Fluorescence Characterization of Quantum Dots for use as Biomarkers

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Abstract

Fluorescence profiles of quantum dots (QDs) were characterized to select the ideal QDs for encapsulation in phospholipids for use as biomarkers to selectively adhere to cancer cells. QDs were synthesized and extracted 0, 30, 60, and 90 seconds after precursor compounds were mixed. These extractions were isolated by extraction time. Portions from each vial were coated in a zinc sulfide shelling procedure, leaving at least half of the QD solution unshelled. These samples were characterized over four days to monitor fluctuations in fluorescence. This was done utilizing an Ocean Optics spectrometer in conjunction with Spectra Suite software. The central wavelength, maximum intensity, and the full width at half maximum (FWHM) were the main focus of the measurements. Ten measurements were taken on each sample at Days 0 (the day of the shelling procedure), 1, and 3. On Day 0, shelled QDs showed significant shifts in central fluorescence wavelength, increases in intensity, and minor increase to FWHM. Over the span of the following 3 days, the unshelled QD’s maintained their central wavelength, relative peak intensity, and FWHM. The central wavelength and FWHM of the shelled quantum dots remained stable after the initial shift as well. In contrast, the shelled quantum dots intensity continued to increase the day after the shelling process, often increasing into the fourth day of characterization. This is likely due to reactants in the solution continuing to bond to the QDs. The higher intensity, shelled quantum dots will be encapsulated with phospholipids for use as biomarkers.

Keywords

Materials, Materials Engineering, Nanotechnology, Nano, Nanotech, Quantum Dots, Biomarkers, Biomedical Materials, Biomedical, Semiconductors, Fluorescence, Cancer
1. Introduction

1.0 Motivation of the Study

Cancer is the second leading cause of death in the United States, exceeded only by heart disease. In 2008, more than 565,000 people died of cancer, and over 1.48 million were diagnosed with cancer.\[1\] Cancer rates are on the rise in the United States. According to the 2013 cancer statistics conducted by the American Cancer Society, it is expected that 1,660,290 new cases of cancer will be diagnosed in 2013. It is also expected that 580,350 of those people living with cancer will die.\[2\] Cancer can grow rapidly if left unchecked, and can metastasize to other areas of the body.\[3\] Diagnosing cancer at later stages has an adverse effect on survival rate.\[4\] If detection is delayed until later stages of development, it is often too late for the patient to survive.\[4\] One especially common form of cancer in the United States is skin cancer. In 2013, it is expected that 76,690 people will be diagnosed with melanoma.\[2\] That does not include other types of skin cancer such as basal and squamous.

1.1 Conventional Methods of Cancer Detection

Early and accurate detection of cancer is critical to patient survival. Early detection of melanoma is especially important as it is the most aggressive of the three types of skin cancer.\[5\] Current methods of detection hold biopsies as the gold standard for cancer detection.\[6\] A skin biopsy removes cells or a sample of
skin from the surface of a patient either with a razor or “punch” device as depicted in Figure 1.\textsuperscript{[7]}

Figure 1: A punch can be used to take a tissue sample at a deeper level in the skin. This tissue sample can then be utilized for a biopsy.\textsuperscript{[8]}

A visual assessment is performed on the sample to see if there are any cells that appear as though they may be cancer cells.\textsuperscript{[7]} The most common error with a biopsy is a false negative which occurs when a medical professional fails to detect cancer when a patient actually does have cancer.\textsuperscript{[9]} A false negative can cause a patient to ignore dangerous cancer progression for prolonged periods of time, allowing the cancer to spread. As cancer progresses undiagnosed and untreated, patients are more likely to die because of the cancer.

\textbf{1.2 Problem Statement}

One of the most prominent and reliable methods of cancer detection is inherently subjective. This subjectivity can lead to false negatives, which delay cancer detection and delay cancer treatment. Delayed detection and delayed treatment
puts patients’ lives at higher risk, which in turn leads to a higher mortality rate to those living with cancer. A more reliable and less subjective method is required.

2. Background

2.0 Quantum Dots

Quantum Dots (QDs) are being designed to act as biomarkers for early, reliable, non-subjective cancer detection. QDs are semiconductor nanocrystals that are typically composed of a hundred to a thousand atoms (2-10nm in diameter).\textsuperscript{[10]} Because of their small size, QDs maintain some characteristics of bulk materials while also retaining characteristics of individual atoms. QDs exhibit properties of both classical and quantum physics. This unique pairing allows a direct influence over fluorescence characteristics by simply changing the size of the QDs, as seen in Figure 2.\textsuperscript{[10]}

The distinctive electronic and fluorescent properties of QDs can be explained by the high surface area to volume ratio as well as a property known as quantum confinement.

