Synthetic strategies and design of highly luminescent cholinomimetic quantum dots

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Synthetic Strategies and Design of Highly Luminescent Cholinomimetic Quantum Dots

by

Maria L. McAtee

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in Chemistry

Dr. Jon R. Kirchhoff, Committee Chair

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The University of Toledo

December 2012
An Abstract of

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The high affinity choline uptake transmembrane protein (CHT) is an important part of the cholinergic transport cycle and yet the structure of this transmembrane protein has still not been determined. The concentration and functionality of choline has been observed to be depleted in neurodegenerative diseases, so obtaining as much data about the cycle is an important part of diagnosis and possible treatment for these diseases, such as Alzheimer’s Disease (AD). Previous research has focused on developing extremely sensitive methods of monitoring acetylcholine and choline throughout the uptake process using capillary electrophoresis with electrochemical detection (CEEC). These quantitative methods have provided nonradiochemical strategies for the measurement of choline transport including measurements under conditions of selective cholinergic inhibition.

A cholinomimetic quantum dot (QD) was designed and synthesized to provide qualitative imaging opportunities for monitoring and observing CHT. These QDs were synthesized using a CdSe/ZnSe/ZnS inorganic core in a colloidal organic matrix with triocylphosphine, triocylphosphine oxide and hexadecylamine as the coordinating ligands and passivating agents to control growth. The QDs were ~6.2 nm in diameter and
exhibited strong and narrow fluorescence intensity and broad UV-absorption properties. The organic ligands were exchanged for water-soluble mercaptosuccinic acid ligands which functionalized the surface with a useful carboxylic acid. An aqueous coupling reaction with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was utilized to create three distinct functionalized quantum dots; one quantum dot with the –OH moiety on the surface, the second with the quaternary ammonium group on the surface and the third with both functional groups on the surface to work together as a cholinomimetic probe, as the quaternary ammonium and the alcohol groups are the two important groups on choline and the mode of interaction with CHT.

The final cholinomimetic QD was a spherical nanocrystal with a diameter of ~ 6 nm and low polydispersity. The final QDs demonstrated higher fluorescence efficiency than either the –OH QD or the quaternary ammonium QD. The UV spectrum demonstrated retention of the broad absorption properties and dynamic light scattering and TEM confirmed their size and shape.
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List of Abbreviations

ACh ............................ acetylcholine
AcCoA ........................ acetyl-coenzyme A
AChE .......................... acetylcholine esterase
ACHR .......................... acetylcholine receptors
BSA ............................ bovine serum albumin
BuCh ........................... butyrylcholine
CE ............................... capillary electrophoresis
Ch ............................... choline
ChO ............................. choline oxidase
CHT ............................. high affinity choline uptake protein
DLS ............................. dynamic light scattering
DMSO ......................... dimethyl sulfoxide
EC ............................. electrochemical detection
EDC ........................... 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride
GABA .......................... gamma-aminobutyric acid
HC-15 ........................... hemicholinium-15 analogue inhibitor
HDA ............................. hexadecylamine
MSA ............................ mercaptosuccinic acid
NHS ............................. N-hydroxysulfosuccinimide
QD ............................... quantum dot
TCR .............................. T-cell receptor
TOP ............................. trioctylphosphine
TOPO ........................... trioctylphosphine oxide
VACHT ......................... vesicular acetylcholine transporter
TGA-DTA ...................... thermogravimetric and differential thermal analysis
Z-STEM ....................... Z-contrast scanning transmission electron microscopy
Chapter 1

1.1 Quantum Dots

1.1.1 Definition and Theory

Quantum dots (QDs) are among the newer innovations in studies utilizing fluorescence to image and monitor biochemical systems.\(^1\)\(^-\)\(^4\) They are currently being used for a wide variety of applications within biological matrixes due to the ability to attach specific functional groups onto the surface of the quantum dot. Quantum dot is the term used to refer to a nano-sized cluster of atoms whose size is within a domain where the electrical properties are dominated by quantum size effects and they no longer share the properties of the bulk material.\(^5\) Semiconductor quantum dot’s electronic bandgap becomes dependent on the size of their nanocluster. The smaller the cluster the more discrete the energy levels become hence the cluster behaves more like a single atom which is why they are sometimes known as an “artificial atom.” The transition from continuous to discrete energy states creates a measurable and size-tunable bandgap for particles that become small enough to exhibit these characteristics.\(^6\) This observable phenomenon is known as the quantum confinement effect and is illustrated in Figure 1-1. As the radius \(a\) of the particle continues to decrease from left to right, and eventually reaches and then becomes smaller than the Bohr Exciton Radius \(a_b^*\) quantum size
effects change the characteristics of the material, changing the energy levels from continuous states to discrete states and providing a measurable and tunable bandgap.\textsuperscript{7}

\textbf{Figure 1-1.} Diagram illustrating the quantum confinement effect.

Because the bandgap becomes a size dependent variable, the radius of the quantum dot can be used to calculate and predict the size of the energy of the bandgap using equation 1\textsuperscript{8}:

\[ E_{NC} = E^B + \frac{\hbar^2 \pi^2}{2R^2} \left( \frac{1}{m_e^*} + \frac{1}{m_h^*} \right) - 1.8 \frac{e^2}{\varepsilon R} \]

(1)
where $R$ is the radius of the nanocrystal, $\varepsilon$ is the size dependent dielectric constant of the bulk form of the material, $m_e^*$ and $m_h^*$ are the reduced masses of the electron and hole. The last term is corrective.

### 1.1.2 Lattice Structure Variances Due to Surface Passivating Agents

Nanocrystals present unique systems for study due to their high surface area to volume ratio. For example, in a 1.2 nm quantum dot, approximately 88% of the atoms are on the surface. While that number obviously will decrease with increasing size of a nanoparticle, it is still an important attribute and characteristic of all nano-sized structures. The other important facet of quantum dot growth is the ligands used as the surfactants and passivating agents. The choice of ligands can both affect the size and uniformity of the crystals grown in a colloidal synthesis. If chosen properly, the colloidal synthesis can yield quantum dots of uniform size and shape, eliminating the need for size selective precipitation which originally was a necessary step for obtaining a sample of highly uniform nanocrystals. The interface dynamics of the crystal with their coordinating ligands offers interesting growth parameters that can be both exploited for optimal control and utilized in understanding the effects of each surface passivating agent upon the fluorescence and perfection of the crystal.\(^9\)

