostic/therapeutic effects simultaneously\textsuperscript{146}. Hence, their importance is growing.
A nanoprobe with bifunctional magnetic and fluorescence properties can take advantage of the high sensitivity and high resolution characteristics of the fluorescence phenomena along with high spatial resolution and noninvasiveness characteristics of MRI. With these properties such a multifunctional nanoprobe will be useful for bioimaging applications.

**Magnetic Fe Doped CdTeS NIR QDs for Bio-Imaging Applications.** The optical, electrical and magnetic properties of bulk semiconductors are strongly influenced by doping. However, despite a few successes many of the efforts to dope semiconducting nanocrystals have failed for unknown reasons. To the best of our knowledge there are no reports available currently on Fe doped NIR emitting QDs synthesized in the aqueous system that have been used for MR imaging of mouse. Here in this dissertation synthesis and characterization of novel water dispersible Fe doped CdTeS QDs that emit in the NIR region and can be detected by MRI inside a mouse are reported.

3.6.1 Hydrothermal Synthesis of Fe Doped Core CdTeS QDs

The Fe doped CdTeS QDs were synthesized in a way which is very similar to the technique used for CdTeS core QDs. In a typical process sodium hydrogen telluride (NaHTe) was prepared from NaBH₄ and Tellurium powder in Argon saturated DI water. The molar ratio of NaBH₄ to Te used was 2:1. The as prepared NaBH₄/Te solution was kept in the refrigerator at 4 °C to keep the hydrogen gas produced during the reaction from expanding. The vial containing the solution might explode if the reaction is allowed to proceed at room temperature. The time period allowed for reaction to be completed was more than 8 hrs. 100 ml solution of a 30 mM solution of CdCl₂ in Argon saturated DIW was prepared separately in a 250 ml round bottle flask. NAC was added to the
solution so that Cd:NAC molar ratio was 1:2.5. FeCl$_2$.4H$_2$O was added so that Cd:Fe is 8.5:1. The solution was Argon bubbled for more than 30 min and then the NaHTe solution was added to this solution. The color of the solution turned wine red. The pH of the precursor solution was adjusted to (8.0 – 8.4) by using 2M NaOH solution with subsequent heating in PTFE lined autoclaves for different time intervals (30 – 100 min) at 180 °C. The autoclaves were cooled by a hydro-cooling process after heating. The QDs thus prepared were taken out and characterized after washing them several times with DI water.

**Effect of reactant concentration.** The CdCl$_2$ concentration was important for obtaining NIR emitting Fe doped QDs. For the undoped core CdTeS QDs, 12.5 mM CdCl$_2$ concentration produced QDs which emitted up to 750 nm. However, for the fluorescent-magnetic Fe doped core CdTeS QDs emitting in the NIR wavelength regime, 30 mM CdCl$_2$ concentration was found optimum. Solution with lower concentration of CdCl$_2$ i.e. 12.5 mM produced QDs that can emit only up to 650 nm. With this concentration, efforts to obtain Fe doped QDs that emitted above 650 nm were unsuccessful. When 30 mM CdCl$_2$ solution was used Fe doped QDs emitting up to 750 nm were obtained. For optimum fluorescence efficiency the Cd:Fe molar ratio was kept constant at 8.5:1. When the amount of Fe added was lower than this value the quantum yield of the Fe doped CdTeS QDs were the same as that of the undoped CdTeS QDs. Addition of more Fe resulted in significant quenching of the QDs.

**Effect of precursor pH, heating temperature and time.** The pH of the precursor solution used for synthesizing Fe doped CdTeS QDs was kept unchanged as was for undoped CdTeS QDs in the range of 8-9. The heating temperature was also kept
unchanged at 180 °C. The QDs produced were stable, had good fluorescence efficiency (40-60%) within the wavelength range of 530 nm – 740 nm when heated for various times within 30 to 100 minutes.

3.6.2 Characterization of the Fe Doped Core CdTeS QDs

The Fe doped core QDs synthesized by the hydrothermal technique were characterized using TEM, SAD, XRD, ICP, EDS, XPS, SQUID and MRI for determining the size, structure, chemical composition, magnetization and relaxivity of these particles.

