Synthesis of Various Sulfur and Selenium Complexes and an Investigation of a Multitude of Biological Applications

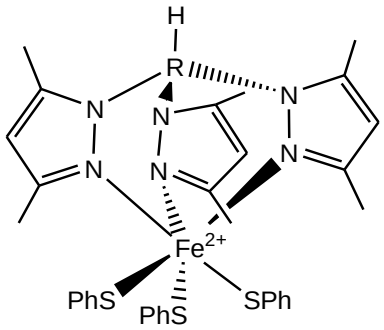
Joseph A. Giesen[*] and Deon T. Miles[#]
Department of Chemistry, Sewanee: The University of the South
735 University Avenue, Sewanee, TN 37383
[*]gieseja0@sewanee.edu, [#]dmiles@sewanee.edu

Christopher Underwood, Martin Kimani, and Dr. Julia Brumaghim
Department of Chemistry, Clemson University
401 Hunter Laboratories, Clemson, SC 29634

Dr. J. Shibata (Chemistry Dept. Chair) _____

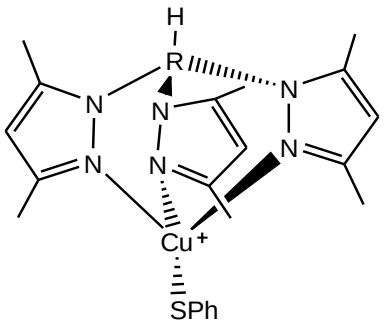
Dr. D. T. Miles (Honors Thesis Advisor) _____

Abstract: Sulfur and selenium have been used with various biological applications. One line of research utilizes the electron scavenging potential of early chalcogens. Sulfur-containing antioxidants have been shown to mitigate stress and damage cause by radical oxygenated species (ROS) through metal binding. To determine the effect sulfur has on chelated Fe(II) and Cu(I) species, trinitrogen ligands tris(3,5-dimethylpyrazolyl)borate (Tp*) and tris(3,5-dimethylpyrazolyl)methane (Tpm*), were chosen since Tp* and Tpm* are commonly used in biometallic complexes. Synthesis of novel iron complexes (**1**) and the copper analogs (**2**) (Figure 1) were attempted and these complexes have since been tested to determine their affect on the reduction potentials of iron and copper via cyclic voltammetry (CV).



1

R = B - Iron(II)tris(3,5-dimethylpyrazolyl) borate
R = C - Iron(II)tris(3,5-dimethylpyrazolyl) methane



2

R = B - Copper(I)tris(3,5-dimethylpyrazolyl) borate
R = C - Copper(I)tris(3,5-dimethylpyrazolyl) methane

Figure 1: Target complexes of iron(II) and copper(I) thiolates chelated by N,N,N-ligands

Secondly, cadmium selenide thiol-protected quantum dots are fluoresencent nanoparticles. Crown ethers (CE) were attached to the semiconducting core through a place-exchange reaction with the protecting thiol. The change in fluorescence was measured with addition of potassium ions (K^+). An expected shift in the emission wavelength was seen upon linking of the QDs by ester linkage and is expected for the sequestering of K^+ by CE groups attached to the QDs.

Table of Contents

A Special Thanks and Notes by the Author

I would like to thank all of the faculty members, both past and present, at Sewanee: the University of the South, for helping to shape my career goal and guiding me throughout my tenure there. I would also like to thank all of the individuals, professors, doctors, research assistants and graduate students, who have taught me more than they know or I could express.

I lastly want to thank my parents and family for always being there for me, and supporting me both morally and financially. My parents have always expected the most from me and have taught me to go for my dreams, never looking back; they have never let me hold myself back, even when they may have needed me the most.

A most sincere thanks to everyone who has impacted the past four years of my educational career.

Chapter 1

Synthesis of Iron(II) and Copper(I) Thiolates Containing Trinitrogen Chelating Ligands

Joseph A. Giesen*, Martin M. Kimani†,
Christopher C. Underwood†, Dr. Julia L. Brumaghim†
**Chemistry Department, Woods Laboratories,*
Sewanee: the University of the South, Sewanee, TN 37383
†Department of Chemistry, Hunter Laboratories,
Clemson University, Clemson, SC 29634

Introduction

The most abundant transition metals in the human body and other biological entities are iron and copper.[1] These two metals have an important role in the Fenton reaction (Equation 1). The Fenton reaction converts hydrogen peroxide into hydroxide and a hydroxyl radical through interaction with iron(II) and copper(I) ions. Hydroxyl is an example of a radial oxygen species (ROS), and hydrogen peroxide is a by-product from cellular respiration in normal environmental conditions.

$$Fe^{2+} / Cu^{+}_{(aq)} + H_2O_{2(l)} \rightarrow Fe^{3+} / Cu^{2+}_{(aq)} + OH^{-}_{(aq)} + {}^{\bullet}OH_{(aq)} \quad (1)$$

Studies have shown that hydrogen peroxide alone, even in high concentrations does not cause DNA damage,[2] meaning the Fenton reaction must yield the ROS required to cause DNA damage. Oxidation of DNA is linked to diseases, such as cancer, neurodegenerative and cardiovascular diseases.[3,4] Antioxidants prevent the formation of ROS, while specific sulfur containing compounds have been shown to inhibit oxidative damage to DNA by specifically attacking the ROS.[5] Cystine has been shown to prevent oxidation at biologically relevant concentrations of 10μM with a half maximal inhibitory concentration (IC_{50}) of 3.4 ± 1.1 μM, while other sulfur compounds provide oxidative

protection in higher concentrations.[2] Studies by Battin have shown the metal coordination is required in order to prevent oxidative damage.[2] This research is focused on the synthesis of iron(II) and copper(I) thiolates containing trinitrogen ligands. The nitrogen ligands selected were tris(3,5-dimethylpyrazolyl) borate (Tp*) and tris(3,5-dimethylpyrazolyl) methane (Tpm*). These were selected because they imitate the histidine molecule found in biological systems.[6]

Since iron and copper are transition metals, they readily convert from inert Fe(III) and copper(II) to the damaging iron(II) and copper(I), leading to the rapid creation of large amounts of $^{\bullet}OH$ for each atom of iron or copper. Studies have shown that even at biological concentrations, severe damage can be caused to DNA, due to oxidation of these metal species.[7]

Experimental

Chemicals

All chemicals were used as received; acetonitrile, methanol, were dried by distillation with potassium metal. All reactions were carried out under argon atmosphere with dry solvents using Schlenck techniques unless otherwise stated. NMR solvents were used as received dry in 0.75 mL ampules.