The crystal structure of a typical CdSe quantum dot is a mixture of both zinc-blende and wurtzite. Although the ratios of the two crystal structures within a quantum dot core can be varied if so desired, creating a core that favors one lattice structure over the other by specifically controlling several parameters (ratios of original
monomers, temperature, ratios of coordinating ligands, etc.) during a quantum dot synthesis. However, with CdSe quantum dots, the very small quantum dot synthesized the higher percentage of zinc blende crystal structure is prominent if not exclusive of the wurtzite structure. As the quantum dot increases in size the more wurtzite structure is again seen. When studying the quantum dots with the wurtzite lattice structure, it was observed that as they are reduced in size, non-uniform bond distortion is seen, as the axial bonds of the tetrahedron are extended and the equatorial bond lengths contract. The authors of this research suggest that this is due to the surface stress of the smaller quantum dots which are affected and controlled by passivation with organic ligands. They specifically researched the effects of trioctylphosphine oxide (TOPO) and hexadecylamine (HDA), which are both widely used passivating agents and work in different mannerisms to change the stresses and surface energies of the nanocrystal. Using Raman spectroscopy and the modified Laplace law for solids, they were able to determine that the HDA passivated quantum dots showed size-dependent compressive stress, which leads to contraction of the lattice and for the TOPO passivated quantum dots the results indicated tensile stress giving rise to lattice expansion.  

The CdSe quantum dots synthesized and grown with only TOPO as the surface passivation surfactant are found to be elongated and non-uniform in both size and shape as compared with both TOPO and HDA as surface passivating agents. When grown in only TOPO the CdSe quantum dots have a higher concentration of Cd atoms than Se atoms within each crystal, and Z-STEM indicates that the excess Cd more heavily cover the surface of the crystal. This method of synthesis leads to more crystal structure defects and more dangling bonds at the surface, which provide more ample surface “traps” for
excited electrons to fall into, overall decreasing the fluorescence efficiency of the quantum dot. However, when synthesized with both HDA and TOPO, the surface was not so unequally distributed with excess Cd, and the surface traps and crystal impurities were minimized.\textsuperscript{11}

For some time throughout the synthesis history of developing an optimal CdSe quantum dot synthetic procedure, it was observed that using pure TOPO resulted in poor crystal growth and development, but using the technical grade increased the growth and uniform size dispersity of the nanocrystals. It was initially referred to as “the magic ingredient” that was an unknown impurity in technical grade TOPO. Peng et. al. determined this magic ingredient to be phosphonic acid, but also discovered that using it in higher quantities than the trace impurity found in TOPO, caused uncontrollable growth and non-uniform size distribution of the quantum dots.\textsuperscript{9} Since more understanding has been obtained regarding the importance of the acids presence in the colloidal growth solution, different acids have been utilized purposefully and their concentrations optimized to obtain good crystal growth without losing control of both size and homogeneity of the final products.

1.1.3 Colloidal Crystal Nucleation and Growth

Time, temperature and concentrations of coordinating ligands are all tunable factors in controlled nanocrystal growth.\textsuperscript{6,12} There are several variations in possible synthetic routes all experimenting with different variables that affect growth rate and size.\textsuperscript{13-16} At higher temperatures larger crystals can be grown faster, but not at a high
enough rate that this is of gross significance overall. However, concentrations of the coordinating ligands seem to be the most critical factor regarding shape and uniformity, while the length of time of crystal growth primarily affects the final size of the crystals and can be optimized and determined by the desired size of the nanocrystal product.

1.1.3.1 Ostwald’s Ripening

Ostwald’s ripening refers to the phenomena of smaller nanoclusters in solution re-dissolving into the solution and becoming free monomers again, and then consequently layering on top of the larger nanoclusters in the solution. This effect creates larger nanoclusters and eliminates the smaller clusters in solution. Ideally growth will be terminated at the chosen size before this occurs by use of temperature controls and surface passivating agents to eliminate the re-dissolving of the smaller particles.

1.1.4 Current Biological Applications

Quantum dots have become very popular for biological tagging and as biological probes because their surface can be covered with a variety of different ligands to be used to interact with specific biomolecules and obtain high resolution fluorescence microscopy images. Chen et al. used near-field scanning optical microscopy of QD-antibody conjugates to study the specific T-cell receptors (TCR) on the surface membrane of T-cells. The QD-labels were used to follow and observe the variations in location and clustering patterns of the receptors after introducing the cells to specific external stimuli. The study performed clearly demonstrates the ability to use specifically
labeled quantum dots to visualize molecular interactions in a live cell with very high molecular resolution. Guoning et al. used CdSe quantum dots modified with poly (D, L-lactide) on the surface to image and label spleen and liver cells (Figure 1-2).

Figure 1-2. Fluorescence microscope images of liver cells (left) and spleen cells (right) labeled and imaged by with CdSe quantum dots (QDs) modified with poly (D, L-lactide) (reproduced with permission).

Chen et al. utilized quantum dots to image and track the uptake of graphene into in vitro Hela cells. Graphene is a useful drug delivery complex due to the π-π interactions, but it is difficult to visually track it’s movement. CdTe quantum dots were coordinated onto the graphene oxide sheets using bovine serum albumin (BSA) as a “bridge” or coordinating ligand. The quantum dots could then be used in fluorescence microscopy to observe the potential drug delivery complex into the cells (Figure 1-3).
Studies have also been performed to show that the cytotoxicity of the CdSe quantum dots is directly related to the coating of the quantum dot and the uniform coverage of the shell and surface modifiers. This is because the CdSe nanocrystal is not exposed to biological matrix if it is completely covered by the ZnS shell, however if the shell does not completely coat the core the CdSe may possibly interact with the biological environment being studied. They have demonstrated that the quantum dots are safe in moderate to low concentrations (the concentrations necessary to perform fluorescent labeling studies) in biological systems as well as being useful for further various studies and investigation imaging.
1.2 Choline

1.2.1 Cholinergic Transport Cycle

Acetylcholine (ACh) was first discovered and identified in 1914 by Henry Hallett Dale and later confirmed to be a neurotransmitter by Otto Leowi in 1921, the first neurotransmitter ever identified. It has become a widely studied substance across many different species. The concentration regulation and intermediate steps in the cholinergic cycle are important to observe and measure as changes throughout the cycle coincide with malfunctions of biological processes and/or brain degenerative and chronic diseases such as Alzheimer’s Disease.\textsuperscript{22}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{choline_acetylcholine.png}
\caption{Choline and Acetylcholine}
\end{figure}

