Determining a Method for Rendering Low Cost CdSe(ZnS) Core(Shell) Quantum Dots Aqueous Soluble via Amphiphilic Polymer Wrapping

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Abstract

DETERMINING A METHOD FOR RENDERING LOW COST CdSe(ZnS) CORE(SHELL) QUANTUM DOTS AQUEOUS SOLUBLE VIA AMPHIPHILIC POLYMER WRAPPING

by Patrick McBride

Herein is described the procedure of two amphiphilic polymer wrapping techniques that may be employed for obtaining aqueous soluble quantum dots (QDs) for use in biological fluorescent imaging applications. The advent of QDs has led to new nanoscale fluorescent materials that exhibit unparalleled quantum yields (QYs), high resistance to photobleaching, tunable emissions, and absorption over a large optical range. However, the QD synthesis employed here at Cal Poly to obtain bright, photostable CdSe(ZnS) core(shell) QDs involves the use of organic solvents and surfactants, leading to hydrophobic QDs. Since all of biology relies on aqueous solubility, this hydrophobicity creates a major problem when trying to use QDs for biological imaging applications. One way to overcome this problem is to employ the technique of amphiphilic polymer wrapping to coat the hydrophobic QDs in an amphiphilic polymer that allows them to disperse in aqueous solutions. This paper describes two procedures for obtaining aqueous soluble QDs here at Cal Poly that fluoresce in the optical range and that can be used for biological imaging at Cal Poly in the future. Both procedures were a success in transferring QDs to an aqueous solvent, but resulted in a 65% decrease in peak emission intensity. Methods of photo-annealing were then used to permanently enhance the QD fluorescence and maintain QD brightness in aqueous solution.

Keywords: Quantum Dot, Biological Imaging, Fluorescence, Quantum Confinement, Semiconducting Nanocrystal, Amphiphilic Polymer Wrapping, Aqueous Soluble Quantum Dot
1 Introduction

1.1 Fluorescence Imaging in Biology

One of the greatest challenges in molecular and cellular biology is the identification and quantification of subtle differences between individual cells as a function of their unique microenvironments. Fluorescence imaging has proven revolutionary for helping to discern some of these differences as evidenced by its tremendous impact on cell biology, cancer research, materials development, and drug discovery [1], [2]. However, the bulk of these research efforts have depended upon the use of small molecule fluorophores, which typically exhibit low quantum yields (QYs), inadequate extinction coefficients, and broad emission spectra [2]–[5]. Small changes in photoluminescence resulting from subtle environmental interactions can therefore be difficult to detect when using these organic fluorescent dyes.

1.1.1 Quantum Dot Fluorophores

Within the past two decades a new type of semiconducting nanocrystal has been developed which is stable for long periods of time and has emission spectra that can be controlled through simple nucleation and growth kinetics [1], [6]. One problem with these so called quantum dots (QDs), however, is that the organic synthesis techniques typically employed leave them with a tendency to agglomerate within aqueous solvents and consequently all applications involving water-based solvents (e.g., all of biology) cannot use these wonderful new fluorescent probes.

1.1.2 Achieving Aqueous Soluble Quantum Dots

One method to combat this problem is to attach certain surface groups to QDs that would allow them to be dispersed in aqueous solvents. While there have been numerous methods for acquiring aqueous soluble QDs, the method of amphiphilic polymer wrapping has been shown to be more employable to a wider range of applications [7]. Rendering QDs aqueous soluble via amphiphilic polymer wrapping
was first performed by Teresa Pellegrino et al. in her 2004 paper using poly(maleic anhydride-alt-1-octadecene) to coat the QDs [8].

While there is already vast amounts of research on the topic of QD fluorescence imaging [1], the area is still quite new, and novel developments in the field are made often. One particular aspect of amphiphilic polymer wrapping that has not been fully expanded upon, however, is a well designed process for producing aqueous soluble polymer wrapped QDs (pQDs) that have the ability to be scaled up into an industrial setting such that pQDs can become more available to both the academic and industrial workforce.

1.2 Problem Statement

Recent advances in the colloidal synthesis of QDs have led to the emergence of entirely new imaging techniques within the field of fluorescence spectroscopy. However, the use of QDs in biological fluorescence imaging at Cal Poly has been hindered due to the hydrophobic nature of the QDs we can cost effectively synthesize. Therefore, the goal of this project is to develop a technique that renders Cal Poly QDs aqueous soluble in a reproducible and cost effective manner. I will do this by coating our QDs with two different biologically compatible amphiphilic polymer coatings to determine a technique that most effectively disperses these QDs into an aqueous solution.

1.3 Quantum Dot Theory

1.3.1 Simple Theory

A result of quantum mechanics is that a single atom will have discrete energies at which an electron can reside. These energies are always degenerate meaning that multiple electrons can reside at the same energy. However, as two atoms begin to interact, these degenerate states begin to split into different energies, which allows for atomic bonding. As more and more atoms interact, all of these electronic degenerate states begin to split and once the bulk is reached, the energy splitting is so fine that it
is essentially a continuum (Figure 1).

Figure 1: Depiction of the effect of molecular orbital splitting. A single atom has a discrete energy spectrum but as other interacting atoms come closer, the degenerate energy levels split into closely spaced atomic orbitals. In the bulk state, these degenerate states are so close that they are essentially a continuum. However as one moves from bulk solids to atomic particles, the spacing between energies widens and this is what gives QDs their controllable properties.