Instrumentation

^{1}H and ^{13}C NMR spectroscopy was carried out on Bruker 300 or 500 MHz NMR spectrometers.

Procedure

Tris(3,5-dimethylpyrazolyl)methane (Tpm*) and iron(II)bis(triflate) ($Fe(OTF)_2$ were synthesized as previously reported.[8,9] Copper tetrakis(acetonitrile) was synthesized as done previously.[10]

Synthesis of sodium phenylthiolate (NaSPh). This was synthesized as stated in the literature with modifications[11] using diphenyl disulfide (PhSSPh). Solid sodium hydride (NaH) (0.84g, 0.01 mol) in 57% dispersion in mineral oil was added to a solution of PhSSPh (2.65 g, 0.01 mol) in THF (80 mL) and refluxed overnight at 60°C to form a white precipitate. The clear solution was separated by cannula filtration, washed with hexane (2 x 20 mL), and dried under vacuum.

^{1}H NMR (DMSO-d_6): δ 7.0 (d, 2H, *o*-Ph), δ 6.6 (t, 2H, *m*-Ph), δ 6.4 (t, 1H, *p*-Ph)

Synthesis of tris(3,5–dimethyl-1-pyrazolyl)methane (Tpm).* This ligand was synthesized as done previously.[1] A mixture of tetra-n-butylammonium bromide (11.44 g, 0.035 mol) and 3,5-dimethylpyrazole (68.0 g, 0.707 mol) were dissolved in ~ 700 mL double distilled water (ddH_2O). This solution was stirred vigorously while sodium carbonate (586 g, 4.20 mol) was slowly added. The solution was cooled, chloroform (300 mL) was added, and the solution was refluxed for 70h. Solid precipitate was separated by Büchner funnel. Dark red solution was removed by separatory funnel from the translucent yellow layer, followed by washing by ddH_2O (3 x 5 mL) and dried with sodium sulfate; the dark solution was then concentrated by rotary evaporation. The resulting solid was sublimed using a water cold finger with heating at 80°C for 48h. Purification was achieved by multiple silica plug chromatography using 2.5% v/v

methanol in dichloromethane and concentrated by rotary evaporation to yield a white solid (95-99% pure from NMR).

^{1}H NMR ($CDCl_3$): δ 8.09 (s, 1H, CH), δ 5.90 (s, 3H, 4-H(pz)), δ 2.20, 2.03 (s, s, 9H, 9H, 3,5-Me)

Synthesis of iron(II)(tris(3,5–dimethylpyrazolyl) borate)tris(acetonitrile) triflate Fe(Tp)(NCCH$_3$)$_3$• SO$_3$CF$_3$.* Preparation of the Fe(Tp*)(NCCH$_3$)$_3$•SO$_3$CF$_3$ complex was followed as reported previously.[12] A solution of KTp* (1.68 g, 5.0 mmol) in acetonitrile (15 mL) was added by addition funnel dropwise over 4 h to a solution of Fe(OTf)$_2$•2CH$_3$CN (1.77 g, 5.0 mmol) in acetonitrile (15 mL) while stirring at room temperature. The resulting translucent pink solution was stirred for 1 h. Diethyl ether (20 mL) was added to the solution and stirred for 1 h to facilitate precipitation. The resulting white solid was separated from the pink solution by cannula filtration and the solid was dried under vacuum overnight.

Synthesis of iron(II)(tris(3,5–dimethylpyrazolyl) methane)tris(acetonitrile) triflate (Fe(Tpm)(NCCH$_3$)$_3$•SO$_3$CF$_3$).* Preparation of Tpm was done in a similar fashion as the synthesis of Fe(Tp*)(NCCH$_3$)$_3$• SO$_3$CF$_3$ using tris(3,5-dimethylpyrazolyl)methane (Tpm) in place of tris(3,5-dimethylpyrazolyl)borate (Tp*).

Attempted synthesis of Fe(Tp)(NCCH$_3$)$_2$(SPh) 1:1 equivalence.* A solution of NaSPh (0.064 g, 0.483 mmol) in methanol (20 mL) was added dropwise by addition funnel to a solution of Fe(Tp*)(NCCH$_3$)$_3$ in dry methanol (15 mL) while stirring. The resulting purple solution rapidly changed to clear solution. The solution was dried under vacuum and diethyl ether (20 mL) was added to precipitate a product, but no Tp*Fe signals were observed by NMR spectroscopy.

Attempted synthesis of $[Fe(SPh)_4][PPh_4]_2$ 1:4 equivalence. Iron(II)tetrakis-(phenylthiolate)bis(tetrapheylphosphonium) was synthesized as done previously with modification.[13] A colorless solution of $FeCl_2 \cdot 4H_2O$ (0.200 g, 1.01 mmol) in acetonitrile (10 mL) was added dropwise to a solution colorless of NaSPh (0.532 g, 0.402 mmol) in acetonitrile (10 mL) while stirring at 50°C for 45 min. The resulting solution turned violet. A solution of PPh_4Cl (0.376 g, 0.106 mmol) in acetonitrile (7 mL) was added by addition funnel while stirring for 15 min. The resulting solution was separated by cannula filtration from the salt and placed in a freezer (-34°C) overnight to produce purple crystals. Crystals (0.171 g, 37%) was separated by cannula filtration and placed under vacuum.

Attempted synthesis of $Fe(Tp^*)(SPh)_x$. A solution of KTp* (0.054 g, 0.16 mmol) in acetonitrile (10 mL) was added by addition funnel dropwise to a solution of $[Fe(SPh)_4][PPh_4]_2$, and the resulting purple solution was stirred for 30 min. The solution was then separated from a light brown solid and placed under vacuum. The solution was then placed in a freezer (-26°C) overnight, but no crystals were produced. To facilitate precipitate, the solution was then placed in an acetonitrile bath with dry ice and thawed in a freezer (-26°C), but no crystals were produced. The solution was then titrated with diethyl ether (15 mL) and stirred for 30 min. The solution was separated from a small amount of solid, and the white solid was placed under vacuum.

Attempted synthesis of Cu(Tp)(SPh).* Under argon atmosphere, a solution of $Cu(NCCH_3)_4$ (0.300 g, 0.955 mmol) in acetonitrile (40 mL) was combined with a suspension of KTp* (0.321 g, 0.955 mmol) in acetonitrile (20 mL) and stirred overnight. A solution of NaSPh (0.130 g 0.955 mmol) in acetonitrile (15 mL) was then added and

the resulting yellow solution was stirred for 4 h. The solvent was removed in vacuo, to produce a yellow solid. Toluene (25 mL) was then added and the resulting light yellow solution was stirred for 4 h. The translucent light blue solution was then separated from the solid, and both were placed under vacuum. White and blue crystals formed from the toluene solution. This resulting blue color is indicative of Cu(II).