ACh is synthesized by choline acetyltransferase (AChT) from the two precursor molecules, choline and acetyl-coenzyme A (AcCoA). This synthesis occurs inside the neurons where ACh is then encapsulated inside synaptic vesicles by the vesicular acetylcholine transporter (VACHT). The vesicle is acidified by way of an ATP-dependent proton pump located in the synaptic vesicle membrane. The location of the pump and the nature of the environment creates a pH gradient which provides the driving
force for the acetylcholine transport as the VACHT exchanges the ACh for protons when it arrives in the appropriate pH atmosphere. The ACh release is $K^+$ dependent and following depolarization, the ACh is released into the synaptic cleft as the synaptic vesicles fuse with the plasma membrane of the axon terminal. The ACh then diffuses into the synaptic cleft where it activates acetylcholine receptors (AChR) and the action of the neurotransmitter is terminated by direct enzymatic hydrolysis of the neurotransmitter in the synaptic cleft by acetylcholinesterase (AChE). This is different than most other neurotransmitters (e.g., GABA, dopamine, serotonin), where the action of the transmitter is terminated by transporter-mediated removal of the transmitter from the synaptic cleft. The choline which is created in the hydrolysis of ACh is then transported out of the synaptic cleft and back into the presynaptic neuron by a high affinity choline transporter (CHT) where the cycle begins anew.\textsuperscript{23}

**Cholinergic Cycle**

![Cholinergic Cycle Diagram](image)

**Figure 1-5.** Schematic of cholinergic transport cycle.
1.2.2 Previous Research Measuring Choline and Acetylcholine

Measuring and detecting biomarkers and neurotransmitters is incredibly important for diagnosing and recognizing neurodegenerative diseases. It is also important for monitoring disease progression and observing the progressive health of patients suffering from such disorders. This is a challenging task however, because of the extremely low concentrations of these important chemicals in the sample volume available for analysis (µL to nL). While previous methods to detect and measure Ch and ACh often involved radiochemical analysis, newer methods have been developed by T.V. Barkhimer et. al. to readily provide accurate and sensitive detection of Ch and ACh using electrochemical methods such as capillary electrophoresis coupled with electrochemical detection.22

Because Ch and ACh are not electroactive, electrochemical detection can only be achieved through indirect means using modified electrodes. Electrodes were modified with choline oxidase (ChO) to detect Ch and AChE and ChO to detect ACh. The modified electrodes provided excellent chemical selectivity in detecting the specific analytes due to the enzyme reactions necessary to interact with the electrode.24-25 A multiple electrode setup was designed to obtain lower detection limits, self-referencing, rapid response and enhanced selectivity. However, this design cannot simultaneously detect both ACh and Ch due to their similar reaction pathways with ChO, so CE was utilized to separate the two analytes prior to their detection at the site of the modified electrode. The developed technique for analyzing ACh and Ch with this method using butyrylcholine (BuCh) as an internal standard proved to provide the lowest detection limits ever reported for both Ch and ACh when compared to all literature values reported
up to that time. The method was used to evaluate transport kinetics of the choline uptake process in vitro using brain synapses harvested from mice as well as to evaluate the effectiveness and mechanisms of several classes of inhibitors, including hemocholinium inhibitors.

1.2.3 Quantum Dots Labeled with Hemocholinium Inhibitor

Gegout et. al. have developed a procedure which attaches a cholinomimetic functional group, specifically an inhibitor hemicholinium-15 analogue, to the surface of water-soluble quantum dots which interacts with the binding sites of choline in a brain synapse. This procedure can potentially both quantify the binding sites and the number of transmembrane proteins in a sample but also provide micro-fluorescence imaging of the sample as well.

The cholinomimetic QDs are an important complement to the CEEC methods of detection of acetylcholine and choline because of their design to interact directly with the CHT. While the CEEC method can monitor the changes in concentration of the choline and acetylcholine, the cholinomimetic QDs will be able to provide qualitative images and insight into the nature of CHT. Utilizing both methods can provide simultaneous information on the concentrations and transport ability of CHT in healthy and diseased synapses.
Chapter 2

2. Project Description

2.1 Project Goals

The current goals of this project are to develop a method to quickly and economically synthesize high quality quantum dots of a variety of sizes and colors. These quantum dots will be modified with biologically active surface ligands to analytically monitor and measure different biological processes involving ACh and Ch, which are important markers in identifying and monitoring neurodegenerative diseases like Alzheimer’s disease.

The synthesis of biologically useful quantum dots includes four steps (1) the semiconductor nano-crystal core synthesis, (2) the coating of the core with a semiconductor with a wider bandgap to raise the fluorescent efficiency of the core and to cover the toxic core with a non-toxic protective shell, (3) the surface ligand exchange to give the quantum dot a water-soluble coating with mercaptosuccinic acid, and finally (4) the attachment of an active ligand which will interact with the biological species of interest to be monitored, measured and studied using the fluorescent properties of quantum dots.

Several methods for the quantum dot core synthesis have been developed. The most practical and efficient method for regular laboratory use is the colloidal
synthesis. This involves arrested nucleation and growth of the semiconductor nanocrystals in a reactive solution mixture. This method uses reverse micelle capping groups to coat and stabilize the nanoclusters at particular sizes by adjusting temperature and concentrations to vary the growth rates of the clusters in solution. The reverse micelle capping groups also serve to passivate the quantum dot by coordinating to the dangling bonds at the abrupt ends of the nanocrystal surface. A “dangling bond” refers to the end of the lattice structure, where there are uncoordinated empty orbitals belonging to an immobilized atom in the crystal structure with an unsatisfied valence. Because the valence is unsatisfied and there are empty orbitals, electrons from a neighboring atom with filled orbitals could potentially move into these positions and form a bond. Unfortunately, the unique properties of quantum dots can also be lost when promoted electrons fall into these dangling bonds on the surface instead of fluorescing as desired. Because of this, it is important to synthesize these nanoclusters in one or more coordinating ligands which will coordinate and in essence smooth the surface of the rough edges of the nanospheres. Trioctylphosphine, trioctylphosphine oxide, hexadecylamine, and lauric acid are four common coordinating ligands and solvents used in CdSe quantum dot synthesis for these purposes.
If these “dangling” bonds are not removed they can lead to defective electrical and optical properties in the nanocrystal. There are a multitude of variables that can be experimented with using the colloidal syntheses including types of reagents to passivate the surface, ratios of the reagents, temperature of nucleation, temperature of growth of the crystal and time allocated for growth of the crystal. Many variations and procedures have been found to produce quantum dots which have monodispersed sizes from one-pot syntheses. To further narrow their size dispersion, size-selective precipitation can follow nearly every colloidal synthesis procedure.