**TEM, SAD and XRD of the Fe doped QDs.** The TEM image of Fe doped QD740 is presented in Figure 3-19. Lattice fringes in the TEM image reveal the crystalline nature of the QDs. The first inset shows the SAD pattern obtained from the QDs. The second inset shows the magnified image of one single QD. The particle sizes of all Fe doped QDs emitting within the wavelength range (530 nm – 740 nm) lie within 3-6 nm. The particles also appear to be elongated and non-spherical. The SAD pattern has three rings that correspond to the [111], [220] and [311] planes. The same three planes were represented by 3 peaks in the XRD of the QDs shown in Figure 3-20. The XRD patterns of the undoped QD and substrate are also provided for comparison. From the figure it is evident that the peaks for Fe doped QDs shifted to the left relative to the 2θ positions of the undoped QDs. Also the peaks of the doped material looks less sharp compared to the undoped samples. Both these effects are probably due to the change in lattice parameter as a result of Fe doping. The calculated d value for the [111] planes of Fe doped QDs is 4.71 Å which is larger than 3.59 Å for undoped CdTeS QDs. For cubic CdTe the 2θ values assigned for [111], [220] and [311] planes are 24.2°, 40.3° and 46.8° respectively while that for cubic zinc-blende CdS the corresponding 2θ values
are 27.1°, 44.8° and 53.4° respectively. Ohata et al.\textsuperscript{142} reported that bulk CdTe\textsubscript{1-x}S\textsubscript{x} type mixed crystals have cubic zinc blende (x < 0.2) and hexagonal wurtzite (x > 0.2) structure. The chemical composition of the Fe doped QDs, QD740 (S = 39.0 atom%) and QD730 (S = 39.4 atom%) as described in the section below have more than 20 atom% S and might possess the hexagonal wurtzite structure assuming that the phase behavior of bulk and QDs is similar. More investigation is required in this area to confirm the phase composition of these magnetic QDs.

**ICP, EDS and XPS of the Fe doped QDs.** The chemical compositions of these particles were determined using ICP. The amounts of S in these QDs were determined by subtraction. For the Fe doped QD740 the compositions in atomic % were Cd (44.4±0.3), Te (11.1±0.2), Fe (5.6±0.1) and S (39.0). For the Fe doped QD730 these numbers are Cd (46.9±1.5), Te (10.6±0.3), Fe (3.1±0.1) and S (39.4). The composition for the Fe doped QD740 was confirmed using EDS. The EDS spectrum in (Figure 3-21) indicates the presence of Fe (4.5±0.7) in the material in addition to Cd (49.4±1.1), Te (10.8±0.7) and S (35.4±0.8). The chemical composition values obtained from EDS for Fe doped QD730 are Cd (51.6±0.8), Te (9.7±1.9) and S (38.8±1.9). Due to small amount of Fe doping (< atomic 5%) in the QD730, which is below the detection limit of EDS, Fe peaks did not show up in the EDS spectra of the QD. The atomic% data for all the other elements Cd, Te, and S from both of these measurements match closely. XPS studies were done to cross check the presence of the elements in the QDs. Fe doped QD710 was analyzed using XPS after etching it with Argon for 10 minutes. The XPS results from the experiment are illustrated in Figures 3-22 (a) – (e). The typical peaks of the elements Cd and Te with the following binding energies: Cd 3d\textsubscript{5/2} (404.9 eV), Cd
3d\(_{3/2}\) (411.8 eV), Te 3d\(_{5/2}\) (572.2 eV) and Te 3d\(_{3/2}\) (582.6 eV) were obtained. The S 2p peak at 161.4 eV indicates the presence of monosulfide (S\(_2^2\)). The appearance of monosulfide peak indicates broken S-S bond which may be present at the Ar etched QD surface due to the breaking of CdS or FeS bonds. The Fe 2p\(_{3/2}\) peak at 708.6 eV signifies the presence of Fe(II)S in the material\(^{147}\).

**Magnetic property measurements of the Fe doped QDs.** Magnetic measurements using SQUID showed that the Fe doped QDs were superparamagnetic at room temperature (Figure 3-23). The temperatures at which the measurements were carried out were 10K and 300K. The saturation magnetization (M\(_s\)) values of Fe doped QD730 (Figure 3-23 (a)) was determined to be 1.7 emu/gm at 10K and at room temperature, 300K. For the Fe doped QD740 (Figure 3-23 (b)) the M\(_s\) values were 3.3 emu/gm at 10K and 2.8 emu/gm at 300K. The M\(_s\) values of the commercial contrast agent, Feridex\textsuperscript{®} I.V. were also measured using SQUID. At 10K and 300K the M\(_s\) values for Feridex\textsuperscript{®} I.V. were 12.3 emu/gm and 7.5 emu/gm respectively as indicated in Figure 3-23 (c). In all the above cases the magnetization values were calculated taking into account the total weight of the particles. However these calculations can also be done with respect to the amount of Fe in these materials. The saturation magnetization values of the Fe doped QDs and Feridex\textsuperscript{®} I.V. NPs with respect to the Fe content are depicted in Figure 3-24. The Fe content in the QDs was determined using ICP and EDS. The results indicated that the amount of Fe in QD730 was 3.1 atomic% (2.2 wt%) and in QD740 was 5.6 at% (3.9 wt%). The manufacturer reported the Fe content of Feridex\textsuperscript{®} I.V. as 11.2 mg/ml. The M\(_s\) values therefore for QD730 is 76 emu/gm[Fe] at 10K and 300K, for QD740 the values are 85 emu/gm[Fe] (10K), 71 emu/gm[Fe] (300K).
and for Feridex® I.V. they are 119 emu/gm[Fe] (10K) and 72 emu/gm[Fe] (300K). The reported Ms value for Feridex® I.V. at room temperature is 64.4 emu/gm[Fe]. Thus we see that the Ms values obtained for the Fe doped QDs are comparable to that of Feridex® I.V. None of the particles tested exhibited coercivity at room temperature indicating their superparamagnetic behavior at room temperature. However, in all of these particles coercivity was found to be present when their magnetic properties were measured at 10K. The 10K magnetization loops show hysteresis curve with a $H_c \sim 240$ Oe for QD740 particle, $H_c \sim 290$ Oe for QD730 and $H_c \sim 90$ Oe for Feridex® I.V. This phenomenon was observed for MNPs. Sun et al. reported the magnetization of 16 nm MNPs of CoFe$_2$O$_4$ with large hysteresis loop at 10K with coercivity of 20 kOe compared to a much smaller loop at 300 K with coercivity of only 400 Oe. However, for Fe$_3$O$_4$ NPs having the same size of 16 nm but without Co doping the coercivity exhibited by the NP assembly was 450 Oe at 10K and zero at 300K. This indicated that the incorporation of the Co cation in the matrix of Fe-O highly enhanced the magnetic anisotropy of the MNPs. For the Fe doped CdTeS QDs reported here the presence of large coercivity at 10K with almost negligible coercivity at 300K indicates similarity of increased magnetic anisotropy as that of the Co doped Fe-O NPs at low temperature.