Synthesis of Cu(Tpm)(NCCH$_3$). In an argon atmosphere glove box, acetonitrile (50 mL) was added to a mixture of Cu(NCCH$_3$)$_4$ (0.300 g, 0.955 mmol) and Tpm* (0.298 g, 0.955 mmol). This solution was removed and stirred overnight on a Schlenck line. The resulting clear solution was placed under vacuum which yielded a white solid on which NMR was carried out.

^{1}H NMR (acetonitrile-d$_3$): δ 7.74 (s, 1H, CH), δ 6.06 (s, 3H, 4-H(pz)), δ 2.51, 2.30 (s, s, 9H, 9H, 3,5-Me), δ 2.17 (s, 3H, NCCH$_3$)

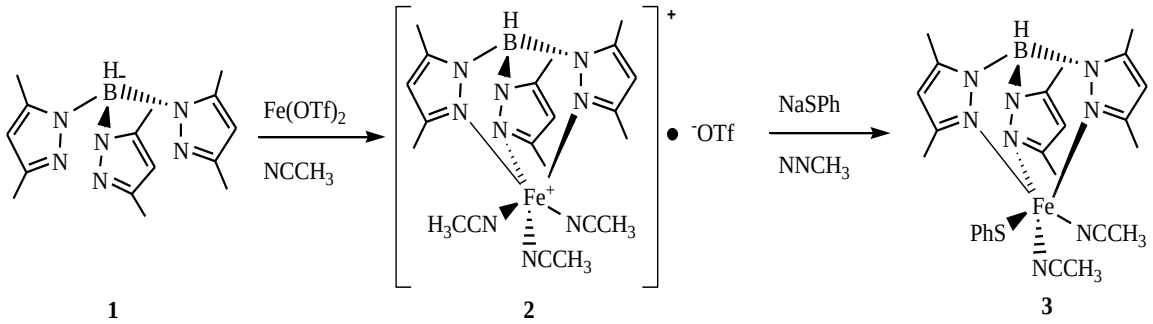
Synthesis of Cu(Tpm)(SPh). The starting material Cu(Tpm*)(NCCH$_3$) was synthesized as reported above. A solution of NaSPh (0.130g, 0.955mmol) in acetonitrile (20 mL) was added by cannula to a solution of Cu(Tpm*)(NCCH$_3$) (0.384g **,** 0.955mmol) in acetonitrile (50mL) and stirred overnight. The resulting translucent solution was placed under vacuum and dried for 24h. Toluene (25mL) was then added to the resulting yellow solid and stirred for 4h, followed by separation from the solid by cannula filtration. The toluene solution was then placed under vacuum to generate a white solid and NMR was taken.

^{1}H NMR (dichloromethane-d$_6$): δ7.55 (d, 2H, o-Ph), δ 7.36 (t, 2H, m-Ph), δ 7.28 (t, 1H, p-Ph), δ 5.79 (s, 3H, 4-H(pz)), δ 2.46, 1.70 (s, s, 9H, 9H, 3,5-Me(pz))

Results and Discussion

To test this hypothesis, iron(II) and copper(I) thiolates containing trinitrogen chelating ligands were synthesized and characterized. The nitrogen ligands selected were tris(3,5-dimethylpyrazolyl) borate (Tp*) (**1**) and tris(3,5-dimethylpyrazolyl) methane (Tpm*) (**4**) because they imitate adenine and guanine bases of DNA, known sites of metal localization.[14]

The iron(II)tris(acetonitrile)tris(3,5-dimethylpyrazolyl)borate, $Tp^*Fe(NCCH_3)_3$ (**2**) (Scheme 1) was synthesized using a procedure similar to that described for the synthesis of Tpm* as observed previously.[15]



Scheme 1: Coordination of Tp* with iron(II) and addition of thiol

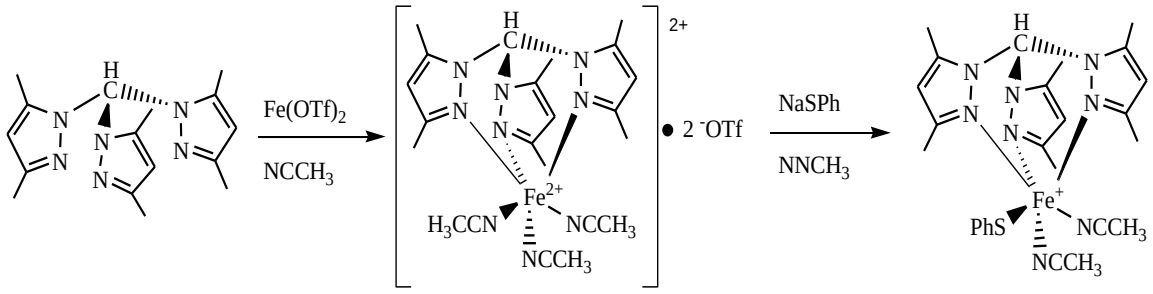
Iron(II) triflate in acetonitrile was treated with a solution of (**1**) in acetone, yielding a white solid (**2**) that was precipitated from solution by the addition of diethyl ether, filtered, dried, and characterized by NMR spectroscopy. ^1H NMR spectroscopy displayed peaks outside of 0-10 ppm at δ 57, δ 40, and δ -13, while ^{19}F NMR showed the presence of non-coordinating (OTf) at δ -79.1.

In order to synthesize the desired iron thiolate complex, a solution of (**2**) in methanol was then treated with sodium phenyl thiolate (NaSPh) in methanol. This reaction did not provide the desired iron(II)bis(acetonitrile)tris(3,5-

dimethylpyrazolyl)borophenylthiolate (**3**). The reaction mixture turned dark purple but changed to a clear yellow as the reaction progressed. The resulting solid was precipitated by titration with diethyl ether and washed with hexane. ^{1}H NMR spectroscopy displayed no chemical shifts and absence of peaks indicating a phenyl group. The solution color shifts and lack of NMR peaks expected for addition of phenyl to iron allowed for a side reaction yielding diphenyl disulfide (PhSSPh), removed by hexane wash, and produced by the reaction of methanol and sodium phenylthiolate.

$$2NaSPh_{(aq)} + 2H^{+}_{(aq)} \rightarrow PhSSPh_{(s)} + H_{2(g)} \quad (2)$$

In the future synthesis of this complex will be attempted in THF to prevent possible proton donation from the methanol to the phenyl thiolate as an initiator to the formation of PhSSPh. Iron(II)bis(acetonitrile)tris(3,5-dimethylpyrazolyl)borophenylthiolate, Tpm*Fe(SPh)$(NCCH_3)_2$, (**4**) was synthesized as done previously (Scheme 2).[4] ^{1}H NMR spectroscopy displayed peaks corresponding with literature outside of 0-10 ppm at δ 52, δ 42, δ 20, and δ 11, similar to (**2**), but lacking the BH peak in the negative region. ^{19}F NMR also displayed a non-coordinating peak for OTf at δ -79.