Size-selective precipitation involves dissolving the quantum dots in a non-polar solvent, such as toluene, then adding a small amount of a polar solvent, such as acetone, followed by centrifugation. The larger quantum dots will precipitate out of solution and can be collected. Another small aliquot of acetone is added to the solution and then the solution is centrifuged again. The precipitate is collected again with these quantum dots being the next largest size that was synthesized in the reaction. These steps can be

Figure 2-1. A reversed micelle surface passivated colloidal synthesis of CdSe QDs.
repeated until all of the synthesized crystals have been collected from the solution separated by their sizes.

A narrow bandwidth semiconductor core like CdSe can have its quantum efficiency for fluorescence increased by confining it within a shell of a semiconductor with a wider bandgap like ZnS. To effectively cover the quantum dot core with an even distribution of ZnS an intermediate ZnSe shell is placed over the core because the lattice mismatch between ZnSe and CdSe is much smaller than the lattice mismatch between ZnS and CdSe. This enables more uniform coverage and maintains the sphere structure more effectively. If the intermediate shell is not grown over the surface uneven distribution of the final ZnS is more likely to occur and the final product is more of a “bullet” shape than a sphere.\textsuperscript{12, 27}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2-2.png}
\caption{Coating the CdSe core with a shell which has a wider bandgap increases the quantum efficiency of the CdSe core.}
\end{figure}
It is also convenient if the quantum dots will be used in biological applications as ZnS is a safer and less toxic substance. The shell also protects the core from photochemical reactions at the core-solvent interface that can possibly inhibit or quench its luminescent ability. The procedure to cap the core quantum dot with the shell is also a colloidal synthetic procedure but concentration and temperature are used to control and prevent the nucleation of ZnS crystals to allow epitaxial surface growth over the nanoclusters already in solution. Surfactants are again used to control and passivate the surface of the nanocrystals.

Figure 2-3. Schematic diagram for the synthesis and method for the growth of the ZnS/ZnSe shell on the CdSe quantum dot core.

For the quantum dots to be useful in biological applications it is necessary for the hydrophobic groups covering the surface to be exchanged or modified with water-soluble groups. The exchange must be controlled and the proper ligands must be chosen so that
the electrical properties of the quantum dot are minimally affected by the process. The
surface ligand shown to have the least detrimental effect to the quantum efficiency of the
quantum dot after being transferred into the aqueous phase has been mercaptosuccinic
acid.\textsuperscript{28-29} This gives a water-soluble carboxylic acid functional group on the surface while
the quantum dot usually loses only 40\% of the quantum efficiency that it had in the non-
polar solvents. There have been many studies on different synthetic routes, showing that
“soft” exchanges, where the quantum dots are not precipitated between reactions, maintain higher quantum efficiencies in the final water-soluble product.\textsuperscript{30}

The variety of methods used to facilitate the exchange in the surfactant molecule
demanded time and attention as to how the surface passivation was initially synthesized
in the original growth of the quantum dot. Much experimentation with the ligand and the
original reagents was necessary to find the type of quantum dot that had the easiest and
most efficient transfer from the organic phase to the aqueous phase. The use of tri-
ocetylphosphine oxide (TOPO) in the original synthesis proved to make the “soft”
exchanges more difficult and they tend to quench the quantum dot of all fluorescence
while the use of tri-octylphosphine (TOP) only in the original synthesis allowed the use
of the “soft” transitions into the aqueous phase with lesser loss of fluorescence.

The final step in the synthesis involves an organic reaction in which the
biologically active ligand is reacted with the carboxylic acid functional group on the
surface of the quantum dot. In this procedure EDC was used to couple the amine group
on the chosen ligand to the carboxylic acid functional group available on the surface of
the quantum dot. Once the quantum dots with different colors and different biologically
active ligands are synthesized comparative analytical studies can be performed using the fluorescent properties of the quantum dots.

![Schematic for EDC reaction](image)

**Figure 2-4.** Schematic for EDC reaction \([R= -C_6H_{12}OH, -C_2H_4N(CH_3)_3]\)

The two functional groups on choline are an alcohol and a quaternary ammonium. These two functional groups are important in binding to and uptake by CHT. Several inhibitors of the choline cyclic process have been synthesized by mimicking the active structure of choline with the alcohol and quaternary ammonium functional groups and structurally placing them close together so as to competitively bind to CHT and prevent choline from interacting with CHT by blocking the active site.\(^{31-32}\) In order to create a QD with ability to selectively bind to CHT both of these functional groups would need to be attached to the outer surface of the QD. Two ligands with an amine and the chosen functional group are used to create individual quantum dots with these functional moieties: one quantum dot with just the alcohol (product 3.a), and one quantum dot with just the quaternary ammonium group (product 3.b), and the third and final product which will contain a 1:1 mixture of both ligands (product 3.c). Product 3.c will have the choline mimetic functional properties to interact with the CHT with both functional groups on the outer surface of the QD.
2.2 Experimental

2.2.1 Materials

The materials used were as follows: (2-aminoethyl)trimethylammonium chloride hydrochloride (99%), trioctylphosphate oxide (TOPO, technical grade 90%), hexadecylamine (HDA, 98%), selenium (-100 mesh, 99.99%), trioctylphosphate (TOP, technical grade 90%), diethyl zinc (Et$_2$Zn, 1.0 M in hexanes), hexamethyldisilathiane, mercaptosuccinic acid (thiomalic acid), and 6-amino-1-hexanol, were purchased from Aldrich. Chloroform, methanol, acetone and hydrochloric acid were purchased from
Fisher as certified ACS reagents. Argon was received as industrial grade from Air Gas and scrubbed of moisture via a drierite column. Ethanol was purchased from Pharmaco-AAPER (200 proof, ACS/USP). Cadmium oxide (Grade 1, for electronic use) was purchased from Johnson Matthey Chemicals limited. Lauric acid was purchased from Matheson Coleman and Bell Manufacturing Chemists. Deuterated solvents, chloroform-D (CDCl$_3$) and dimethyl sulfoxide-d$_6$ (DMSO-d$_6$) for NMR analysis were purchased from Cambridge Isotope Laboratories, Inc. High purity chloroform (≥ 99.8%) was purchased from Fluka. TOP, Et$_2$Zn and hexamethyldisilathiane were stored under argon. All other reagents were used as purchased.