The M vs H curves discussed above were obtained by measuring the magnetization of the NPs with varying magnetic field at a constant temperature. Now, the variation of magnetization of the NPs with varying temperature at a fixed magnetic field will be discussed. While the MH curves provided information on the saturation magnetization and coercivity of NPs, the field-cooled (FC) and zero field-cooled measurements provided information on the blocking temperature ($T_B$) of the NPs.
(Figures 3-25 (a) – (c)). In the ZFC measurements the QD and Feridex® I. V. samples were cooled without any applied magnetic field to a temperature much below the anticipated blocking temperature, $T_B$. Then the temperature of the system was raised and the magnetization measured as a function of temperature at a relatively low fixed magnetic field of 80 Oe. The FC magnetization measurements were carried out by cooling the sample in an applied magnetic field (80 Oe) and magnetization measured as a function of increasing temperature. The temperature dependent magnetizations, $M$ versus $T$ curves for both FC and zero-field-cooled ZFC cases were plotted for all the samples viz QD730, QD740 and Feridex® I.V. NPs. The blocking temperatures ($T_B$) for the NPs were determined from the ZFC curves. As the samples were heated the transition from ferromagnetism to superparamagnetism occurred at the blocking temperature. The free movements of the magnetic moments in the samples were ‘blocked’ at the temperature below $T_B$ by the anisotropy leading to their ferromagnetic behavior. Above $T_B$ the NPs exhibit super paramagnetic behavior. From the curves, $T_B$ for QD730 was determined to be 190K whereas, for QD740 it was 150K. By fitting the Bloch’s $T^{3/2}$ equation in the region $T << T_C$:

$$M = M_s[1 - \left(\frac{T}{T_c}\right)^{3/2}]$$

(3-3)

where,

$M_s$ – Saturation magnetization (emu/gm)

$T_c$ – Curie temperature (˚C)

$T_C \sim 570$ K for QD740, $T_C \sim 720$ K for QD730 nm particle and $T_c \sim 283$ for Feridex® I.V. were also obtained. For both of these QDs curie temperatures, $T_c$ is above the room temperature. From the magnetization data we conclude that Fe doped QDs show
ferromagnetism at low temperatures which is evident from their magnetization loops with non-zero $H_c$ values and low field saturation.

**MR imaging of magnetic Fe doped QDs and Feridex® I.V. NPs.** The $T_2$ weighed images of Fe doped CdTeS QDs (QD740) and those of Feridex® I.V. NPs are depicted in Figures 3-26 (a) and (b). In both cases serial dilutions of the particles were loaded into glass capillaries and imaged using a 750 MHz 17.6 T 89 mm bore MRI. The Fe doped QDs were well dispersed and hence they were imaged after diluting them with DI water. However, the Feridex® I.V. NPs were found to be poorly dispersed and agglomerated. Hence they were imaged after diluting them with 0.25% agarose solution. The Fe doped QD740 sample concentrations used for capillaries numbered 1, 2, 3, 4, 5 and 6 (Figure 3-26 (a)) were 8.6, 4.3, 2.15, 1.08, 0.54 and 0.27 mg/ml respectively. DIW was used for glass capillary numbered 7 and was used as a control sample. For the commercial Feridex® I.V. samples (in 0.25% agarose solution) imaged in Figure 3-26 (b) the sample concentration used were 8.6, 4.3, 2.15, 1.08, 0.54, 0.27 and 0.14 mg/ml for capillaries numbered 1-7 respectively. The 8th capillary was filled with distilled water and was used as the control sample. From the figures it is evident that various concentrations of the contrast agent, Fe doped particles generated different contrast with respect to distilled water.