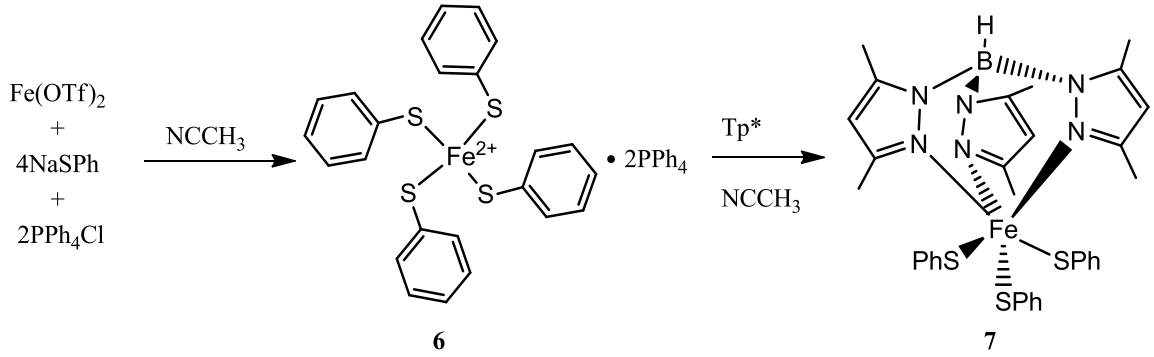


4 **5**

Scheme 2: Coordination of Tpm with iron(II) and addition of thiol

The difficulty of synthesizing complexes (**3**) and (**5**) was later proven to be due to the bis addition (or two ligand addition) of Tp* (**1**) or Tpm (**4**).[16] This effectively utilizes the six

coordination/bonds to the iron core preventing further experimentation on this species. During the synthesis of the ligand iron complex, a pink hue could be observed due to more rapid addition (more than a drop per minute). It was believed that this pink color was due to the bis coordination of the trinitrogenated ligand. However, upon further analysis, the white precipitate that formed was shown to be the bis production consisting of $Fe(Tpm)_2$ or $Fe(Tp^*)_2$. The resulting pink or white color was due to the transition of the iron core from iron(II) to iron(III), with iron(III)$(Lig)_2$ yielding the observed pink color. Due to the iron(II) target thiolate complexes forming the bis-complex, the synthesis was then attempted in reverse synthetic order (Scheme 3), by first synthesizing iron(II)tetrakis(phenylthiolate) (**6**), according to a modified procedure.[17]



6 **7**

Scheme 3: Synthesis of $Fe(Tp^*)(SPh)_x$ via reverse synthesis

This was done in hopes that by saturating the system with thiol ligands, we could prevent the formation of the bis complex, which would require the loss of all of the bound phenyl thiolate.

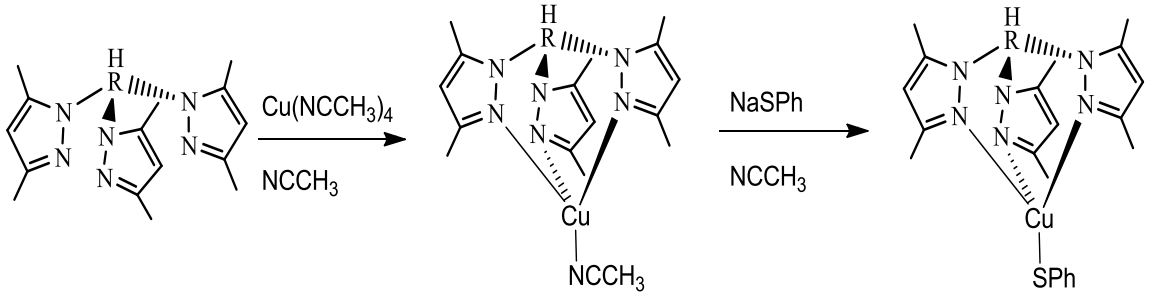
A solution of hydrated iron(II) chloride in a solution of acetonitrile was reacted with a solution of NaSPh in acetonitrile. The resulting solution was violet in color. A solution of tetraphenylphosphonium chloride in acetonitrile was then added as a counter ion. ^{1}H NMR spectroscopy peaks at δ 22.5, δ 21.5, δ 11, δ 6.7-8, and δ -24 that are slight

chemical shifts from the iron(II)tetrakis(phenylselenalate), but the equivalents found from the integrations displayed excess PPh_4. The resulting product, (**6**), was isolated by cooling at -20°C overnight to yield purple crystals. A solution of (**1**) in acetonitrile was added to a solution of (**6**) in acetonitrile and stirred for 30 min. Ether was added to the resulting to precipitate a small amount of white solid, [Tp*Fe$(SPh)_2$][PPh_4] (**7**). This reaction shows promise, with NMR chemical shifts of the trinitrogen ligand present. However, due to the impurity of the starting material, the product could not be completely and accurately characterized. Manipulation of the procedure to produce a greater yield and additional purification of starting material are required.

The copper(I) target complexes were synthesized by addition of the trinitrogen ligand followed by the thiolate (Scheme 4). [Tp*Cu(SPh)][Na] (**9**) was synthesized under argon in a glove box by the addition of a solution of Tp* to a solution a [Cu$(NCCH_3)_4$][BF_4] in acetonitrile, forming copper(I)(acetonitrile)-tris(3,5-dimethyl-pyrazolyl)borate (**8**) a light translucent blue solution. A solution of NaSPh in acetonitrile was added to the solution of Cu(Tp*)($NCCH_3$) in acetonitrile stirred overnight. The solutions were then placed under vacuum to form a yellow solid and then washed with toluene. ^{1}H NMR spectroscopy display the expected equivalents and the peaks that could be defined at δ7.55 (d, 2H, *o*-Ph), δ 7.36 (t, 2H, *m*-Ph), δ 7.28 (t, 1H, *p*-Ph), δ 5.79 (s, 3H, 4-H(pz)), δ 2.46, 1.70 (s, s, 9H, 9H, 3,5-Me(pz)).