2.2.2 Instrumentation

Ultraviolet visible absorption measurements were performed on a Thermo Electron Corporation Nicolet Evolution 300 spectrophotometer. Quartz cuvettes with either two or four polished faces (pathlength 1 cm) were used to hold the samples. Room temperature measurements of the original QD were recorded in CHCl$_3$ while that of the water soluble quantum dots were recorded in PBS buffer (pH = 6.7).

Fluorescence measurements and luminescence spectroscopy were obtained and performed with an Aminco-Bowman Series 2 (SLM Instruments, Inc.) spectrophotometer. Quartz cuvettes with four polished faces (pathlength 1 cm) were used to hold the samples. All solutions were absorbance matched (0.03 au) at 450 nm (equivalent solutions were obtained from UV studies). NMR spectra were obtained with a Varian 400 MHz instrument. The solvent used for nonaqueous soluble NMR samples was DMSO and all the aqueous spectra were obtained in D$_2$O.
A PerkinElmer Spectrum GX FT-IR spectrometer was used to collect the IR spectra. Samples were prepared using a traditional cast method on a KBr salt plate. The precipitate was dispersed in acetone, shaken or sonicated to disperse throughout the solution. The solution was then dropped onto a salt plate and the solvent evaporated.

Thermogravimetric and differential thermal analysis experiments were carried out on a SDT 2960 DTA-TGA from TA Instruments, in nitrogen, at a constant gas flow of approximately 100 mL/min. The program used a ramp of 10 °C/min until the temperature reached 600 °C. The sample (between 10 and 20 mg) was placed in platinum pans.

Dynamic light scattering (DLS) studies were carried out on a DynaPro Titan instrument with temperature control. A 1 cm pathlength quartz cuvette was used for the DLS experiments. The study was conducted at room temperature, with an acquisition time of 10 s and a laser wavelength of 829.3 nm.

Transmission electron microscopy (TEM) images were obtained on a Hitachi HD-2300 transmission electron microscope with an accelerating voltage of 200 kV. Copper grids with a Formvar carbon film (200 square mesh) were purchased from Electron Microscopy Sciences. The grids were prepared by dispersing ~1 mg of functionalized QDs in 5 mL of 2-propanol by sonication for 5 minutes. One drop of the solution was placed in the center of the TEM grid and a flow of argon gas was used to evenly disperse the drop across the grid and evaporate the solvent. Remaining solvent evaporation was performed by placing the grid on a warm hot plate until the solvent evaporated.
2.2.3 Methods

2.2.3.1 Synthesis of CdSe/ZnSe/ZnS Quantum Dot

The synthetic procedure was modified and adapted from Liu et. al.\textsuperscript{27} A solution of 48 mg of CdO and 800 mg lauric acid was dissolved in a hot coordinating solvent mixture of 6 g of TOPO and 6 g of HDA to form a CdO-HDA complex. The solution was heated to 320 °C with stirring until the color changes from orangish-red to clear. Then, 320 mg Selenium powder was dissolved in 8 mL TOP. The SeTOP solution was injected rapidly (within 2 s) into the CdO-HDA solution with vigorous stirring. CdSe nanocrystals were grown for desired length of time at 280 °C after mixing. The solution was then cooled to ~200 °C to prevent further growth/Ostwald ripening.

A ZnSe shell solution (0.05 mL diethyl zinc, 15.8 mg Se, 1.0 mL TOP) was injected slowly over a 10 min period. The temperature was reduced to 160 °C. A ZnS shell solution (0.1 mL diethyl zinc, 0.1 mL hexamethyldisilathiane and 1 mL TOP) was injected slowly over 10 min. The CdSe/ZnSe/ZnS nanocrystal mixture was then annealed for 1-2 h at 110 °C. The solution was cooled to 70 °C and anhydrous methanol was added to precipitate the nanocrystals. Nanocrystals were then collected by centrifugation, rinsed several times with methanol and dispersed in anhydrous toluene or chloroform.

2.2.3.2 Mercaptosuccinic Acid Ligand Exchange

Method adapted from Chen et. al.\textsuperscript{33} Quantum dots (150 mg) was dissolved in 20 mL methanol with 150 mg mercaptosuccinic acid. Tetramethylammonium hydroxide
was added dropwise to bring the pH above 10. The solution was refluxed overnight. The MSA capped quantum dots were precipitated with ether. They were then centrifuged and rinsed multiple times with methanol and ether. They can be stored either dry in vacuum or in PBS to retain fluorescence. Quantum dots stored in solution retain their fluorescence indefinitely long term, whereas quantum dots stored dry as a precipitate tend to lose their fluorescence over time despite storage in a vacuum. How quickly the fluorescence is lost over time may be related to how efficiently the capping steps cover all of the crystal imperfections in the core allowing or disallowing the surface of the core to oxidize whenever exposed to air.

2.2.3.3 EDC Organic Coupling Reaction

Method adapted from Pandey et. al. A 25 mg sample of QDs were dispersed in 5 mL of phosphate buffer (pH = 6.7) solution containing 0.02 M EDC and 0.01 M NHS for about 2 h with continuous stirring (for activation of the COOH group). The selected ligand containing the -NH₂ group (5 mg/mL) was added to the solution. The CO-NH amide bond was formed with the selected ligand group on the external surface of the QDs. The two ligands selected for study were (a) 6-amino-1-hexanol to provide the alcohol moiety for the surface functional group, and (b) (2-aminoethyl)trimethylammonium chloride hydrochloride to provide the quaternary ammonium moiety for the surface functional group. The third experiment (c) would include using both ligands (a) and (b) in a 1:1 ratio to put both functional groups onto the quantum dot in an even surface coverage to produce a quantum dot with both choline functional groups.
2.3 Results and Discussion

2.3.1 Organic Coated Quantum Dots

The CdSe core was synthesized with relative ease following established procedures from literature for colloidal synthesis of CdSe quantum dots. The cores were highly fluorescent with narrow emissions band (FWHM <50 nm) (Figure 2-6). The UV-visible spectrum demonstrates narrow size distribution with the sharp and distinct peak at 528 nm and broad absorbance from 350-550 nm (Figure 2-7). The cores could be synthesized in a variety of colors depending on the length of time the clusters were allowed to grow in solution. Different colored cores were synthesized and observed (Figure 2-8).

![Figure 2-6. Fluorescence spectrum of CdSe QD core.](EmLRefm1217a3.dat)
Figure 2-7. UV spectrum of CdSe QD.