Because the spacing between energies can be controlled as a function of size, it is possible to alter the electronic states of a material simply by changing its dimensions. This is of particular interest for solids with a band gap that lies at the Fermi level (i.e. semiconductors). This will then allow one to alter the band gap size by changing the spatial dimensions of the semiconducting material. This is the technique that is employed in QDs to alter their fluorescence profile (Figure 2). Since the energy of light emitted is determined by the size of the band gap, controlling QD size distributions will help to control their fluorescence profile. With a smaller (larger) size there is a larger (smaller) energy band gap, which leads to higher (lower) energy photon emitted during absorption and re-emission of incoming light.
1.3.2 Complex Theory–Particle in a Box

In order to provide a more technical model of quantum confinement, it is easiest to begin with the example of non-interacting free particles in a 3D box. Employing the time independent Schrödinger equation and using separation of variables, it is possible to obtain the eigenstates of $|\psi_{xyz}\rangle$:

$$|\psi_{xyz}\rangle = A \sin\left(\frac{n_x \pi}{a} x\right) \sin\left(\frac{n_y \pi}{b} y\right) \sin\left(\frac{n_z \pi}{c} z\right); \quad (n_{xyz} = 1, 2, 3, \ldots)$$  \hspace{1cm} (1)

with the energies

$$E_{xyz} = \frac{\pi^2 \hbar^2}{2m} \left(\frac{n_x^2}{a^2} + \frac{n_y^2}{b^2} + \frac{n_z^2}{c^2}\right)$$  \hspace{1cm} (2)

where $A$ is an arbitrary normalization constant and $a$, $b$, and $c$ are the lengths of the box in the $x$, $y$, and $z$ directions, respectively. Now if $a = b = c$, then Equation 2 reduces to

$$E_{xyz} = \frac{\pi^2 \hbar^2}{2ma^2} \left(\frac{n_x^2 + n_y^2 + n_z^2}{a^2}\right)$$  \hspace{1cm} (3)

$$E_{xyz} = \frac{\pi^2 \hbar^2 n^2}{2ma^2}$$  \hspace{1cm} (4)
where if we define \( \mathbf{n} \equiv (n_x, n_y, n_z) \), we obtain

\[
\| \mathbf{n} \| = \sqrt{\frac{2ma^2 E_{xyz}}{\pi^2 \hbar^2}}
\]  

which describes the length of the vector \( \mathbf{n} \) in "\( n \)-space," where each point is actually an energy state with a defined \( n_x, n_y, \) and \( n_z \) (Figure 3).

---

**Figure 3:** Depiction of the surface of a sphere in \( n \)-space with radius \( n \) and thickness \( dn \). This representation can then be used to calculate the density of states for the 3D particle in a box.

Since \( n_x, n_y, \) and \( n_z \) are only positive integers, this space only fills one octant of a 3D cartesian \( n \)-space. Now if \( a >> n \), then the points in \( n \)-space can be treated as a continuum and the number of states, \( dQ \), available at a certain \( n \) can then be described as 1/8 the volume of a small spherical shell of radius \( n \) and thickness \( dn \), or \( dQ(n) = (1/8)4\pi n^2 dn \). By integrating this expression from 0 to \( n \), multiplying by 2 for the spin 1/2 degeneracy in electrons, and substituting Equation 5 in for \( n \), the total number of available states as a function of \( E \) is given by

\[
Q(E) = \frac{\pi}{3} \left( \frac{2mE_{xyz}}{\pi^2 \hbar^2} \right)^{3/2} a^3
\]  

and by dividing out the volume of the solid, \( V = a^3 \), we obtain the electron concentration:

\[
q(E) = \frac{\pi}{3} \left( \frac{2mE_{xyz}}{\pi^2 \hbar^2} \right)^{3/2}
\]  

---
In order to determine the number of available states per volume in the energy range $E + dE$, we can differentiate $q(E)$ with respect to energy to obtain the density of states:

$$g_{3D}(E) = \frac{dq}{dE} = \frac{\pi}{2} \left( \frac{2m}{\pi^2\hbar^2} \right)^{3/2} E_{xyz}^{1/2}$$

(8)

It is important to note that in the 3D particle in a box model, the density of states, or states per unit volume per unit energy, increases with $E^{1/2}$.

In quantum well structures, one of the dimensions in Equation 2, say $c$, is no longer much greater than $n_z$, and therefore states along the $\hat{n}_z$ direction can no longer be described as a continuum. This creates quantized available energy levels in the $\hat{n}_z$ direction, while a continuum of states is still available in the $\hat{n}_x$ and $\hat{n}_y$ directions. Therefore by treating quantum wells as 2D non-interacting particles in a box and employing the method used to obtain Equation 9, the density of states in 2D can be shown to be constant with respect to energy:

$$g_{2D}(E) = \frac{m}{\pi\hbar}$$

(9)

and by similar reasoning for quantum wires, the 1D density of states can be shown to be proportional to $E^{-1/2}$:

$$g_{1D}(E) = \left( \frac{2m}{\pi^2\hbar^2} \right)^{1/2} E^{-1/2}$$

(10)

However, Equations 8 and 9 must be modified to account for the extra degeneracies brought about when $E > E_i$, where $E_i$ is the $i$th energy at which a quantized direction of $n$ is allowed to jump up to the $i$th integer value. To account for this, Equations 8 and 9 can be re-written:

$$g_{2D}(E) = \frac{m}{\pi\hbar} \sum_i H(E - E_i)$$

(11)

$$g_{1D}(E) = \left( \frac{2m}{\pi^2\hbar^2} \right)^{1/2} \sum_i h_i H(E - E_i) (E - E_i)^{1/2}$$

(12)

where $H(x)$ is the Heavyside function and $h_i$ is a constant that accounts for multiple degeneracies with one $E_i$.