Copper(I)(Tpm*)(SPh) was synthesized using the same procedure as that of the Cu(Tp*)($NCCH_3$), followed by the addition of NaSPh. Cu(Tpm*)($NCCH_3$) was characterized by ^{1}H NMR spectroscopy with peaks of δ 7.74 (s, 1H, CH), δ 6.06 (s, 3H,

4-H(pz)), δ 2.51, 2.30 (s, s, 9H, 9H, 3,5-Me), δ 2.17 (s, 3H, $NCCH_3$). Cu(Tpm*)(SPh) was attempted to be characterized following synthesis via the procedure for thiolate



8 **9**

R = C = Cu(Tpm*)(SPh) R = B = Na•Cu(Tp*)(SPh)

Scheme 4: Synthesis of trinitrogen chelated copper thiol complex

addition to Cu(Tp*)($NCCH_3$). The ^{1}H NMR however, yields very broad peaks that could not be characterized, but were near the expected peaks, which could indicate intraconversion between two products. A bulkier ligand was attempted for a Se analog to the Cu(Tpm*)(SPh) and will be attempted here.

The syntheses of both of the copper complexes were only attempted a few times. The Schlenk techniques appear to have been fruitful for the formation of the Tpm complexes and NMR data was gathered for the thiol complex. However, due to lack of chance to obtain the Tp* Cu complex a blue color resulted during the synthesis at various steps, with the last occurring as stated in the Experimental Section. This is no doubt caused by the introduction of air or water during the step prior to the appearance of the blue color, as the blue color is due to the oxidation of copper from Cu(I) to Cu(II), which is not useful as a reductant in biological systems. Further synthesis with these solutions were not attempted as the resulting product would not yield useful cyclic voltammetry (CV) data.

Synthesis of the iron complexes was attempted via Scheme 1 and 2, attempting to yield compounds (**2**) and (**5**) with purity. However, the addition of the thiolate ligand was proven to be unsuccessful. The initial purple color indicates that the initial target complexes may have formed, but due to the reactivity of the thiolate and the labiality with iron, the thiolate instead formed the yellow PhSSPh. Synthesis of the iron thiolate complex (**5**) via Scheme 3 resulted in impure product, due to the formation of the bis-chelated iron. This bis-chelation formed six bonds to the iron core, decreasing the effectiveness of further reaction as a result of the three bonds securing the ligand to the metal core. However, this does not explain the formation of the purple crystals. The product crystals were prepared for analysis using X-ray diffraction (results are still pending). Copper complexes are believe to have been achieved, however, further characterization by NMR spectroscopy and purification of the desired complexes is required, before CV can be performed to quantify the effect of sulfur as an antioxidant.

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Chapter 2:

Effect of Crown Ether Addition to Thiol-protected Cadmium Selenide Quantum Dots and the Effect of Dilution on the Fluorescent Intensity

Joseph A. Giesen[*] and Deon T. Miles[#]
Department of Chemistry, Sewanee: The University of the South
735 University Avenue, Sewanee, TN 37383
[*]*gieseja0@sewanee.edu,* [#]*dmiles@sewanee.edu*

Introduction

Nanomaterials are of ever growing interest as optoelectronic, photovoltaic devices and potential in-vitro fluorescent biolabels.[18,19,20] Quantum dot (QDs) are nanoparticles, with a diameter of 1-10 nm, thus confined to the Bohr radii of an exciton (electron-hole pair) producing their unique fluorescent nature. The fluorescent wavelength maximum (λ_{max}) of the QDs is a product of the band gap width of the QDs and this distance becomes smaller for larger particles. Thus the emission λ_{max} is a function of particle size, where large particles emit light at longer wavelengths and smaller particles emit light at shorter wavelengths.

Various thiols can be used to increase the stability and solubility of the CdSe core and preventing Ostwald ripening. Ostwald ripening is a process in which a smaller particle loses outer shell molecules to a larger particle in order to form a more stable system with an overall lower energy state. This decrease in energy of the system results from better packing arrangements in larger molecules because of the smaller surface-to-volume ratio.[21]

Thiolated crown ethers (CE) can be added to the protective monolayer through a place- exchange reaction (Figure 1).

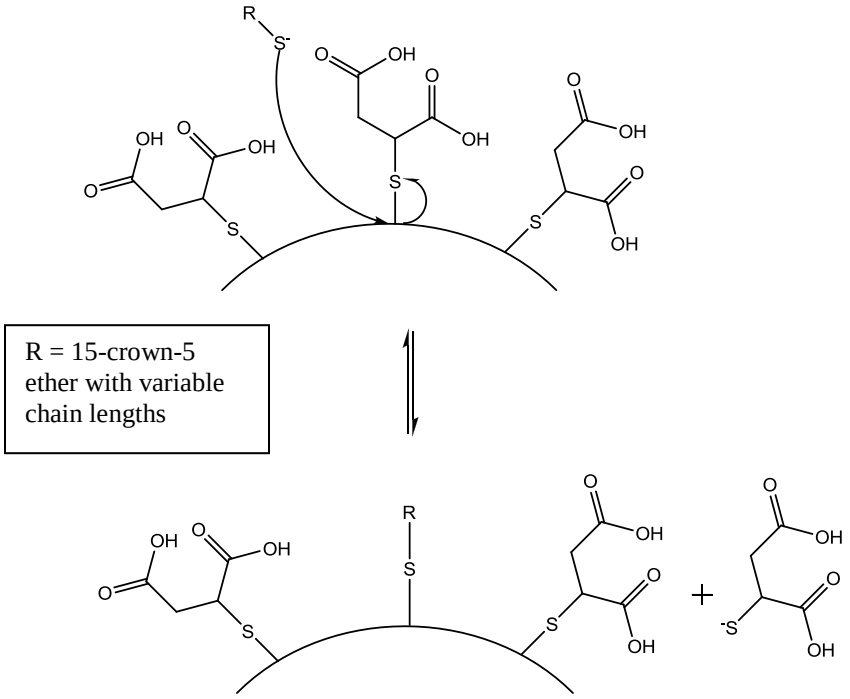


Figure 1: Place exchange reaction of R constituent CE for MSA

A place-exchange reaction substitutes one of the existing thiols with a thiolated CE of some variable chain length. Using this place exchange, two or more QDs can be linked through complexating/sandwiching with an addition of an alkaline ion. In this investigation, 15-crown-5 ethers were used and linked using K^+ (Figure 2).