Figure 2-8. CdSe core quantum dots of different sizes in TOPO/TOP matrix.
2.3.2 CdSe/ZnSe/ZnS Quantum Dots and Fluorescence Changes

The ZnSe shell was grown over the surface of the quantum dots to encourage uniform growth and coverage of the core. This enhances the quality of coverage of the final ZnS shell to enable complete encapsulation of the core. The protected and isolated core has a higher fluorescence efficiency and is not as easily influenced by reactions from the surrounding matrices or solution changes. The fluorescence changes with the addition of each shell was measured and compared to show the change in fluorescence efficiencies at each step throughout the synthesis (Figure 2-9). As the quantum dots are grown their subsequent fluorescence efficiencies change at each step. The initial CdSe core is protected by two shell layers, first the ZnSe and then the ZnS. After the first capping a decrease in fluorescence efficiency is observed as well as a minor redshift. Then after the final coat is applied a dramatically large increase in the fluorescence is observed as well as a more noticeable redshift. After one hour of annealing a slight increase in fluorescence intensity is observed with a slightly more narrow peak.
Figure 2-9. Comparison of intensity and red shifts that occur throughout the initial core synthesis and the following shell capping steps followed by the one hour annealing of the final product.

Although the final products were cleaned in several washing and centrifugation steps, the products could never be isolated from the majority of the synthetic solution matrix. A thermal gravimetric analysis (TGA) was performed to see how much non-quantum dot material was in the solid material obtained even post washings. The TGA obtained from the organic coated QD products always showed an extremely large amount of organic residue in the products, often close to 90% of the product was organic residue, presumably mostly HDA and TOPO (Figure 2-10). The TGA shows extreme mass loss and the boiling of the solvents between ~250 °C and ~365 °C, which correlates to the boiling points of the solvents used in the synthesis (the boiling points are HDA: 330 °C, TOPO : 201-202 °C, TOP: 284-291 °C). Subsequent washings with different solvents did
not prove to be much more efficient at isolating the quantum dot product as the coordinated organic ligands seemed to cling to the organic residue from the reaction mixture. The most effective way of removing the excess organic matrix from the quantum dots was to do a ligand exchange and move the quantum dots into an aqueous solution.

![Figure 2-10. TGA-DTA of TOPO/TOP covered quantum dots.](image)

### 2.3.3 Size Growth with Each Shell Step

The original CdSe core was spherical with an average diameter of about 4 nm in (Figure 2-11). Each subsequent shell added 1 nm in diameter indicating an average shell thickness of 0.5 nm across the surface of the sphere. The CdSe/ZnSe QD were ~5 nm in diameter (Figure 2-12) and the final CdSe/ZnSe/ZnS quantum dot usually being ~6 nm in diameter (Figure 2-13). TEM imaging was difficult in the organic matrix as the organic ligand and any organic residue would quickly decompose and melt the surface of the
TEM grids as the images were being taken. Due to the excess organic matrix blurry images were obtained, however the average size and diameter at each shell step could be approximated.

Figure 2-11. CdSe quantum dots in organic matrix. Diameter ~4 nm.
Figure 2-12. CdSe/ZnSe QD in organic matrix with diameter ~5 nm.

Figure 2-13. CdSe/ZnSe/ZnS in organic matrix. Diameter ~6 nm. (Z-contrast mode)
2.3.4 UV-visible Spectra Changes With Each Shell Step

UV-visible spectra were obtained for each step of the core/shell procedure. The initial spectrum of the CdSe core gave the characteristic peak at ~550 nm which indicates a narrow size distribution and relative size of the QD core can be extrapolated by peak position (Figure 2-14). After the first shell was grown on the surface the peak position shifted red slightly as the quantum dots grew ~1 nm in diameter with the addition of the 0.5 nm thick shell layer was deposited (Figure 2-15). The peak was still observable and distinct despite the slight shift. With the second shell layer however, the size-characteristic peak was no longer distinct and easily observable although the broad absorption capabilities of the QD was still demonstrated by the UV-vis spectrum (Figure 2-16). After one hour of annealing there were no noticeable differences between the UV-vis spectra of the CdSe/ZnSe/ZnS QD before and after annealing process were complete (Figure 2-17).
Figure 2-14. UV-visible spectrum of CdSe core dispersed in CHCl$_3$.

Figure 2-15. UV-visible spectrum of CdSe/ZnSe QD core/shell dispersed in CHCl$_3$. 
Figure 2-16. UV-visible spectrum of CdSe/ZnSe/ZnS quantum dots dispersed in CHCl₃.

Figure 2-17. UV-visible spectrum of CdSe/ZnSe/ZnS QD in CHCl₃ after 1 h of annealing.
2.3.5 Isolation of CdSe Core Confirmed with Cyclic Voltammetry

Cyclic voltammograms were obtained to observe possible interactions of the quantum dots with electrodes. When the CdSe/ZnSe/ZnS QDs were adsorbed onto the surface of a glassy carbon electrode, there was no significant change in the voltammogram from the background (Figure 2-18 and Figure 2-19).

Figure 2-18. Cyclic voltammogram of glassy carbon electrode in 0.2 M (pH = 7) phosphate buffer.
CdSe QD cores were then transferred into an aqueous media by coating with MSA and then cast onto the surface of a glassy carbon electrode (Figure 2-20). A small peak was observed that was not previously observed in the voltammogram of CdSe/ZnSe/ZnS MSA-QD. A square-wave voltammogram was then obtained to further isolate, amplify and examine the peak (Figure 2-21). The peak was identified as the reduction of Cd\(^{2+}\) on surface of the electrode occurring around -0.85 V vs Ag/AgCl. The fact that this peak does not show up for the CdSe/ZnSe/ZnS QDs when they are deposited on the surface of the glassy carbon electrode demonstrates that the two shell covering completely and fully isolated the Cd inside the CdSe core and it does not interact with the solution matrices. This is an important fact to note when continuing with the QD studies because it ensures that any future biological matrix will be prevented from dissolving or absorbing the toxic Cd\(^{2+}\) into solution during biological fluorescent tagging studies.
Figure 2-20. Cyclic voltammogram of glassy carbon electrode modified with MSA capped CdSe MSA-QDs absorbed onto the the surface of the electrode in 0.2 M (pH = 7) phosphate buffer.