Quantum dots would then be 0D quantum structures with energy levels quantized in
all three dimensions of \( n \)-space. This causes the density of states to then become distinctly spaced dirac delta functions of the form:

\[
g_{0D}(E) \sim \sum_i h_i \delta(E - E_i)
\]  

These various distributions are graphically depicted in Figure 4.

![Figure 4: Depiction of \( g(E) \) vs. \( E \) for 3D, 2D, 1D, and 0D quantum structures.](image)

1.3.3 Experimental Verification of Quantum Theory

Some of the quantum mechanical effects in QDs can be directly observed through optical spectroscopy (Figure 5). As is expected, the CdSe bulk semiconductors have an absorbance graph that has a fairly distinct increase in absorbance near the band edge. However, the CdSe nanocrystals contain distinct bands where the exciton energies are allowed. These distinct energy bands in the nanocrystals can be seen as peaks in the absorbance data in Figure 5. The initial absorbance peak is typically referred to as the first excitonic absorption peak because it is the energy at which the lowest energy electron-hole pair (\( i.e. \) exciton) can be generated. The peaks are not as distinct as the Dirac delta spike depicted in Figure 4 because there is a distribution of QD sizes, which causes a smearing of the density of states function.
1.4 Amphiphilic Polymer Wrapping

One of the methods used for achieving aqueous solubility with hydrophobic QDs is to wrap them in an amphiphilic polymer [8]. Amphiphilic polymer wrapping is typically the most employable and yields robust, stable dispersed polymer wrapped QDs (pQDs) in aqueous solvents [7]. Here at Cal Poly, I attempted to reproduce two of the polymer wrapping procedures seen in literature: that of T. Pellegrino et. al. (hereafter referred
Both the Pellegrino procedure and the Anderson procedure employ the same basic principles in how they disperse QDs into aqueous solutions. First, the QDs with their hydrophobic ligands are mixed together with a type of amphiphilic polymer to form pQDs (Figure 6). Once wrapped, the pQDs can then be dispersed into aqueous solvents.

Figure 6: Depiction of the basic principle behind amphiphilic polymer wrapping with QDs. The QD is added into a solution of the amphiphilic polymer and the hydrophobic side chains of the polymer intercalate with the hydrophobic ligands of the QD to form a type of micelle coating. This allows the QDs to disperse in water or aqueous solvents as pQDs. The circular nature of the polymer shown is used for simplicity, actual polymers are typically linear polymer and multiple polymers adhere to each QD in the pQD constructs [8], [7].

Typical polymer chains are on the order of 5–10 mer units and what is not shown in Figure 6 is that there are typically 100–200 mer units per pQD. There are also multiple steps in between synthesizing the QDs, wrapping them in polymer, and dispersing them in an aqueous solution.

1.4.1 Pellegrino Procedure

Wrapping Procedure

The Pellegrino procedure employs the use of poly(maleic anhydride-alt-1-octadecene) (PMA) as the amphiphilic polymer used to wrap the QDs (Figure 7). The hydrophobic side chains of the PMA interact with the surface ligands of the QDs through hydrophobic interactions, or Van der Waals forces. The hydrophilic anhydride functional groups will then lie on the surface of the pQD and point outward into the

9
Figure 7: Scheme of the wrapping procedure employed by T. Pellegrino et. al. The surfactant molecules on the QD surface can intercalate with the hydrophobic side chains of the PMA to form a bond through hydrophobic (Van der Waals) interactions. The anhydride side chains will then lie on the surface, pointing outward. This is what allows the pQDs to disperse within an aqueous solution. Picture borrowed from reference [8].

**Charging the Anhydride Groups**

Once these PMA wrapped QDs (PMA-QDs) are mixed with an aqueous solvent, the polar anhydride rings can then interact with the solvent molecules to allow the PMA-QDs to fully disperse in the solution. This hydrophilic interaction can be made even stronger by using a basic aqueous solvent. This allows the anhydride rings to split into a carboxylate anion and a carboxylic acid group (Figure 8). While the original Pellegrino procedure used a TBE buffer solution, I used sodium hydroxide as the base for my aqueous solution. Once the anhydride groups react in a basic aqueous solution, both the sodium carboxylate and the carboxylic acid will dissociation into charged carboxylate anions. This extremely polar functional group will then drive an even greater hydrophobic interaction between the intercalated alkyl chains and the QD surface ligands, further stabilizing the PMA-QD construct.
Figure 8: Reaction of the anhydride rings in basic solution to produce a sodium carboxylate group and a carboxylic acid group. In a basic aqueous solution, both the sodium carboxylate and the carboxylic acid groups will dissociate into a charged carboxylate anion, which is extremely polar and helps drive the hydrophobic alkyl chains into the QD to maintain its stability and allow greater dispersion.

1.4.2 Anderson Procedure

Wrapping Procedure

The Anderson procedure uses an alkylamine modified poly(acrylic acid) (mod-PAA) as the amphiphilic polymer coating in the pQD construct. This type of polymer is a great alternative to the PMA employed by Pellegrino et. al because poly(acrylic acid) (PAA) is a much more widely available polymer than PMA and is a much more stable product in the commercial industry [7]. After obtaining PAA as a starting material, one can obtain mod-PAA in straightforward synthesis that is detailed in the 2008 paper by R.E. Anderson and W.C. Chan [7].

The basic chemical process for obtaining mod-PAA is through an amidation reaction between a long chain alkylamine and the PAA carboxylic acid groups (Figure 9). This is achieved through using the dehydrating agent N,N'-Dicyclohexylcarbodiimide (DCC) to drive the amidation reaction forward between the alkylamine and the PAA carboxylic acid groups.
Figure 9: Reaction of PAA with an alkylamine in the presence of DCC to obtain the mod-PAA. DCC acts as a dehydrating agent in order to drive the reaction forward between the PAA and the alkylamine. The final product will have randomly distributed alkyl chains extending from the main PAA backbone. These alkyl chains will be able to intercalate with the QD surface ligands in order to form the amphiphilic polymer coating. Borrowed from reference [7].