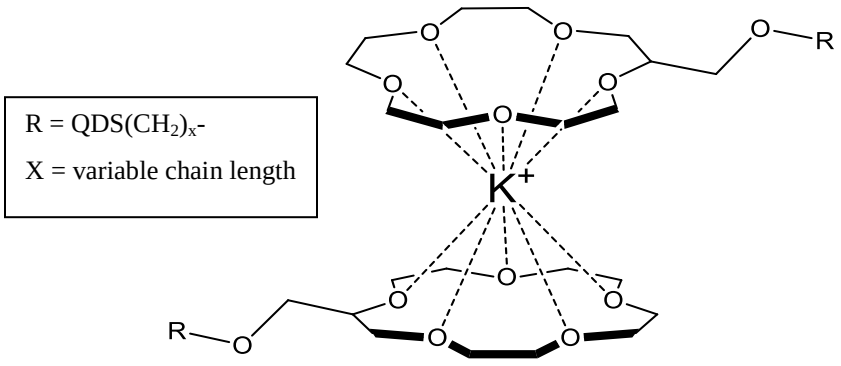


Figure 2: Complexating linking of two QDs using potassium ion

Potassium ions are used in conjunction with the 15-crown-5 ether due to the size of the ether ring and the ion allow for complexing. Similarly, the 15-crown-5 ether is insensitive to other ions, reducing potential interference and increasing the ease of the reaction.[22] This complexation links two QDs and in this confined arrangement the QDs may be able to communicate on the electronic level. This interaction can yield a theoretically larger QD size, thus producing a red shift in the fluorescence λ_{max}. This project concentrates on this phenomenon and the synthetic steps necessary to reach those goals.

The quantum yield of a fluorescent particle compares that efficiency of the particle by measuring the quantity of photons absorbed and the subsequent photons emitted as the particle transition between the excited and ground states. The number of photons it absorbs is usually greater then those fluoresced out, however this value has been found to be as large as 80% for some of the QDs.

Experimental

Chemicals

All reagents were used as received unless otherwise stated. All synthesis of QDs was done under inert atmosphere, while subsequent reactions were carried out under ambient conditions. Dilute 15-crown-5 ether (5.0 μL) obtained from the Kittredge Lab at Siena College with Type 1 water in a 100-mL volumetric flask, in order to form a dilute CE solution. Mass and dissolve potassium chloride (74 mg, 0.05 mmol) in 100-mL volumetric flask with Type 1 water to produce a $1x10^{-4}$ M solution K^+.

Instrumentation

Type I water was obtained from a Millipore Direct-Q® 3 UV with pump. Fluorescence spectra were obtained using a quartz cuvette on a Jobin Yvon Fluoro-Max P® spectrofluorimeter with the following parameters: scan range of 400 to 660 nm and excitation wavelength of 350 nm.

Procedure

Synthesis of CdSe QDs. Monolayer-protected CdSe QDs were prepared as done previously with modification.[23] Mass and place cadmium perchlorate hydrate (0.73 g, 2.35 mmol) and a water-soluble thiol (5.7 mmol) in a 250-mL 3-neck 14/20 round bottom flask. Dissolve mixture in ~100 mL Type I water and adjust the pH with NaOH (1 M) to a pH greater than 11. Transfer the solution to a 250-mL three-neck round bottom flask and deaerate for 30 min with N_2 gas and atmosphere (Figure 3). Mass and place Al_2Se_3 lumps (0.20 g, 0.46 mmol) in a 100-mL three-neck round bottom flask and deaerate for 5-10 min. Setup apparatus as shown in Figure 3 and add H_2SO_4 (0.5 M, ~18 mL) to the Al_2Se_3 to produce H_2Se gas, which is bubbled through the Cd-thiol solution (Figure 3). Allow solution to react for 5-20 min., depending on thiol, until the solution obtains a slight yellow tint. Disconnect apparatus and store solution under nitrogen in a refrigerator (~2 ˚C) to prevent further Ostwald ripening.

Determination of effect of Ostwald ripening. Equip CdSe-thiol solution synthesized in above procedure with a reflux condenser. Reflux the solution with stirring, remove aliquot ever 10 min or at color change. Obtain fluorescent spectrum and record λ_{max} and full width at half maximum (fwhm) and the corresponding intensities.