Figure 2-21. Square-wave voltammogram of glassy carbon electrode modified with MSA capped CdSe MSA-QDs absorbed onto the the surface of the electrode in 0.2 M (pH = 7) phosphate buffer.
2.4 Results and Discussion of Aqueous Quantum Dots

2.4.1 MSA capping

The overnight reflux in methanol was the most effective method of exchanging the organic coordinating ligands with an aqueous coordinating ligand. Although much fluorescence efficiency was lost, the MSA ligand has been proven to be one of best choices for an aqueous ligand due to the double carboxylic acid per coordination point which provides a more thorough coverage of the surface of the quantum dots eliminating more possibilities of the quantum dot surface interacting with the solutions. IR spectra were obtained to observe the carboxylic acid as a functional group on the surface of the isolated quantum dots (O–H stretch from 3300 cm\(^{-1}\); C=O stretch from 1700 cm\(^{-1}\)) (Figure 2-22).

![Figure 2-22. IR spectrum of MSA-capped QD.](image-url)
2.4.2 NMR Challenges with Quantum Dots

An NMR spectrum was attempted to be collected to clearly see the MSA as a molecule on the surface of the quantum dot. The sample was cleaned using repeated methanol washings and centrifuging followed by drying in a vacuum. The NMR solution was run in D$_2$O. The sample was extremely soluble in small amounts, but in higher concentrations would slowly fall out of solution if allowed to sit over a period of time. The sample was highly fluorescent in aqueous solution. However, it was initially extremely difficult to see the MSA on surface of the water-soluble quantum dots. NMR was run on pure MSA (Figure 2-23) and then on the obviously water soluble quantum dots (Figure 2-24) and the distinct peaks and splitting patterns observed for MSA were not easily observable on the water soluble quantum dots (acetone impurity is visible at 2.1 ppm, and ether impurity is visible at 3.4 ppm). The quantum dots were further washed with methanol and centrifuged and dried but the only observable peaks were for the solvents, not the quantum dot products. It was then observed that the peaks were in fact in the spectrum, but down near the baseline and hidden among the impurities. It was then determined that the amount of small molecules that were in the ligand were relatively unnoticeable compared to the extremely large amounts of inorganic material they were coordinated to. To obtain the NMR spectra for the small surface ligand, the amount of quantum dots in the NMR tube had to be increased more than tenfold. Once the amount of product in the sample size was increased, the NMR spectrum of MSA could be more easily observed (Figure 2-25). However, increasing the sample size also
increased the noticeability of the impurities still in the compound (it is believed to be ether showing up at ~3.4 ppm in the spectra, as it was used to precipitate the QDs after the ligand exchange). It must be taken into account, that these impurities are still in very small and trace amounts; however, the sample size had to be increased so that MSA on the surface of the quantum dot could be observed with NMR. NMR spectrum have been used in specific ligand studies \textsuperscript{36-37} but are not routinely collected as a characterization for functionalized quantum dots in the literature, probably due to this very reason. It is difficult to get a clear and interpretable NMR spectrum when the sample size has to be increased and solubility may be low in the solvents used. Also, the trace impurities make the spectra more difficult to interpret when the sample size is so large.

Once an NMR was obtained in which the MSA surface ligand was observable, it showed an unpredicted splitting pattern and twice as many peaks as predicted between 2.6-2.5 ppm. This is attributed to possible different 3-dimensional orientations of MSA on the surface of the quantum dot. Or it could possibly be impurity interference with the spectra due to the fact that impurities become a more significant problem when the sample sized is increased to such levels.
Figure 2-23. NMR spectrum of mercaptosuccinic acid.

Figure 2-24. Mercaptosuccinic acid functional group as the coordinating ligand on the surface of the quantum dot, spectrum taken in D$_2$O. Low QD concentration.
Figure 2-25. NMR spectra after increased concentration (approximately ten times more than that of an average NMR sample) of mercaptosuccinic acid covered quantum dots in D$_2$O.

2.4.3 UV-visible Spectra from ZnS capping and MSA Exchange Procedure

UV-visible spectrum obtained after ZnS capping procedure and mercaptosuccinic acid exchange lost characteristic size distinguishing peaks but retained broad absorption (Figure 2-26).
2.4.4 TGA of MSA Capped Quantum Dots

Due to the difficulties in observing the aqueous ligand with NMR spectroscopy, a TGA was performed to try to obtain an accurate estimate of the mass of the ligand to the mass of the inorganic quantum dot. A TGA of pure MSA was performed. The melting point is observed in the DTA between 150-154 °C and the boiling point of mercaptosuccinic acid can be observed at 256 °C (at 760 mm Hg) and the decomposition of the small organic molecule shortly thereafter (Figure 2-27).
The TGA of the MSA-QD was then performed and showed a much cleaner picture of ligand:quantum dot (Figure 2-28) than was observed with the organic TOPO/TOP – QD which was seen in the previous step (compare to Figure 2-10). The TGA demonstrated that the percentage of non-bound organic material contained in the precipitate is much lower than before the ligand exchange. The initial mass decrease is suspected to be non-coordinated ligand that was still in the precipitate as it shows a similar boiling point although a much less drastic loss of mass in the amount of time as the pure sample due to
the steric hindrance of the surrounding quantum dot material. The mass decrease that begins after 400 °C is suspected to be the ligand that is actually directly coordinated to the inorganic quantum dot, and the exothermic heat reaction is attributed to the fast decomposition of the ligand. This would indicate that the mass ratio of the ligand:inorganic quantum dot to be approximately 5:75 or the mass percentage of the MSA to the MSA covered quantum dot would be ~6.25%.

**Figure 2-28.** The TGA of quantum dots with mercaptosuccinic acid as the coordinating ligand.
2.4.5 The Effects of pH on Water Soluble Quantum Dots

The water soluble quantum dots were fluorescent in water, and were stored in a PBS buffer (pH = 7.3). The fluorescence was not lost over time when stored in solution, however, dry quantum dots lost fluorescence efficiency and water-soluble properties over time. The fluorescence of the QDs in aqueous solution were pH dependent (Figure 2-29). While higher pH showed stronger photoluminescence than lower pH, the fluorescence efficiency has a maximum quantum yield around a pH of 7.

Table 2-1. Relative PL Intensity as a Function of Variable pH

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Figure 2-29. Fluorescence efficiency of water soluble quantum dots varies with pH of the solution.
2.4.6 Size Analysis of MSA Quantum Dot

2.4.6.1 TEM Image

TEM analysis of the MSA-coated QDs demonstrated high size homogeneity with an average diameter of the water soluble quantum dots of 6.2 +/- 0.5 nm and 8.2% polydispersity (Figure 2-30).