The long chain alkylamine used in all of my reactions was octadecylamine (ODA), and I followed the exact procedure listed in [7] in order to produce my ODA modified PAA (ODA-PAA). The ODA provide the long alkyl side chains in the ODA-PAA that are analogous to the octadecene side chains found in the PMA used by Pellegrino et al. Therefore, when QDs are mixed together with the ODA-PAA in solution, the hydrophobic ODA side chains will intercalate with the QD surface ligands to create mod-PAA wrapped QDs (PAA-QDs) as depicted in Figure 10.
Figure 10: Scheme of the wrapping procedure employed by Anderson and Chan. The QDs are added to the mod-PAA in solution and the QD surface ligands intercalate with the alkylamine side chains of the mod-PAA to create the PAA-QDs. The PAA-QD carboxylic acid groups then point outward to form a polar surface that may be dispersed into aqueous solvents. Borrowed from reference [7].

Charging the Carboxylic Acid Groups

Once PAA-QDs are formed, it is possible to further stabilize the PAA-QD structure by charging the PAA-QD surface groups. This is done through a process similar to the Pellegrino procedure when splitting the anhydride rings. When PAA-QDs are added to a strong basic solution and given energy in the form of heat, the carboxylic acid surface group can become deprotonated and form a charged ionic surface (Figure 11).
1.5 Broader Impacts

With the advent of QDs, a new class of nanoscale fluorescent materials has emerged that exhibits unparalleled QYs, high resistance to photobleaching, and absorption over a large optical range. These new inorganic semiconducting nanocrystals exhibit size dependent emissions that can be tuned over a wide range of wavelengths in the visible range, making them ideal in biological imaging and sensing applications [4]–[6], [10], [11].

QDs are also being employed in solid state technologies due to the high degree of electronic tunability in these constructs. This stems from the electronic structure of quantum dots being highly dependent upon their shape, size, and orientation with respect to excitation media (e.g. polarization of light, electric field) [12]. By varying these three parameters in different amounts, it is possible to control the electronic structure of these semiconducting materials to a much higher degree than is allowed through variations in composition and crystal structure [13]. However, most processing techniques for solid state QDs do not employ colloidal assembly because the desired quantum effects are difficult to achieve with this synthesis method [12].

1.5.1 Use in Cellular and Molecular Biology

Inorganic QDs are currently under intense study for use in biological sensing applications that employ various methods of fluorescence spectroscopy, especially
fluorescence resonance energy transfer (FRET) and single molecule imaging [1], [2], [14], [15]. Cellular and molecular biologists require great sensitivity and spatial resolution in order to image the molecules and cellular pathways within a biological microenvironment. Therefore QDs need to be stable, have high signal to noise ratio, and be implementable into a biologically relevant environment while not significantly disrupting cellular or molecular behavior.

1.5.2 Use in the Commercial Industry

Once QDs are further developed and biological imaging techniques employing them become mainstream, there will be a need for them in commercial applications. One possible application for QDs is in the pharmaceutical industry where fluorescence labeling is quintessential in the discovery of many new drugs and cures. However, industries such as this cannot always afford to purchase expensive fluorophores such as polymer wrapped QDs. It is therefore necessary to design a polymer wrapping process that renders QDs aqueous soluble, but does so in a cost effective manner.

2 Methods and Materials

2.1 Spectroscopic Characterization

Fluorescence and absorbance measurements were taken in either CHCl$_3$ or aqueous NaOH solution, pH 11, on an Ocean Optics USB4000 fiber optic spectrophotometer. All fluorescence spectrums were taken with a LED excitation source with a center wavelength 400 nm. All analyses were carried out at California Polytechnic State University, San Luis Obispo, CA.

2.2 Quantum Dot Preparation

Two batches of CdSe(ZnS) core(shell) QDs were synthesized to be wrapped in an amphiphilic polymer. The first batch of QDs (QD#1s) were used in the Pellegrino
procedure and the second batch of QDs (QD#2s) were used in the Anderson procedure. Both batches were synthesized in octadecene (ODE) by Cal Poly graduate student Josh Angell. The CdSe QD synthesis procedure was based on a previous study by E.M. Boatman et al. [16] and developed further by senior Aaron Lichtner in his 2009 senior project. The ZnS coating procedure was based on the procedure used by Pellegrino et al. [8] and was refined by Josh Angell during his 2011 master’s thesis work.

In order to exchange ODE for CHCl$_3$, one part CdSe(ZnS) core(shell) QDs in ODE were mixed with 4 parts tech grade ethanol (99.5% pure) and centrifuged at 4000 rpm for 10 min. The supernatant was discarded and the remaining QD pellet was dissolved in CHCl$_3$. Fluorescence and absorbance measurements were then taken for each of the QD batches to determine the initial QD optical properties before addition of the amphiphilic polymer coating. The peak absorbance wavelength could then be used to approximate the average particle size using the following empirical equation derived by Yu et al. [15]:

$$D = (1.6122 \times 10^{-9})\lambda^4 - (2.6575 \times 10^{-6})\lambda^3 + (1.6242 \times 10^{-3})\lambda^2 - (0.4277)\lambda + 41.57$$ (14)

where $D$ is the diameter of the CdSe QD in nm and $\lambda$ is the wavelength of the first excitonic absorption peak in nm. Using the average nanoparticle diameter from Equation 14 and the known density of bulk CdSe ($5.67 \times 10^{-21}$ g/nm$^3$), it is possible to calculate the molar mass for an arbitrary batch of CdSe QDs. This enabled me to calculate how much amphiphilic polymer to add to a given sample of QDs.