\textsuperscript{[11]} Figure 2: As QDs increase in size, the frequency of fluoresced light drops.
2.1 Molecular Orbitals and Band Theory

Electrons exist in orbitals surrounding an atom’s nucleus. The orbitals closest to the center of the nucleus have the lowest energy, while shells further from the nucleus have increasingly higher energies. Electrons have a strong tendency to occupy the lowest energy state possible, so long as no two orbitals have the same energy. This property also extends into bulk materials. As more atoms come into interaction with each other, orbital energies will shift to accommodate each other. Eventually, these shifts become so small that they can be regarded as continuous bands of energies. In bulk semiconductors, a valence band and a conduction band form. QDs are in a unique limbo between the atomic and bulk properties. They maintain discrete energy levels for electrons to occupy (as in atoms), while starting to act more like a bulk material by forming more spaces for electrons to occupy in two “bands” as seen in Figure 3.

Figure 3: Quantum dots exhibit characteristics of both atoms and bulk solids with discrete energy levels forming in two unique bands.
The majority of electrons stay in the valence band, while few electrons move into the conduction band. When electrons are stimulated with enough energy, they may cross the distance between the valence and conduction band, known as the bandgap.

### 2.2 Quantum Confinement and Bohr Exciton Radius

When electrons jump from the lower energy levels to the higher energy levels, they leave behind a “hole”. A hole is the positively charged area left behind when an electron moves locations. The pair of an electron and the hole are known as an exciton. The distance between an exciton’s electron and its hole is known as the exciton Bohr radius. In a bulk material the exciton Bohr radius is miniscule in comparison to the material as a whole. As the material’s size decreases to the nanoscale, as it does with QDs, the exciton Bohr radius is constricted. The electron and the hole are placed closer together than they would be in a bulk lattice, yielding higher electrostatic forces between the two.

When the material is confined in one dimension, it creates a quantum well. Confinement in two dimensions creates a quantum wire. Total confinement yields a quantum dot. Each of these progressions alters the density of states in comparison to the energy as seen in Figure 4. The electron energy levels move from continuous to discrete energy states as they lose degrees of freedom.
2.3 Fluorescence

Once electrons are excited into the conduction band, they want to fall back to a lower energy state, as described with the Aufbau principle.\cite{12} When electrons fall back to the ground state, the energy needs to leave the material in a new form. This can be achieved in a combination of non-radiative decay (loss of energy through heat) and radiative decay (loss of energy through photon emission) as seen in Figure 5.\cite{17} While nonradiative
relaxation occurs between energy states that are close to each other, radiative decay occurs most often in the final transition from the conduction band back down to the ground state of the valence band. Ultraviolet light is used to excite the QD electrons into excited states. The radiative relaxation is what causes the visible fluorescence of QDs. Smaller quantum dots will have a larger bandgap and produce light of a higher frequency.\textsuperscript{18}

Fluorescence can be characterized several ways. Three main factors of fluorescence important to potential QD biomarkers are intensity, full width at half maximum (FWHM), and central wavelength. Intensity is a measurement of how many photons are being released. A brighter light will have a higher intensity. FWHM indicates the width of a waveform at half of the maximum intensity. A narrow FWHM in fluorescing QDs indicates similarly sized QDs. A narrower spectrum of fluoresced light would also be more distinct and easier to detect. Central wavelength indicates the most common wavelength of emitted photons from the QDs. It is a good way to predict what color QDs will fluoresce.

\textbf{2.4 QD Core Synthesis}

Quantum dots can be grown by multiple methods using multiple materials. This paper will focus on organometallic colloidal growth of CdSe QDs. In this process a precursor solution containing selenium (Se) is created and added into another precursor solution containing cadmium (Cd). Once the two precursors are mixed, Se and Cd ions begin to build up until they begin to overcome the energy barrier required for nucleation. This creates a multitude of small CdSe particles.
After an initial burst of nucleation, the newly formed QDs will then begin to grow to larger and larger sized as the reaction continues. Extracting the quantum dots from this process should yield different sizes of QD cores. A representation of a QD core can be seen in Figure 6.