![TEM Image of MSA QD and particle size histogram.](image)

**Figure 2-30.** TEM image of MSA QD and particle size histogram.

2.4.6.2 Dynamic Light Scattering

The DLS analysis indicated the radius to be ~3.0 nm with a percent polydispersity of ~13.9%. Although the relative intensity for the signal was low due to the small particle size, the mass percent indicates 94.8% of the sample analyzed to be quantum dots (Figure 2-31). Further samples were run to test variance in sample preparation and analysis, the second sample results indicated the radius to be ~3.4 nm with a percent polydispersity to be ~0%. Although the relative intensity for the signal is low due to the small particle size, the mass percent indicates 93% of the sample analyzed to be quantum dots (Figure...
DLS was determined to be useful in size approximation and in confirming radius size determined through the TEM images, but the result variances between samples did not provide confidence to base exact size determination solely on this method.

![Graph 1](image1.png)

**Table 2.31.** DLS of MSA-QD.

<table>
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<th>Item</th>
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**Figure 2.31.** DLS of MSA-QD.
Figure 2-32. Second DLS analysis of MSA QD.

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2.5 Cholinomimetic Quantum Dots

2.5.1 EDC Reaction

Once the carboxylic acid functional group was incorporated on the surface of the quantum dots, a multitude of possibilities are available for further derivatives to be synthesized. An aqueous coupling reaction with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was utilized to create three distinct functionalized quantum dots; one quantum dot with the –OH moiety on the surface, the second with the quaternary ammonium group on the surface and the third with both functional groups on the surface to work together as a cholinomimetic probe (Figure 2-33), as the quaternary ammonium and the alcohol groups are the two important groups on choline and the mode of interaction with CHT (see Figure 1-4).

![Figure 2-33. The three water soluble quantum dots synthesized by use of the aqueous EDC coupling reaction.](image-url)
2.5.2 Fluorescence Studies on the Functionalized QDs

Each quantum dot was measured for fluorescence efficiency to compare how the ligand on the surface affected the core’s ability to fluoresce. While the hydroxyl moiety decreased the fluorescence even more than the carboxylic acid group did, the quaternary ammonium functional group quenched almost all visible fluorescence. However, the hybrid quantum dot proved to have more fluorescence stability and had a higher intensity than either of the two individual ones and was even of a comparable fluorescence to that of a single step ligand exchange performed by another member of the lab. (Figure 2-34)

![Overlaid fluorescence spectra of the water soluble QD products synthesized, with the addition of a thiol-choline product synthesized by a labmate (a direct ligand exchange which skips the MSA capping step).](image)

**Figure 2-34.** Overlaid fluorescence spectra of the water soluble QD products synthesized, with the addition of a thiol-choline product synthesized by a labmate (a direct ligand exchange which skips the MSA capping step).

The fluorescence decreased significantly among all of the functionalized water-soluble quantum dots when compared to the original synthesized products in the organic matrix.
of TOPO/TOP/HDA as can be seen in Figure 2-35. However, it is shown that the final hybrid quantum dot lost less fluorescence intensity than the other water-soluble products in the successive steps after the MSA-capping. This is suspected to be caused by more uniform coverage and possible charge balance, to keep the core more isolated from reacting with either the surface ligands or the aqueous environment from charge withdrawing effects or interactions with crystal imperfections. The comparison of the fluorescence intensity peak integrations for all water-soluble products can be seen clearly in Figure 2-36.

![Fluorescence spectra of every step of the synthesis from the original core/shell products and every water-soluble functionalized QD.](image)

**Figure 2-35.** Fluorescence spectra of every step of the synthesis from the original core/shell products and every water-soluble functionalized QD.
Figure 2.36. Chart comparing the integrated peak value of the intensity of fluorescent continues to decrease in each successive step.

2.5.3 UV-Visible Comparisons of the Functionalized QDs

The UV-visible spectra for the three final functionalized QDs did not vary too much from the UV-vis spectrum of the MSA-capped quantum dot. Although it is interesting to note that the size-characteristic peak which mostly disappears after watersolubilization of the product becomes slightly more prominent in the final cholinomimetic quantum dot (Figure 2-39) than it is in either of the other two functionalized quantum dots (Figures 2-37 and 2-38).
Figure 2-37. UV-visible spectrum of OH-QD in 0.2 M PBS buffer (pH = 6.7)

Figure 2-38. UV-visible spectrum of QA-QD in 0.2 M PBS buffer (pH = 6.7)
Figure 2-39. UV-visible spectrum of Hy-QD in 0.2 M PBS buffer (pH = 6.7)

2.5.4 Size Studies of the Cholinomimetic Quantum Dots

2.5.4.1. TEM analysis

TEM analysis showed spherical nanoparticles with a radius of ~6 nm which confirms the supposition that no further growth or degradation occurs during the EDC coupling procedure which is performed at room temperature. No apparent change in size from the MSA-capping step can be seen with TEM analysis (Figure 2-40).
Dynamic light scattering (DLS) on cholinomimetic QD demonstrates the radius to be \( \sim 6.9 \) nm with a percent polydispersity of \( \sim 13.9 \% \). Although the relative intensity for the signal is low due to the small particle size, the mass percent indicates 94\% of the sample analyzed to be quantum dots. It is suspected that these are the quantum dots aggregating in solution, as the radius is approximately twice that which was observed in the other two samples, and also observed by the TEM images. It is suspected that the change in surface ligands encourages more aggregation of the quantum dots in solution that was not observed in the MSA-capped quantum dots. The polydispersity matches that of the first MSA sample obtained which is confirmation that the size of the quantum dot was not affected by the EDC coupling procedure (Figure 2-41).
2.6 Conclusion

Cholinomimetic QDs were synthesized and characterized through UV-vis and fluorescence spectroscopy, DLS and TEM. The hybrid cholinomimetic quantum dots proved to have higher fluorescence intensity than either of the individual precursors with only the –OH and quaternary ammonium functional group on the surface, although the fluorescence did decrease from the MSA water-soluble precursor. The TEM and the DLS showed spherical nanocrystals with high size homogeneity and no core structural change from the MSA precursor step.

**Figure 2-41.** DLS of Cholinomimetic QD.

<table>
<thead>
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<th>Item</th>
<th>R (nm)</th>
<th>%Pd</th>
<th>MW-R (kDa)</th>
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<td>13.9</td>
<td>306</td>
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<td>Peak 2</td>
<td>33.4</td>
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<td>12406</td>
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<td>Peak 3</td>
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<td>232117</td>
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<td>748201000</td>
<td>17.2</td>
<td>2.0</td>
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