### 2.3 Pellegrino Procedure

The PMA-QDs were obtained through a slightly modified procedure by Pellegrino et al. [8]. Equal volumes of QD#1 dissolved in CHCl$_3$ and of PMA (Sigma-Aldrich #419117) dissolved in CHCl$_3$ were mixed together in a closed reaction flask under constant stirring for 2 h to form a PMA-QD solution in CHCl$_3$. The molar ratio of PMA to QD#1 used was determined by using 100 PMA mer units per nm$^2$ of QD#1 surface area. The PMA-QDs in CHCl$_3$ (25 $\mu$M) were then allowed to air evaporate in a fume
hood to leave behind a yellow colored gel that fluoresced red under UV radiation. Under constant stirring, H$_2$O and NaOH were then added to the reaction flask until a pH of 10–11 was reached (PMA-QD concentration at 25 $\mu$M was maintained). PMA-QDs were then heated to 80$^\circ$C under constant stirring for 45 min, sonicated for 15 min, heated to 80$^\circ$C for another 45 min, and sonicated again for 5 min. The final solution was a red murky color and fluoresced red under UV radiation (Figure 12). PMA-QDs were then cooled to room temp before spectroscopic characterization.

![Figure 12: Picture of both the as synthesized PMA-QDs (left) and the PMA-QDs after 20 min 400 nm radiation. The change in photoluminescence after radiation is difficult to see directly.](image)

After initial fluorescence characterization, it was noticed that the integrated fluorescence intensity would increase under continuous UV radiation. The PMA-QDs were then exposed to 400 nm UV radiation until the peak emission intensity began to level off around 20 min. The final PMA-QD change in brightness was only slightly noticeable to the naked eye (Figure 12).
2.4 Anderson Procedure

mod-PAA Synthesis

The ODA modified PAA was synthesized through a slightly modified procedure by Anderson and Chan [7]. To create a 40% ODA modified PAA, 0.0695 mol (5 g) of PAA (Sigma-Aldrich #323667) was dissolved in 150 mL of 1-methyl-2-pyrrolidinone (MPD, Sigma-Aldrich #328634) at 60°C for 24 h under constant stirring. In a separate vessel, 0.0278 mol (5.74 g) of N,N'-dicyclohexylcarbodiimide (DCC, Sigma-Aldrich #D80002) was dissolved in 10 mL of MPD. The DCC-MPD solution was then added to the PAA-MPD solution and allowed to react for 1 h at 60°C under constant stirring. During the reaction, 0.0278 mol (7.49 g) ODA was dissolved in 10 mL MPD. The 10 mL of ODA-MPD solution was then added to the PAA-DCC solution and allowed to react for 24 h at 60°C under constant stirring. The solution was then cooled to room temperature and filtered through a Whatman filter paper to remove any excess dicyclohexylurea crystals formed during the reaction. The mod-PAA was then precipitated out in a bath of H₂O and methanol at 0°C. A small addition of NaOH also helped to stabilize the precipitate during centrifugation (4000 rpm, 10 min). Supernatant was then discarded and excess solvent was evaporated off before polymer wrapping.

Polymer Wrapping

The PAA-QDs were obtained through a slightly modified procedure by Anderson and Chan [7]. Equal volumes of QD#2 dissolved in CHCl₃ and of mod-PAA dissolved in CHCl₃ were mixed together in a closed reaction flask under constant stirring for 2 h to form a PAA-QD solution in CHCl₃. A polymer to QD#2 molar ratio of 2000:1 was used to achieve the greatest QD#2 transfer efficiency as per reference [7]. The PAA-QDs in CHCl₃ (30 μM) were then allowed to air evaporate in a fume hood to leave behind a yellow colored gel that fluoresced green under UV radiation. Under constant stirring, H₂O and NaOH were then added to the reaction flask until a pH of 10–11 was reached (PAA-QD concentration at 30 μM was maintained). PAA-QDs were then sonicated for 30 min, heated to 75°C under constant stirring for 15 min, and then sonicated for
another 5 min. It was observed that excessive sonication after heating would sometimes induce an irreversible exothermic reaction that resulted in PAA-QD flocculation. The final solution was a murky white color and fluoresced a faint green under UV radiation (Figure 13). PMA-QDs were then cooled to room temp before spectroscopic characterization.

Figure 13: Picture of both the as synthesized PAA-QDs (left) and the PAA-QDs after 20 min 400 nm radiation. The change in photoluminescence after radiation is visibly noticeable.

After initial fluorescence characterization, it was noticed that the integrated fluorescence intensity of the PAA-QDs also increased under continuous UV radiation. The PAA-QDs were then exposed to 400 nm UV radiation until the peak emission intensity began to level off at around 20 min. The final PAA-QD change in brightness was noticeable to the naked eye (Figure 13).
3 Results

3.1 Fluorescence Characterization

3.1.1 ZnS Coating

Fluorescence spectroscopy was used to quantitatively determine the brightness of the synthesized CdSe QDs before and after coating them with a ZnS shell. This helped to determine if a ZnS coating would be necessary for a pQD fluorophore. The QD fluorescence was first characterized both before and after the ZnS coating process for QD#1 and QD#2.

First, the QD#1 fluorescence was characterized as can be seen in Figure 14. After ZnS coating of QD#1, the fluorescence intensity is increased by 3.4x, which allows for a greater signal to noise ratio when trying to analyze the pQDs in aqueous solvents. The center wavelength of the QD#1s also shifts after coating from 556 nm for the bare CdSe QD to 621 nm for the ZnS coated QD.
Figure 14: Normalized emission intensity of the CdSe QD#1s both before (blue) and after (red) ZnS coating. This shows that there is a slight red shift in the center wavelength after coating from 556 nm to 621 nm. There is also 3.4x fluorescence increase in the emission intensity, making the ZnS coated QDs much more viable as fluorescence probes. The small secondary bump around 480 nm is due to scattering from the excitation source.