2.5 Shelling QDs

After creating the QD cores, the cores can be shelled with another compound. Shelling is the encapsulation of QD cores in a layer of new elements. This experiment used zinc sulfide for the shell material. Shelling the QD cores creates a “charge separation” in the QDs. The electrons want to stay in the core of the QD, but the holes want to go to the shell. This separation changes the properties of the QD. Of two electrons in the low energy state, one needs a significantly larger energy increase than the other, so it typically stays at the low level. As the other electron excites to the high-energy
state and forms an exciton, it recombines in the presence of a photon and generates two photons to leave the material. Because more photons should be produced, the fluorescence of shelled quantum dots should appear brighter than unshelled quantum dots, even when exposed to the same energy.\[20\]

2.6 Project Goal

The goal of this project is to characterize the fluorescence of both shelled and unshelled QDs. This project will analyze the changes in fluorescence as QDs are processed from an unshelled to a shelled state. It will further characterize the stability of fluorescence in QDs in both a shelled state and an unshelled state over the course of four days. The shifts in fluorescence will be compared to standard deviations of the measurements for statistical significance.

3. Experimental Procedures

3.0 Realistic Constraints

There were two significant factors that influence the experimentation process. The first factor was the manufacturability of the quantum dots. The other factor was the precautions required and for the health and safety of individuals interacting with the QDs and the synthesis and shelling chemicals.

The amount of samples that could be produced was limited due to the manufacturability of the QDs. Producing a batch of unshelled samples took approximately 50 minutes to complete. This was done twice. The most time-
consuming process was the shelling of the samples. Each vial of unshelled QDs was shelled separately. This took 45 minutes and was performed eight times. One major time-consuming aspect of both processes was the purging of chemical solutions with nitrogen gas to reduce the possibility of oxygen interfering with the QD synthesis and shelling processes. Along with this, the mixing of precursor solutions maintained high tolerances that were difficult to attain. Limits of equipment and imperfect transfer of chemicals between measurement devices and mixing chambers may have caused further error. To reduce the effect of high tolerances, precursors were produced in 4x scale amounts. By doing this, small discrepancies in measurements had less of an effect on the precursor as a whole.

The chemicals involved in the manufacturing process were often hazardous. All synthesis and shelling took place beneath a fume hood. Nitrile gloves were used whenever handling chemicals or quantum dots. Eye protection, closed-toed shoes, and long pants were used as well. This provided protection from carcinogenic, corrosive, toxic, and pyrophoric chemicals used in the process. All hazardous waste had designated disposal units. There were separate waste containers used for liquid waste, solid waste products, and a third specifically for needles and other sharp objects. The work area was cleaned after manufacturing procedures using acetone.
3.1 Sample Preparation

Quantum dots were synthesized utilizing an organometallic colloidal growth method. This process involves mixing two precursors, and allowing a chemical reaction to occur for a designated period of time before the solution (now containing quantum dots) is removed from the reaction vessel. Four extraction times were used in the experiment. Extractions were taken at 0 seconds, 30 seconds, 60 seconds, and 90 seconds. This procedure yielded four vials of unshelled quantum dots as seen in Figure 8.

At this point in the process, the unshelled vials were split into eight vials. Half of the quantum dot solutions from each vial were set aside to remain unshelled for testing. The corresponding half of the extractions were then subjected to a shelling procedure. The unshelled quantum dots were mixed with a zinc sulfide solution to encapsulate the cadmium-selenium quantum dots in a zinc-sulfur shell. This was done twice, to two separate batches of QDs, creating a total of 16 samples for analysis. There were eight unshelled samples and eight shelled samples.
3.2 Testing Samples

The area of interest in this testing was the fluorescence characteristics of the quantum dots. The fluorescence characteristics measured were the intensity, the FWHM, and the central wavelength produced. The fluorescence characteristics of these 16 samples were analyzed in two main ways. Fluorescence characteristics of corresponding pairs of unshelled and shelled quantum dots were compared (e.g. a 30-second-extraction of unshelled QDs from Batch A compared to their 30-second-extraction shelled QDs from Batch A counterparts). Along with the direct comparison of individual pairs, the unshelled and shelled quantum dots were compared as two groups to analyze how their fluorescence values changed over the course of four days. The fluorescence values of these samples were measured utilizing the testing setup shown in Figure 9.