The efficiency of the synthesized Fe doped QD740 and the Feridex® I.V. NPs, both $T_2$ agents in generating MRI contrast can be evaluated by comparing their relaxivity coefficients ($r_2$), which is related to $T_2$ through the following equations\(^{150}\).

$$\frac{1}{T_2} = \frac{1}{T_2^0} + r_2 C$$  \hspace{1cm} (3-4)
\[ R_2 = R_2^0 + r_2 C \]  

where, \( C \) is the concentration of the contrast agent

\( T_2 \) is the observed relaxation time in the presence of the contrast agent and

\( T_2^0 \) is the relaxation time for pure water

\( R_2 \) or \((1/T_2)\) is the relaxation rate in the presence of contrast agent

\( R_2^0 \) or \((1/T_2^0)\) is the relaxation rate of pure water

From Equation 3-4 it is evident that \( T_2^0 \) and \( r_2 \) being constants the relaxation time is inversely proportional to the concentration of the contrast agent. Thus, as the concentration of the contrast agent increases the relaxation time decreases and the MRI image appears darker. Also, the higher the value of \( r_2 \) for a contrast agent, smaller the concentration required to generate the same contrast.

Plots represented in Figures 3-27 depict the (a) \( T_2 \)-weighted relaxation rate, \( R_2 \) and (b) \( T_2^* \)-weighted relaxation rate, \( R_2^* \) versus metal ion concentration (Fe) for Fe doped QD740 and Feridex® I.V. NPs. The relaxivity co-efficient, \( r_2 \) for both contrast agents was determined from the slope of the plots. The \( r_2 \) values obtained for the Fe doped QD740 and Feridex® I.V. sample were 732.36 (mM\(^{-1}\)s\(^{-1}\)) and 389.18 (mM\(^{-1}\)s\(^{-1}\)) respectively whereas the \( r_2^* \) values for the samples were 730.75 (mM\(^{-1}\)s\(^{-1}\)) and 438.11 (mM\(^{-1}\)s\(^{-1}\)) respectively. Thus the relaxivity coefficient \( r_2 \) for Fe doped QD740 is 88% higher than that of Feridex® I.V. NPs. This phenomenon is unusual and the underlying reason behind the higher relaxivity exhibited by the Fe doped QDs when compared to Feridex® I.V. NPs was not understood and needs further investigation.

From the data it can be concluded that these particles are magnetic and can have useful applications as MRI contrast agents. As discussed before these particles are also
fluorescent unlike the Feridex® I.V. particles. Thus these particles can have novel bimodal fluorescent and magnetic applications and can be tracked more efficiently compared to Feridex® I.V. which can only generate magnetic contrast.

3.6.3 Performance Assessment of the Magnetic Fe Doped QDs

Experiments were performed to evaluate the performance of the magnetic QDs as \textit{in-vitro} and \textit{in-vivo} MRI contrast agents. J774 macrophages were incubated with Feridex® I.V. NPs and Fe doped QDs separately at 37 °C for 6 hours. Prior to incubation, Fe doped QD740 and Feridex® I.V. NPs were added to J774 macrophages of concentration $1 \times 10^6$ cells per ml. The concentration achieved for both particles was 0.2 mg/ml. Macrophages containing the particles were then loaded into glass capillaries and imaged with 14 T MRI at 600 MHz. Macrophages containing Feridex® I.V. NPs were used as control for the experiment. Some of these macrophages were then injected into a live mouse to ascertain the efficacy of the Fe doped QDs as \textit{in-vivo} optical and MRI contrast agent. The macrophages containing the QDs were injected into the left leg of the mouse while the Feridex® I.V. particles were injected into the right leg of the mouse. The mouse was imaged using Xenogen IVIS® imaging system for assessing the \textit{in-vivo} fluorescent contrast generating efficiency of the QDs. MR imaging was also performed on the mouse to find out the suitability of the magnetic QDs as \textit{in-vivo} MRI contrast agents.

**MR Imaging of magnetic Fe doped QDs and Feridex® I.V. NPs after injecting them into J774 macrophages.** MR imaging of the QD labeled macrophages was performed using 14T MRI at 600 MHz. Figure 3-28 shows the MRI images of capillaries filled with cells attached to both the QDs and the Feridex® I.V. NPs. There is also a capillary containing just the cells without any particles as control sample.
From the images depicted in Figure 3-28 the contrast generated by the two capillaries containing both the QDs and the Feridex® I.V. particles relative to the capillary containing cells without any of the particles is significant and can be easily detected by the unaided eye. The Feridex® I.V. NPs generated more contrast than the QDs. This was due to the higher iron content (10.4 wt%) of the Feridex® I.V. NPs relative to the Fe doped QDs (3.9 wt%) as discussed earlier.

**Optical and MR imaging of mouse after injecting it with cells loaded with Fe doped QDs and Feridex® I.V. particles.** About 10 μl of the J774 macrophages containing Fe doped QDs (emitting at 740 nm) was injected into the left leg of a mouse while the right leg was injected with cells containing Feridex® I.V. NPs. The mouse was then imaged using Xenogen IVIS® imaging instrument. As depicted in Figure 3-29 the fluorescence from the QDs inside the left leg of the mouse could be seen whereas there was no detectable fluorescence from the Feridex® I.V. NPs (non-fluorescent) in the right leg.