QD#2 was then characterized using the same method as QD#1 (Figure 15). As can be seen from Figure 15, the ZnS coated QD#2s are much brighter than the uncoated CdSe QD#2s. This allows for a much higher signal to noise ratio when attempting to characterize the pQDs after the transfer to water. It can also be seen from the fluorescence spectrum that the CdSe QD#2 center wavelength is 530 nm while the CdSe(ZnS) center wavelength is shifted to 568 nm.
Figure 15: Normalized emission intensity of the CdSe QD#2s both before (blue) and after (red) ZnS coating. This shows that there is a slight red shift in the center wavelength after coating from 530 nm to 568 nm. There is also 10x fluorescence increase in the emission intensity, making the ZnS coated QDs much more viable as fluorescence probes. The small secondary bump around 480 nm is due to scattering from the excitation source.

3.1.2 Pellegrino Procedure

Fluorescence of the QD#1s was characterized after the transfer to CHCl₃ and after addition of PMA to form the PMA-QDs in CHCl₃ (Figure 16). The PMA displays fluorescent behavior across the visible spectrum and this can be seen as a slight bump in the emission spectrum around 500 nm. This PMA fluorescence also causes the peak emission intensity to increase a small amount (6.8%) and to slightly blue shift by 11 nm.
Figure 16: Normalized emission intensity of the QD#1s in CHCl₃ (red) and when combined into the PMA-QD construct (green). This shows that there is a slight blue shift in the QD center wavelength from 614 nm to 603 nm after PMA wrapping in CHCl₃. The peak emission intensity also increased by 6.8%. The small secondary bump around 500 nm is due to fluorescence emission from the PMA.

Once the PMA-QDs are dissolved in CHCl₃, they were transferred into a basic aqueous solution. This was done by first evaporating off the CHCl₃ until a gel was formed. Under constant stirring, H₂O with NaOH at 80° was then added to the PMA-QD gel for 1 hour. Afterward, the solution was cooled and its fluorescence was characterized (Figure 17). Here it can be seen that there was a 65% decrease in peak emission intensity after the transfer to basic H₂O, which is indicative of a lower PMA-QD concentration.
Figure 17: Normalized emission intensity of the PMA-QD in CHCl\textsubscript{3} (blue) and after transfer to basic H\textsubscript{2}O (red). The peak emission intensity decreased by 65%, indicating incomplete transfer of the PMA-QDs. The small secondary bump around 500 nm is due to fluorescence emission from the PMA.

After initial fluorescence characterization, it was noted that the integrated fluorescence intensity began to increase and leveled off after about 20 min of radiation at 400 nm (Figure 18). Since this is a permanent increase in fluorescence and can be attributed to a rearrangement of surface molecules and a passivation of the surface layer [17], I will refer to it as photo-annealing. The peak emission intensity of the PMA-QDs increased by 1.7x, which resulted in an overall fluorescence decrease after transfer of 40.5%.
Figure 18: The effect of permanent photoenhancement after 20 min of radiation at 400 nm. The peak emission intensity of the PMA-QDs increased by 1.7x after the transfer to H₂O.

3.1.3 Anderson Procedure

Fluorescence of the QD#2s was characterized after the transfer from ODE to CHCl₃ (Figure 19). Figure 19 also shows the fluorescence spectrum of the PAA-QDs in CHCl₃. Transfer to CHCl₃ from ODE significantly reduced the peak emission intensity of the QD#2s. The PAA displays fluorescent behavior across the visible spectrum, and this can be seen as a slight blue shift in the peak emission intensity from 564 nm to 559 nm. Addition of the PAA also seems to cause the peak emission intensity to decrease by 25%, which is due to the PAA absorbing and scattering the excitation source.
Figure 19: Normalized emission intensity of the QD#2s in ODE (blue), in CHCl$_3$ (red), and when combined into the PMA-QD construct (green). This shows that there is a slight blue shift in the QD center wavelength from 564 nm to 559 nm after PAA wrapping in CHCl$_3$. The peak emission intensity also decreased by 25%. The small secondary bump around 480 nm is due to scattering from the excitation source.

Once the PAA-QDs are dissolved in CHCl$_3$, they can be dissolved into a basic aqueous solution. This is done by first evaporating off the CHCl$_3$ until a gel is formed, and then H$_2$O with NaOH is added. This solution is sonicated for 30 min, stirred for 30 min, heated to 75°C for 10 min, and then sonicated for another 5 min. Afterward, the solution was cooled, and its fluorescence was characterized (Figure 20). Here it can be seen that there was a 65% decrease in peak emission intensity after the transfer to basic H$_2$O. There is also an increase in the scattering of the excitation source that can be seen at 400 nm, which partially explains the decrease in PAA-QD emission intensity during the transfer to H$_2$O. The small secondary bump around 490 nm is due to fluorescence emission from the PAA.
Figure 20: Normalized emission intensity of the PAA-QD in CHCl₃ (blue) and after transfer to basic H₂O (red). The peak emission intensity decreased by 65%, indicating incomplete transfer of the PAA-QDs or increased scattering. The mod-PAA fluorescence increases by 3.6% after the transfer and can be seen as a small secondary bump around 500 nm.