Figure 9: Incident ultraviolet light (400-100nm) made QDs fluoresce. This fluorescing light was picked up by a fiber optic at 90° to the light, and led to a spectrometer. The spectrometer sent fluorescent data to a computer to be analyzed.
The quantum dots were loaded into cuvettes and placed in a holding chamber. In this chamber, they were exposed to an ultraviolet beam to make them fluoresce. This fluorescence was then conveyed down a fiber optic cable placed at 90° the incident light, to an Ocean Optics spectrometer. This spectrometer relayed fluorescence values to a nearby computer, where the output was measured utilizing Spectra Suite software. In order to reduce experimental error, ten fluorescence measurements were taken per sample per day. This allowed for analysis of the quantum dots to be compared to a typical range of a day’s measurements. This would later help indicate if changes in fluorescence values were statistically significant against the variances of a single test. Fluorescence data was taken on Day 0 (the day the QDs were shelled), Day 1 (the day after the QDs were shelled), and Day 3 (three days after the QDs were shelled).

The resultant data was then stored in a Microsoft Excel workbook. This raw data was then processed into more useful and more easily analyzed daily-averages. Along with the averages, standard deviations were generated so that the measurements between days could be observed to be similar to a single day’s measurements or not.

The unshelled to shelled assessment compared the differences in the averages of individual unshelled samples and their shelled counterpart sample. These differences could then be averaged themselves, revealing the average shift of fluorescence characteristics when QDs transitioned from unshelled to shelled state.

A simultaneous study also monitored the unshelled QD fluorescence
characteristics over the four days. Over the same timeframe, the shelled QD fluorescence values were monitored. This data set allowed for the stability of unshelled QD fluorescence to be compared to the stability of shelled QDs.

4. Results

4.0 Unshelled to Shelled State

In going from their unshelled to the shelled state, QDs experienced an average increase in intensity of 3407 counts. The population standard deviation of the unshelled and shelled intensity was only 71 counts. The observed increase is far outside the range of a day’s error. Further calculations revealed this shift in intensity was a 278% increase from their unshelled state.

The central wavelength also experienced shifts. Changes in central wavelength were not as simple as changes in intensity. While the central wavelengths of 30 second, 60 second, and 90 second samples increased an average of 12.3nm, the 0 second samples experienced negative shifts in wavelength of -81nm and -109nm. All of these shifts were statistically significant, as the population standard deviation was roughly 1.8nm. By excluding the more variable 0second measurements, the population standard deviation drops even further to 1.2nm.

There was also a shift in QD full width at FWHM going from the unshelled to a shelled state. Once again excluding outliers of the 0second extractions, the FWHM had an average increase of 2.66. The population standard deviation for FWHM was only 0.52. This means that the average increase in FWHM,
excluding the 0 second outliers, was 6.6%. The 0 second FWHM were excluded because of the difficulty of using the software to get an accurate FWHM. As seen in Figure 10, the FWHM spans multiple peaks. The multiple peaks are most prominent in the 0 second values. While multiple peaks were seen in other extraction times, there was always one especially prominent peak which overwhelmed the others in measurement. This allowed for the other FWHMs calculation to provide a better representation of the actual FWHM of the dominant peak.

Figure 10: The arrows point to the full width at half maximum peak height. Due to the secondary peak, the distance is not representative of the peak and creates errors. The observed waveform is from an unshelled 0 second extraction.
4.1 Change Over 4 Days

There was relatively little change in either the unshelled or the shelled QDs over the observational period of four days. The unshelled QDs experienced no statistically significant change in any of the fluorescent characteristics observed (intensity, central wavelength, FWHM). That is to say that the average fluorescence values all fell within one population standard deviation. Similarly the average central wavelength and FWHM of shelled quantum dots showed no statistically significant changes.

The only statistically significant change over the four days was the intensity of the shelled quantum dots. The intensity of the shelled quantum dots increased an average of another 4088 counts. The population standard deviation of these measurements was just slightly shy of 100 counts. This translates to an additional 71% increase in intensity compared to the Day 0 shelled values, or a 531% increase compared to the original unshelled sample counterparts.

5. Analysis

5.0 Unshelled to Shelled State

All three fluorescence characteristics observed experienced changes going from the unshelled to shelled state. As literature predicted, the intensity of the fluorescence increased. This is likely due to the charge separation created with shelling. This helped validate that the shelling procedure was effective. The general small increase in central wavelength can be attributed to the subtle
changes in electron excitation pathways as well as the increase in QD size. The second samples likely reacted differently than the other QDs because the nucleation and growth of the QDs was in a significantly earlier stage compared to the other QD samples. The final altered fluorescence property of FWHM did experience an undesirable increase. Because the increase was small, and because the intensity increased so drastically, a shelled QD is more desirable after the initial shelling.