The MRI of the mouse was carried out at 600 MHz. As shown in Figure 3-30 both the Fe doped QDs as well as the Feridex® I.V. particles generated contrast with respect to the surrounding tissue. The contrast generated by the Feridex® I.V. NPs was more than that generated by the Fe doped QDs. This phenomenon is due to the stronger magnetic property of the Feridex® I.V. NPs because of its higher Fe content (10.4 wt%) compared to the Fe doped QD740 (3.9 wt%). However the important point to note here is that the QD particles have enough magnetic property that can generate MRI contrast when injected into animals. Thus these QDs show potential for dual fluorescent and magnetic bimodal contrast agents.
The amount of Fe content of the QDs can be increased by coating the Fe doped core QDs (emitting at 730, 740 nm) with Fe doped CdS shells. The formation of Fe doped shells on magnetic core QDs will not only increase their iron content, thus improving their magnetic property but also help in tuning the emission wavelength toward 800 nm. Better magnetic properties and NIR emission around 800 nm are both desirable in these QDs for bio-imaging applications.
Table 3-1. Particle size comparison (Measured versus calculated)

<table>
<thead>
<tr>
<th>Serial</th>
<th>Quantum Dot</th>
<th>Measured ($D_m$) (nm)</th>
<th>Calculated ($D_c$) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>QD580</td>
<td>3.8±0.4</td>
<td>3.3</td>
</tr>
<tr>
<td>2</td>
<td>QD630</td>
<td>4.0±0.5</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>QD700</td>
<td>4.2±0.4</td>
<td>4.6</td>
</tr>
</tbody>
</table>

QD580, QD630 and QD700 – CdTeS quantum dots emitting at 580 nm, 630 nm and 700 nm respectively.

Table 3-2. Chemical composition of the core QD670, core QD750 versus core/shell QD750 determined by EDS.

<table>
<thead>
<tr>
<th>Element</th>
<th>Core 670 nm</th>
<th>Core 750 nm</th>
<th>Core/shell 750 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>49.8</td>
<td>53.3</td>
<td>49.4</td>
</tr>
<tr>
<td>Te</td>
<td>15.5</td>
<td>11.2</td>
<td>3.3</td>
</tr>
<tr>
<td>S</td>
<td>34.7</td>
<td>35.5</td>
<td>47.3</td>
</tr>
</tbody>
</table>

Table 3-3. Surface chemical composition of the core QD670, core QD750 versus core/shell QD750 determined by XPS.

<table>
<thead>
<tr>
<th>Element</th>
<th>Core QD670</th>
<th>Core QD750</th>
<th>Core/shell QD750</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>55.1 ± 0.1</td>
<td>57.2 ± 0.7</td>
<td>59.5±2.0</td>
</tr>
<tr>
<td>Te</td>
<td>12.5 ± 0.3</td>
<td>8.8 ± 0.1</td>
<td>3.0±1.1</td>
</tr>
<tr>
<td>S</td>
<td>32.5 ± 0.2</td>
<td>34.0 ± 1.1</td>
<td>37.9±1.6</td>
</tr>
</tbody>
</table>
Table 3-4. Surface chemical composition (atomic %) of the core QD670 determined using XPS after etching with Argon for 0, 5 and 10 minutes.

<table>
<thead>
<tr>
<th>Element</th>
<th>Cd</th>
<th>Te</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min Ar etching</td>
<td>55.1±0.1</td>
<td>12.5±0.3</td>
<td>32.5±0.2</td>
</tr>
<tr>
<td>5 min Ar etching</td>
<td>53.4±0.7</td>
<td>16.8±0.4</td>
<td>29.8±1.1</td>
</tr>
<tr>
<td>10 min Ar etching</td>
<td>51.8±0.2</td>
<td>18.0±0.1</td>
<td>30.2±0.3</td>
</tr>
</tbody>
</table>

Table 3-5. Surface chemical composition (atomic %) of the core QD750 determined using XPS after etching with Argon for 0, 5 and 10 minutes.

<table>
<thead>
<tr>
<th>Element</th>
<th>Cd</th>
<th>Te</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min Ar etching</td>
<td>56.3</td>
<td>8.9</td>
<td>34.8</td>
</tr>
<tr>
<td>5 min Ar etching</td>
<td>55.1</td>
<td>12.4</td>
<td>32.5</td>
</tr>
<tr>
<td>10 min Ar etching</td>
<td>54.1</td>
<td>13.0</td>
<td>33.0</td>
</tr>
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</table>

Table 3-6. Surface chemical composition (atomic %) of the core/shell QD750 determined using XPS after etching with Argon for 0, 5 and 10 minutes.