After initial fluorescence characterization, it was noted that the integrated fluorescence intensity began to increase and leveled off after about 20 min of radiation at 400 nm (Figure 21). The peak emission intensity of the PAA-QDs increased by 3.4x, which resulted in an overall fluorescence increase of 19%.
Figure 21: The effect of permanent photoenhancement after 20 min of radiation at 400 nm. The peak emission intensity of the PMA-QDs increased by 3.4x after the transfer to H₂O.

### 3.2 Absorbance Characterization

Absorbance spectroscopy was used to quantitatively determine the absorptivity of the synthesized CdSe(ZnS) QDs before and after combining them with an amphiphilic polymer as well as after the transfer to an aqueous solvent. Characterizing the QD vs. pQD absorptivity in CHCl₃ was used to determine any adverse effects that the polymer itself had on the QDs ability to absorb incoming light. Characterization on the absorbance was then measured in H₂O to determine how well the transfer to an aqueous solvent worked.

The absorbance of a solute is an empirical parameter that can be used to determine the concentration of that solute in a solution by using the Beer-Lambert Law:

\[ A = ebc \]  

where \( A \) is the absorbance, \( e \) is the molar absorptivity in M\(^{-1}\)cm\(^{-1}\), \( b \) is the path length of light in cm, and \( c \) is the concentration of solute in M. Since \( b \) was unchanged
for all measurements and the extinction coefficient is not dependent upon the type of solvent for CdSe QDs [15], any changes in absorbance can then be attributed to a change in concentration of solute.

### 3.2.1 Pellegrino Procedure

Initial characterization of the PMA-QDs involved measurements of the QD#1 absorbance in CHCl₃ both before and after addition of PMA (Figure 22). Comparison between the absorbance of QD#1 vs. PMA-QD from Figure 22 shows that the value of the primary absorbance peak at 575 nm decreases by 63% after addition of PMA. This can be attributed to a reduction in QD#1 concentration upon addition of the PMA solution.

![Absorbance of QD#1 vs. PMA-QD](image)

Figure 22: Absorbance of QD#1 before (blue) and after (red) addition of PMA to the solution. There is a 63% reduction in the primary QD#1 absorbance peak located at 575 nm. This can be attributed to a reduction in QD#1 concentration during addition of the PMA solution.

The PMA-QDs in CHCl₃ were then transferred into a basic aqueous solution using the method described above. Once dissolved in H₂O, the PMA-QDs were allowed to cool, and absorbance measurements were taken to compare to the PMA-QDs in CHCl₃.
(Figure 23). It can be seen from Figure 23 that the absorbance of the PMA-QDs decreases by 57% when transferred to H$_2$O. This is most likely due to a decrease in the concentration of PMA-QDs when transferring to the basic H$_2$O solution.

Figure 23: Absorbance of PMA-QD before (blue) and after (red) transfer to basic H$_2$O. There is a 57% reduction in the primary PMA-QD absorbance peak located at 580 nm. This can be attributed to a reduction in QD#1 concentration as a result of incomplete PMA-QD transfer.

3.2.2 Anderson Procedure

Initial characterization of the PAA-QDs involved measurements of the QD#2 absorbance in CHCl$_3$ both before and after addition of PMA (Figure 24). Comparison between the absorbance of QD#2 vs. PAA-QD from Figure 24 shows that the value of the primary absorbance peak at 550 nm decreases by 50% after addition of PAA. Furthermore, the entire characteristic QD absorption profile disappears so that the exciton peaks of QD#2 can no longer be seen. This is indicative of an absorption interference by the PAA.
Figure 24: Absorbance of QD#2 before (blue) and after (red) addition of PAA to the solution. The characteristic QD absorption profile can no longer be seen after addition of the PAA. This indicates that the PAA is strongly interfering with the ability of the QD#s to absorb incoming light.

The PAA-QDs in CHCl$_3$ were then transferred into a basic aqueous solution using the method described above. Once dissolved in H$_2$O, the PAA-QDs were allowed to cool and absorbance measurements were taken to compare to the PAA-QDs in CHCl$_3$ (Figure 25). It can be seen from Figure 25 that the PAA-QD absorbance at 550 nm increases by 5.6x when transferred to H$_2$O. This is most likely due to the large amount of undissolved particulate matter in the aqueous solution, which results in high amounts of light scattering.
4 Discussion

4.1 Analysis of Pellegrino Procedure

The fluorescence of the QD#1 in CHCl₃ (Figure 16) showed a slight blue shift and increase in fluorescence intensity upon addition of PMA. This is partially due to the fact that PMA slightly fluoresces across the optical spectrum as can be seen by the slight bump in fluorescence around 500 nm after addition of the polymer. However, this does not account for such a dramatic increase (6.8%) as is seen in Figure 16. By looking at the absorbance data for QD#1 in Figure 24, it is possible to see a large amount of absorbance of wavelengths far greater than the first excitonic absorption peak (575 nm). Since this region typically has negligible absorbance, the high amount of absorbance seen is most likely due to light scattering caused by a high concentration of colloidal particles. If too concentrated, the QDs will actually scatter light instead of absorbing and re-emitting it through fluorescence. Once the PMA is
added to the QD#1s, the solution of PMA-QDs is actually at a lower concentration than before, and this allows the QD#1s in solution to absorb more incoming photons. This scattering effect is confirmed in the absorbance curve for the PMA-QD solution, which has half the concentration of just the QD#1s in solution due to a 1:1 mixing with the PMA solution.