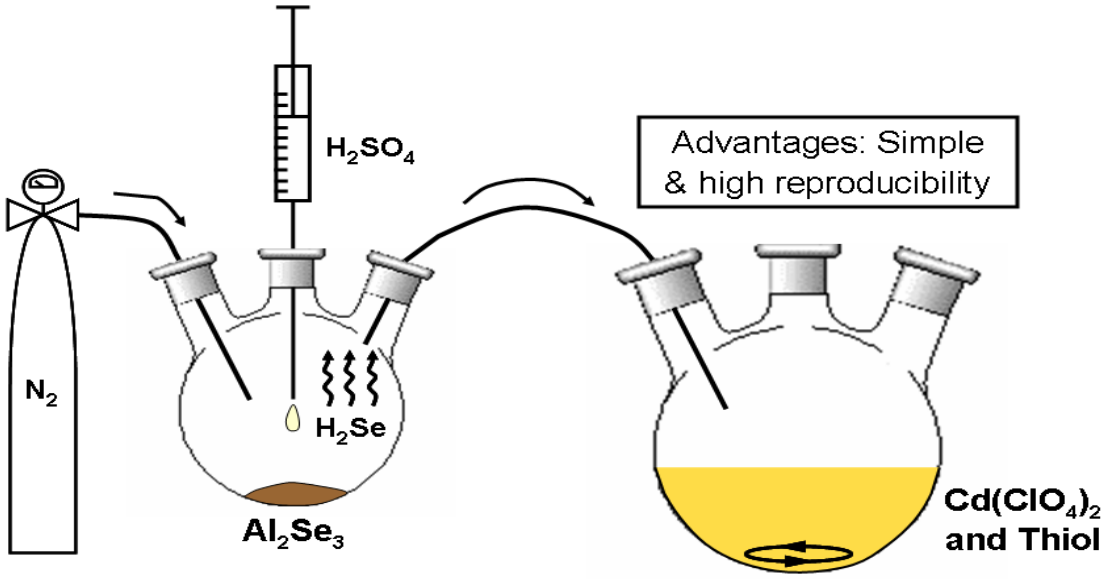


Figure 3: Setup of apparatus for synthesis of CdSe QDs

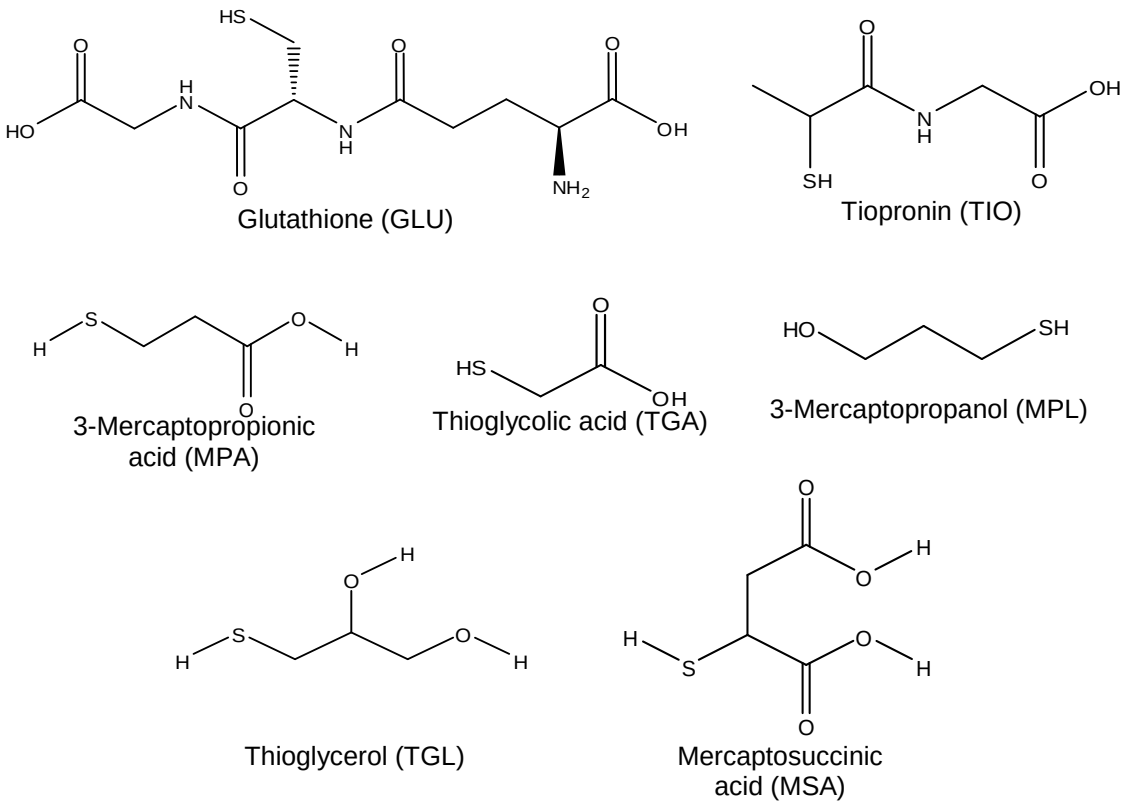


Figure 4: Water-soluble thiols

In situ extraction of Na^+ with EDTA. Add sodium ethylenediaminetetraacetate (2.6 g) in a 1:2 molar ration EDTA:Na^+ to the reaction solution following reaction with H_2Se. Stir the resulting solution for 30 min and filter out solid using a glass fritted funnel. Note: *The glass frit turned bright yellow in color with orange solid covering it. Multiple washes with Type 1 H_2O were needed to remove and suspend QDs trapped in frit. Solution became very dilute and produced reduced fluorescence.*

Post-reaction extraction of Na^+ with EDTA. Rotovap an aliquot (10 mL) of the QD solution to dryness and add EDTA (0.026 g) and dissolve solution in minimal amount of ethanol. Sonicate the solution as necessary to suspend it into solution. Decant off the liquid portion and obtain fluorescence. Note: *Minimal fluorescence, decanting proves to be very difficult and reduction in appreciable fluorescence within a few days.*

Alternate post-reaction extraction of Na^+ with EDTA. Mass and add EDTA (0.023g) to an aliquot of QD solution (~10 mL) and stir. Place resulting solution in dialysis tubing (MWCO 3500) and dialyze in RO H_2O for 72 h. without exposure to light. Note: *Dialysis in pure RO water precipitated QDs, yielding a colorless transparent solution with pink precipitate.*

Synthesis of QDs with NH_4OH as base. Carry out similar synthesis as reported above, substituting concentrated NH_4OH (20-40 mL) as base. Perform Ostwald ripening process as stated above and determine fluorescence changes. Note: *No further need for removal of Na^+ because 15-crown-5 ether insensitive to NH_4^+.*

Proof of concept for $\Delta\lambda_{max}$ by esterification of MSA and TIO QDs. TIO-CdSe QDs and MSA CdSe QDs were synthesized previously at stated above. Pipet MSA-CdSe QDs (10 mL) into a prepared cellulose dialysis membrane (3,500 MWCO) and pipet TIO-

CdSe QDs (3.5 mL) into a separate dialysis membrane (3,500 MWCO). Add Type I H_2O (~800 mL) to two 2 1-L beakers and add NH_4OH to achieve a basicity of >11.0 to create two basic dialysis chambers. Place one dialysis chambers containing the QDs into each of the dialysis chambers and let stir overnight in refrigerator (~4 °C). Remove the dialysis chambers and pipet all of the QDs solution into two labeled vials. Volumetrically add the MSA and TIO QDs (2 mL each) into a 30-mL beaker. Mass and add 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (38 mg, 0.20 mmol), and N-hydrosulfosuccinimide (sulfo-NHS) (2 mg, 0.01 mmol) to a 20-mL beaker and dilute with Type I H_2O. Add the sulfo-NHS and EDC solution to the QD solution with stirring and let stir at room temperature for 48 h. The resulting solution was place into dialysis tubing (3,500 MWCO) and place in a third basic dialysis chamber, created similarly to those above and stirred for 24 h. Remove the dialysis chamber and place the resulting solution into a vial and obtain fluorescence and UV-visible data. Note: *This synthesis was also done using MPA and TGL QDs using the same procedure.*

Determination of place exchange by ferrocene carboxylic acid. Neat or reaction dilute MPA QDs were placed into 5 separate flasks and TGL was added accordingly (Table 1). Add ferrocene carboxylic acid (0.250 g), EDC (0.193 g) and sulfo-NHS 0.0102 g) to a 20-mL scintillation vial and dissolve with stirring in 100% ethanol (15.0 mL),

Table 1: Addition of stock TGL to MPA QDs

Solution	Vol. MPA QDs Added (mL)	Vol. TGL Added (µL)	Mass TGL Added (mg)
1	10.0	5.0	6.25
2	10.0	8.0	10.00
3	10.0	25.0	31.00
4	10.0	40.0	50.00
5	10.0	80.0	100.00

yielding a red transparent solution. Volumetrically pipet an aliquot of the ferrocene solution (3.0 mL) into each of the 5 solutions in Table 1 and place a stir bar in each solution. Place all of these solutions in the refrigerator (~4 ºC) and let stir overnight. Upon removing the solution place each into a separate dialysis cell (3,500 MWCO) and place each into an individual a pH neutral dialysis chamber (~450 mL each). The dialysis chamber was refreshed 3 times over a week yielding an orange dialysis bath each time and a yellow solid precipitated in the dialysis chambers. Remove the supernatant from each of the dialysis tubing chambers and analyze by UV-visible spectroscopy and electrochemistry.