<table>
<thead>
<tr>
<th>Element</th>
<th>Cd</th>
<th>Te</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min Ar etching</td>
<td>59.9</td>
<td>3.1</td>
<td>37.9</td>
</tr>
<tr>
<td>5 min Ar etching</td>
<td>58.4</td>
<td>3.5</td>
<td>38.1</td>
</tr>
<tr>
<td>10 min Ar etching</td>
<td>56.9</td>
<td>4.0</td>
<td>39.2</td>
</tr>
</tbody>
</table>
Table 3-7. Characteristics of clinically used imaging modalities (Basilion et al.\textsuperscript{145})

<table>
<thead>
<tr>
<th>Modality</th>
<th>Resolution</th>
<th>Depth</th>
<th>Cost</th>
<th>Sensitivity</th>
<th>Imaging agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET</td>
<td>++</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
<td>Radioisotope</td>
</tr>
<tr>
<td>SPECT</td>
<td>+</td>
<td>++++</td>
<td>++</td>
<td>+++</td>
<td>Radioisotope</td>
</tr>
<tr>
<td>MRI</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>Paramagnetic ion (Gd\textsuperscript{3+}, Mn\textsuperscript{2+}), Paramagnetic nanoparticles, Superparamagnetic nanoparticles( iron oxide)</td>
</tr>
<tr>
<td>Optical</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>Organic dye, Fluorescent protein, QDs, RE materials, Carbon nanotube</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>Microbubble, Perfluorocarbon, nanoparticles</td>
</tr>
</tbody>
</table>
Figure 3-1. (a) Fluorescence from Vis-NIR QDs when excited with UV light (b) PL intensity versus wavelength plot for the QDs (some wavelengths are omitted for clarity)
Figure 3-2. Emission wavelength versus heating time for core QDs. Heating temperature was kept constant at 180 °C for the entire heating period.
Figure 3-3. TEM image of core CdTeS QDs emitting at 700 nm. Insets show the SAD pattern and the size of a single QD.
Figure 3-4. EDS spectra for the (a) core 560 nm and (b) core 700 nm emitting QDs.
Figure 3-5. Chemical composition variation in QDs determined using EDS spectra analysis.
Figure 3-6. Determination of extinction coefficient of core QDs (a) QD630 and (b) QD710.
Figure 3-7. (a) Energy diagram of CdTeS/CdS core/shell QDs represented schematically, adapted from Ref. 124 (b) Red-shift in emission wavelength with increasing CdS shell thickness.
Figure 3-8. Emission tunability in core/shell quantum dots (a) core quantum dots (b) and (c) change in wavelength of the core dots due to CdS shell coating on the CdTeS core QDs.
Figure 3-9. Zeta potential variation for core CdTeS 690 nm emitting QDs as a function of pH.
Figure 3-10. TEM image of core/shell QDs emitting at 720 nm. Insets show the SAD pattern and the size of a single QD.
Figure 3-11. XRD of core and core/shell QDs. The standard diffraction lines for cubic CdTe are shown at the bottom axis while for cubic CdS are shown at the top axis of the plot.
Figure 3-12. EDS spectra for (a) core CdTeS QDs emitting at 750 nm and (b) core/shell CdTeS/CdS QDs emitting at 750 nm.
Figure 3-13. (a) XPS of the core QD670, core QD750 and core/shell QD750 (b) XPS showing the Cd 3d$_{5/2}$ and Cd 3d$_{3/2}$ peaks for all the samples.
Figure 3-14. XPS showing the (a) Te 3d$_{5/2}$ & Te 3d$_{3/2}$ peaks and (b) S 2p & S 2s peaks in core QD670, core QD750 and core/shell QD750 samples.
Figure 3-15. Normalized PL decay curves for core 620 nm and core/shell 800 nm emitting QDs.
Figure 3-16. Normalized PL decay curves for core 670 nm, core 750 nm and core/shell 750 nm emitting QDs.
Figure 3-17. (a) J774 mouse cells labeled with core/shell CdTeS/CdS QDs emitting at 800 nm (b) unlabeled DAPI stained cells as control.
Figure 3-18. Xenogen IVIS® Spectrum Biophotonic imaging of mouse with core/shell CdTeS/CdS QDs emitting at 800 nm with different excitation wavelengths and emission filters (a) excited at 640 nm and imaged with 720 nm filter (b) excited with 710 nm and imaged with 780 nm filter (c) excited with 710 nm and imaged with 840 nm filter.
Figure 3-19. TEM image of Fe doped CdTeS QDs having emission wavelength of 730 nm. Insets show the SAD pattern and the size of a single QD.
Figure 3-20. XRD of the magnetic Fe doped and undoped CdTeS QDs.
Figure 3-21 EDS spectrum for the Fe doped QD740.
Figure 3-22. XPS of the Fe doped QD710 sample etched with Argon for 10 minutes showing the (a) Cd, Te, Fe, S, O and C peaks and (b) Cd 3d\textsubscript{5/2} and Cd 3d\textsubscript{3/2} duplet peaks.
Figure 3-22. Continued. XPS of the Fe doped QD710 sample etched with Argon for 10 minutes showing the (c) Fe 2p$_{3/2}$ and Fe 3p$_{1/2}$ duplet peaks (d) Te 3d$_{5/2}$ and Te 3d$_{3/2}$ duplet peaks.
Figure 3-22. Continued. (e) XPS of the Fe doped QD710 sample etched with Argon for 10 minutes showing the S 2p peak.
Figure 3-23. Magnetometry measurements using SQUID at 10K and 300K for (a) 730 nm and (b) 740 nm emitting Fe doped CdTeS QDs and (c) commercial Feridex® I.V. NPs normalized with respect to particle mass.
Figure 3-24. Magnetometry measurements using SQUID at 10K and 300K for (a) 730 nm and (b) 740 nm emitting Fe doped CdTeS QDs and (c) commercial Feridex® I.V. NPs normalized with respect to Fe content.
Figure 3-25. Magnetization versus temperature plot at a constant magnetic field of 80 Oe for (a) Fe doped QD730 and (b) Fe doped QD740.
Figure 3-25. Continued. (c) Magnetization versus temperature plot at a constant magnetic field of 80 Oe for commercial Feridex® I.V. particles.
Figure 3-26. MRI in-vitro $T_2$-weighted images of serially diluted (a) Fe doped QDs (in DI water) and (b) Feridex® I.V. particles (in 0.25% agarose solution), loaded into glass capillaries.
Figure 3-27. (a) $R_2^*$ and (b) $R_2$ versus Fe concentration plots for Fe doped QD740 (in water) and Feridex® I.V. NPs (in 0.25% agarose solution).
Figure 3-28. Fe doped QDs and Feridex® I.V. labeled J774 macrophages were loaded into glass capillaries and imaged using 14T MRI at 600 MHz.
Figure 3-29. J774 macrophages labeled with Fe doped QD740 and Feridex® I.V. were injected into the left and right leg of the mouse respectively and excited with 710 nm light.