After PMA-QDs were transferred to an aqueous solution it was noticed that the final solution was slightly cloudy, which is indicative of particle flocculation. Fluorescence spectroscopy showed that there was a 65% decrease in peak emission intensity (Figure 17). Since the fluorescence of QDs is highly dependent on the concentration of the QDs in solution, it is likely that there was an incomplete transfer of PMA-QDs to H₂O. The absorbance curve for the PMA-QDs in CHCl₃ vs. H₂O (Figure 23) shows that the first excitonic absorption peak decreases by 57% after the transfer. Using the Beer-Lambert Law (Equation 15), the 57% decrease in absorbance can be interpreted as a 57% decrease in concentration. This strongly suggests that a decrease in concentration due to an incomplete transfer is the main factor contributing to such a large decrease in fluorescence intensity.

4.2 Analysis of Anderson Procedure

The fluorescence of the QD#2 in CHCl₃ (Figure 16) showed a slight decrease in fluorescence as well as a small blue shift. The blue shift in fluorescence can be attributed to the fluorescent nature of the mod-PAA, which emits wavelengths across the visible spectrum. This can be seen by the slight bump in emission intensity around 480 nm. The 25% decrease in peak emission intensity, however, is due to absorption and scattering from the polymer in solution. This effect can be seen from the absorbance data for QD#2 vs. PAA-QD (Figure 24). The characteristic absorption curve for colloidal QDs completely disappears after addition of the mod-PAA, which indicates that addition of the polymer is strongly interfering with the QD#2 absorbance. It can also be seen that the absorbance does not go to zero as it levels off in the near-IR region, which implies that addition of the polymer also increases scattering of light as well. This is most likely due to the polymer not completely dissolving in the CHCl₃, which is due to the amphiphilic nature of polymer [7]. This increased scattering...
can also be seen in Figure 19 where the increase in emission intensity around 400 nm is due to light scattering from the excitation source after addition of the polymer. With more light being directly scattered, less light can reach the QD#2s, and this disallows them from emitting photons.

An even further increase in excitation light scattering can be seen around 400 nm when the PAA-QDs are transferred into H₂O (Figure 20). Here there is also an even further decrease in QD#2 peak emission intensity while the mod-PAA emissions actually increase by 3.6%. The increase in mod-PAA emissions is most likely due to the large increase in scattered excitation emissions around 400 nm. This is because the excitation source actually emits a distribution of wavelengths around 400 nm, and as the scattering of the source increases, the tail end of these emissions begins to become more prominent between 400–500 nm, thereby increasing the apparent fluorescence of the mod-PAA. The decrease in PAA-QD peak emission intensity can also be partially attributed to light scattering. With more light being scattered, the PAA-QDs cannot absorb and reemit (through fluorescence) as much light, thereby decreasing their fluorescence intensity.

The effect of scattering can also be seen in the absorbance data for the PAA-QDs in H₂O vs. CHCl₃ (Figure 25). It can be seen that there is a major increase in absorbance over the entire visible spectrum and that it never goes to zero. Since this is not characteristic for either QDs [15] or organic molecules [18], it can be inferred that this absorbance behavior is a result of high amounts of light scattering. The solution of PAA-QDs also became more cloudy after the transfer to H₂O, which can account for the high amount of light scattering. With the uncharacteristic nature of the absorbance data, it is difficult to determine what the transfer efficiency of the PAA-QDs was, and therefore the effect of transfer efficiency on fluorescence intensity was impossible to determine.

4.3 Comparison of Wrapping Procedures

Both the amphiphilic polymer wrapping procedures were a success to a certain degree. It can be seen from the fluorescence results in Figures 17 and 20 that there is
definitely fluorescence being exhibited by both of the pQDs in an aqueous solvent. Both of the pQDs, however, showed a significant decrease in emission intensity (65%) after the transfer to an aqueous solvent. Interestingly, this decrease in emission intensity was identical for both the Pellegrino and the Anderson procedure. This shows that both of the PMA and mod-PAA are viable options for amphiphilic polymer wrapping in the future. Multiple wrapping procedures were carried out for both polymers to try and refine the method most effectively (Figure 26).

Figure 26: Picture of all the successful wrapping syntheses for both the Pellegrino procedure (left) and the Anderson procedure (right). The PMA-QD solutions were much brighter and were typically less cloudy than the PAA-QD solutions.

The disparity in the brightness of the two solutions can be accounted for by comparing the original QD fluorescence spectrums in CHCl₃ (Figure 27). From the fluorescence data, it can be seen that the QD#2s had 33% of the peak emission intensity of the QD#1s, which explains why the PMA-QD solutions are much brighter than the PAA-QD solutions.
Figure 27: Comparison of the fluorescence between QD#1 and QD#2. The peak emission intensity of QD#2 is 33% of the peak emission intensity for QD#1. This explains the difference in the brightness of PMA-QDs vs. PAA-QDs after their transfer to H$_2$O.

5 Conclusion

This project detailed the results of two amphiphilic polymer wrapping processes to obtain aqueous soluble quantum dots. The Pellegrino procedure obtained aqueous soluble quantum dots that emit at a center wavelength of 603 nm. During this process the peak fluorescence intensity of the polymer wrapped quantum dots decreased by 65%, which can be attributed to a decrease in quantum dot concentration due to an incomplete transfer to the aqueous solution. Irradiation of polymer wrapped quantum dots in H$_2$O lead to a 1.7x permanent fluorescence enhancement, giving an overall fluorescence decrease after transfer of 40.5%. The Anderson procedure obtained aqueous soluble quantum dots that emit at a center wavelength of 559 nm. During this process the peak fluorescence intensity of the polymer wrapped quantum dots decreased by 65%, which can be attributed to an increase in scattering by undissolved particulate matter. Irradiation of polymer wrapped quantum dots in H$_2$O lead to a 3.4x permanent fluorescence enhancement, giving an overall fluorescence increase after
transfer of 19%. Both methods of amphiphilic polymer wrapping show promise for obtaining aqueous soluble quantum dots that may be used for biological functionalization and imaging purposes.
References