5.1 Change Over 4 Days: Shelled

It is likely that the intensity continued to increase on average over the course of the four days as reactants in the solution continued to slowly bond to the QDs. Along with this, the shelled QD structure may have reached a more stable state. In the initial shelling process, bonds may have been imperfect and in partially-stable bonds. These bonds had time to move to more stable states where there was less stress on the lattice, making the QDs more uniform, and improving the shell by bonding more directly with the cores. The central wavelength and FWHM remained statistically the same as the reactions were small enough as to not alter the size of the quantum dots to where these properties would fluctuate.

5.2 Change Over 4 Days: Unshelled Cores

Unlike the shelled QDs, the unshelled QDs may have experienced only few more unshelled QDs forming and even some QDs decomposing. Unlike the QD cores
solution, the shelling precursor already had nucleation points in the forms of QD cores, imperfectly-shelled QDs, and shelled QDs. To increase intensity for unshelled QDs, more QD cores would need to form without interfering with existing QDs’ absorption and emission of photons. Nucleation requires high energy so that the Cd and Se ions can begin to bond. This was not present when the QDs were at room temperature between tests.

5.3 Extractions at 0 Seconds

The 0 second extractions often acted differently than the other extraction times. While there were only four samples of 0 second extractions in total, their fluorescence characteristics were often so drastically different than the other QDs that it warranted notice. More time is needed for QD core nucleation and growth. This will help a uniform size of QD core to form. The 0 second QD cores often experienced multiple similar-size peaks. This means that there were multiple sized of QDs present. There was too much variability in fluorescence compared to the other samples. The shelling procedure often amplified the undesirably unique nature of the 0 second extraction fluorescence. The 0second extraction proved to be too unpredictable and unreliable to produce consistent fluorescence trends.
6. Conclusion

6.0 Overview

This experiment provided a multitude of useful information. The 0 second extraction will be omitted from future synthesis due to its unreliable and unpredictable nature. While there was a slight undesirable increase in FWHM when shelling QDs, it is relatively small and would not drastically affect biomarker quality. The shelled QDs will be used in further experimentation because of the significantly higher intensity than the unshelled QD cores. The increased intensity would be easier to detect either by visual observation or by a scanning device.

No particular time interval was observed to output a specific wavelength by any statistically valid means. There was not enough replicates to detect, with any significant amount of certainty, the differences in extraction times. There were only two samples per extraction time of unshelled and shelled. Any observed differences could potentially be within the scale of measurement error.

6.1 Recommendations

If this experiment were to be repeated, it could be improved. An increase in replicates past two batches would simultaneously improve accuracy of results as well as allow for testing of individual time intervals. It would also be useful to test a wider range of extraction times, as it appeared that QD cores did change in fluorescing central wavelength as the extraction times reached greater
differences when assessed visually. This is further backed in literature. Along with this, the potentially larger QDs could react differently to the shelling process. A wider range of time may reveal further changes to both the unshelled and shelled QDs.

7. Future Work

7.0 Testing of Flow-Cell QDs Shelling Over 7 Days:

Recently a flow cell model for QD core synthesis has been produced on Cal Poly's campus. The same shelling process as used in this project will be performed on the QD cores produced by the new method. The same observations will be performed over the longer time period of a week. If fluorescence characteristics are the same or better, flow-cell QD cores may be used in further experiments to be used as biomarkers. The flow-cell QDs can be produced faster, and possibly with greater control when compared to the present organometallic colloidal growth method.

7.1 Encapsulation with Phospholipids

Once the appropriate shelled QDs are selected, they will be encapsulated with phospholipids. Phospholipids make QDs water soluble. This will allow for the QDs to attach to cells. It is a specific goal to get the QDs to adhere only to the outside of keratinocyte cells. Previous experiments have had QDs which were
drawn into the interior of the cells, which in turn killed the cells. If this can be achieved, then further work adhering unique proteins to the phospholipids will be done. These proteins will adhere specifically to cancer cells, allowing for detection of cancer specifically.

7.2 Broader Impacts

In the future, QD biomarkers may be used for faster, more accurate, and less subjective forms of cancer detection. There is also potential for using QDs for localized therapy. If QDs can reliably adhere to only cancer cells, drugs to treat the cancer can be attached to quantum dots. This will provide surgical precision to destroy cancer cells specifically, unlike present methods which can damage healthy cells and tissue. Cancer could be detected and cured with only a brief set of QD therapy without damaging the rest of the body.
8. References


es+by+general+pathologists%3A+diagnostic+discrepancy+rate+measured +by+blinded+review>.