Fluorescence from J774 macrophages labeled with magnetic Fe doped QDs emitting at 740 nm.

No fluorescence from J774 macrophages labeled with magnetic Feridex® I.V NPs.
Figure 3-30. Fe doped QDs and Feridex® I.V. labeled J774 macrophages were injected into the left and right leg of the mouse respectively and imaged with MRI.
4.1 Conclusions

In this research we demonstrate the synthesis and characterization of two kinds of QDs by the hydrothermal synthesis technique (a) core and core/shell CdTeS/CdS QDs emitting in the visible - NIR wavelength regime of 530 – 820 nm and (b) novel bimodal Fe doped CdTeS QDs that exhibit dual fluorescent and magnetic properties. Either types of QDs were capped with NAC and were highly dispersible in water. Through the hydrothermal synthesis process the emission wavelength of the QDs could be easily tuned by changing the size of the QDs while keeping the heating temperature constant. Longer heating times (30 – 100 min) produced larger particles with higher emission wavelengths in the 530 - 750 nm regimes. Particle agglomeration with QDs emitting above 750 nm was observed. Decomposition and degradation of the dispersing ligand (NAC) in high amounts is the probable reason behind QD agglomeration.

Wavelength tunability above 750 nm was achieved by coating the core CdTeS QDs with a CdS shell. Maximum emission tunability of 200 nm was achieved by coating CdS layer on core CdTeS QDs (emitting at 620 nm) which produced 820 nm emitting CdTeS/CdS core/shell QDs. The sizes of core and core/shell QDs varied within 3-6 nm and their fluorescence QY ranged from 20 to 60%. The QY for the vis-NIR core 530 – 700 nm emitting QDs varied between 40-60% while that of core/shell 700 – 820 nm emitting QDs varied within 20-40%. Fe doped QDs exhibited similar values for size and QY. The core CdTeS QDs possessed a gradient alloy composition where the amount of S increased while that of Te decreased with increasing QD size. The amount of Cd remained constant at around 50% for the entire QD size range of 3-6 nm. The suitability
of these core/shell QDs as optical contrast agents for NIR fluorescence imaging was assessed by attaching the QD800 with J774 macrophages and imaging them using NIR detecting camera. The QDs were bright enough to be imaged by the camera.

The Fe doped magnetic QDs were highly fluorescent and emitted within the wavelength range of 530 – 750 nm. The amounts of Fe in the 730 – 740 nm emitting QDs were determined to be within 4-5 atomic% and they exhibited ~40% QY. The magnetic properties of these QDs were evaluated by measuring the saturation magnetization \( (M_s) \) using SQUID and the values were found to be in the range of 1.5 – 4.0 emu/gm. J774 macrophages incubated with QD740 were further injected into the left leg of a mouse and imaged using Xenogen IVIS\textsuperscript{®} to find out the suitability of these QDs for \textit{in-vivo} imaging. The QDs inside the mouse were visible when imaged with Xenogen IVIS\textsuperscript{®} and they were also successfully imaged by MRI. The relaxivity coefficient, \( r_2 \) obtained for QD740 (732.4 mM\textsuperscript{-1}s\textsuperscript{-1}) was 88% higher than that of Feridex\textsuperscript{®} I.V. NPs (389.2 mM\textsuperscript{-1}s\textsuperscript{-1}). The underlying reason for the higher relaxivity exhibited by the Fe doped QDs per unit Fe compared to Feridex\textsuperscript{®} I.V. is not understood. This is an unusual phenomenon and needs further investigation. In summary, these particles are highly fluorescent and magnetic in nature and they have potential as bimodal contrast agents for biological applications.

\textbf{4.2 Suggestions for Future Work}

In this research it was demonstrated that fabrication of magnetic QDs, as small as 3-6 nm in diameter with significant fluorescence and magnetic properties for bimodal biological imaging applications is possible by aqueous hydrothermal synthesis techniques. This research can be further extended in various ways. Few of them are reported here:
The QDs have elements Cd and Te which are considered to be toxic to animals. QDs can be synthesized with elements that are minimally toxic/non-toxic to animals using processes similar to the hydrothermal techniques described in this research. One of the elements contained in the QDs need to have thiol affinity for the hydrothermal synthesis with thiol stabilizers to be successful. For e.g. CuInS$_2$ is a good potential candidate for NIR imaging of biological materials. These QDs have already been synthesized by the organometallic route and they exhibit good fluorescence property in the NIR wavelength regime of the electromagnetic spectrum around 800 nm. Li et al.\textsuperscript{151} reported the synthesis of CuInS/CdS QDs (emitting 709 nm) having QY > 80%. Cu is known to be relatively less toxic to human beings than Cd and also has affinity for thiol. Indium has some toxicity however the amount of Indium used in CuInS$_2$ QDs was relatively smaller than Cd used for Cd based QDs and thus these Cu/In based QDs should be much less toxic compared to Cd based QDs. Also, these Cu based QDs can be made bimodal with optical and magnetic properties by doping them with paramagnetic ions similar to the Fe doped CdTeS QDs reported here.

The QDs in this research were doped with Fe, which is a T$_2$ contrast agent for MRI. However, paramagnetic ions such as Mn can be doped in the CdTeS QDs in a similar way. Mn is a T$_1$ contrast agent and has complementary MR imaging characteristics to Fe. Thus the hydrothermal process described here can also be utilized to develop T$_1$ contrast agents for bio-imaging applications.

The amount of Fe/Mn content in the QDs can be increased by coating these core QDs with Fe/Mn doped CdS shells. The formation of paramagnetic ion doped shells on core QDs will not only increase the amount of paramagnetic ion content of the QDs,
thus improving their magnetic property, but also help in tuning the emission toward the 800 nm regime, both of which are desirable for bio-imaging applications.

The QDs synthesized can be coated with silica or a polymer to reduce toxicity and improve their biocompatibility. He et al.\textsuperscript{138} demonstrated that CdTe QDs prepared by aqueous synthesis techniques can be made bio-compatible by coating them with protein amino groups. The authors used crosslinkers, N-(3-dimethylaminopropyl)-N-ethylcarbodiimde hydrochloride (EDC) and N-hydroxysuccinimide (NHS) as conjugates for coating the QDs.
APPENDIX
DETERMINATION OF QUANTUM DOT MOLECULAR WEIGHS

The MWs of QDs were calculated by a method that is similar to the one reported by Quall et al.\textsuperscript{152}. Discussions on the extinction coefficient are available in section 3.2.4.

**Molecular weight of QD630.**

\[ \lambda_{\text{max}} = 600 \text{ nm} \quad \text{Diameter, } d = 3.66 \text{ nm} \]

CdTe bond length = 0.28 nm

The number of Formula units across the QD diameter is given by:

\[ \text{FU} = \frac{\text{Diameter}}{\text{CdTe bond length}} = \frac{3.66 \text{ nm}}{0.28 \text{ nm}} = 13.07 \text{ units} \]

The number of CdTe units present in a CdTe spherical particle of size \( d = 3.66 \text{ nm} \) is:

\[ (4/3) \times \pi \times (\text{FU}/2)^3 = (4/3) \times \pi \times (13.07/2)^3 = 1168.44 \]

Therefore, Molar Mass of QD630 with diameter 3.7 nm = (1168.44 units of CdTe) \times (molar mass of Cd + Molar mass of Te) = 1168.44 \times (112.41 + 127.60) = 280436 g/Mol

**Molecular weight of QD710.**

\[ \lambda_{\text{max}} = 650 \text{ nm} \quad \text{Diameter, } d = 4.34 \text{ nm} \]

CdTe bond length = 0.28 nm

The number of Formula units across the QD diameter is given by:

\[ \text{FU} = \frac{\text{Diameter}}{\text{CdTe bond length}} = \frac{4.34 \text{ nm}}{0.28 \text{ nm}} = 15.5 \text{ units} \]

The number of CdTe units present in a CdTe spherical particle of size \( d = 3.7 \text{ nm} \) is:

\[ (4/3) \times \pi \times (\text{FU}/2)^3 = (4/3) \times \pi \times (15.5/2)^3 = 1948.83 \text{ units} \]

Therefore, Molar Mass of QD630 with diameter 4.6 nm = (1948.83 units of CdTe) \times (molar mass of Cd + Molar mass of Te) = 1948.83 \times (112.41 + 127.60) = 467738 g/Mol
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