Place exchange of 15-crown-5 ether. Volumetrically add QDs (2, 4, 6, 8 mL) to several 25 mL round bottom flasks. Add the dialyzed CE solution (8, 6, 4, 2 mL) volumetrically to form final volumes of 10 mL. Wrap each flask in aluminum foil to prevent degradation of CE by UV light. Note: *Large volumes of CE 6, 8 formed precipitate that could be placed back into solution by adding NH_4OH to achieve pH greater then 11.*

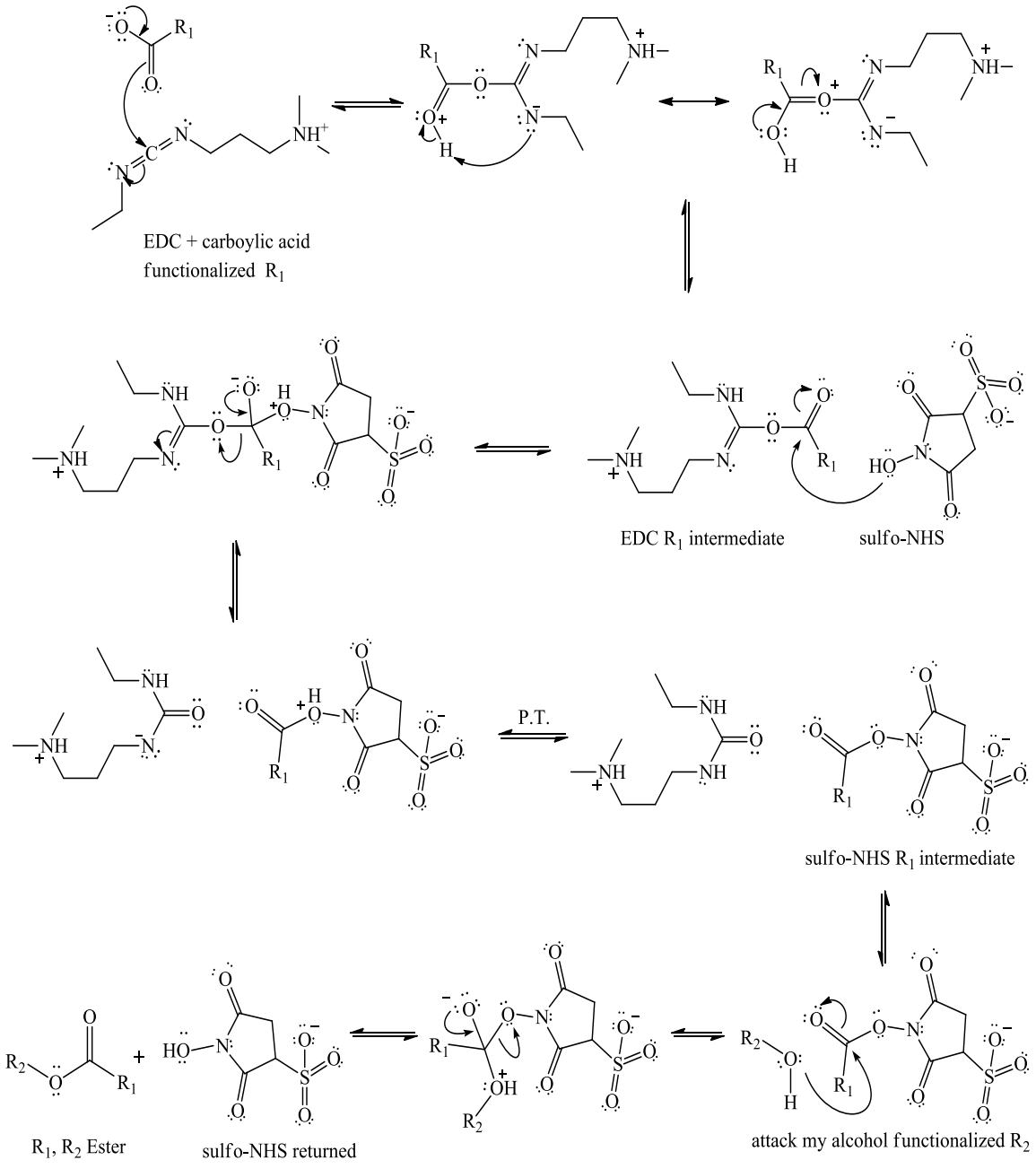
Determination of effect of addition of K^+. Add $1x10^{-4}$ M (0.1 mM) K^+ solution (0.100 mL) with stirring to the CE-QD solution, wait 1 min. Place aliquot in cuvette, determine fluorescence and record λ_{max} and fwhm. Repeat steps until total addition of K^+ is 2.0 mL. Note: *Some intensity changes shows promise and seems to be a related to mixing time.*

Results and Discussion

A primary proof of concept experiment was performed using TIO, which has an alcohol functional group and MSA which has a carboxylic acid functional group to determine if there is a shift in the fluorescent λ_{max}. This reaction was performed using two types of QDs, with similar λ_{max} (about 580 nm) and through reaction with EDC and sulfo-NHS, produces an ester linkage between the TIO- and MSA-CdSe QDs. This acid-catalyzed esterification yields a non-labile bond, or irreversible link between two QDs (Scheme 5).[24]

This was seen in a red shift from the yellow staring material to an orange-red slurry of QDs, that were non colloidal due most likely to the increased size and aggregation of multiple QDs. It does appear that there should be a shift, while only briefly, in the λ_{max} shifts from 580 nm to 594 nm (Figure 5). This reaction was attempted again with MPA and TGL, with a similar shift was observed briefly in the λ_{max} with a shift of about 5 nm, reverting back from 573 nm at time 30 min to the original 568 nm at time 1 hr. This shift and subsequent degeneration is most likely due to a hydrolysis of the ester linkage in the still basic solution. While, there is only a slight increase in the λ_{max} the reversion back to the original λ_{max}, this evidence does seem to support the conclusion that the red shift is due to the linkage of two QDs. The reversion of the λ_{max} back to the original wavelength suggest that this is due to a reversible reaction and not from a physical enlargement of the QD's core corresponding to Ostwald ripening effects.

This conclusion of the shift should be further tested through the use of various thiols of varying chain lengths. Varying the distance constraint placed on the two cores could lead to an increase in the electronic interaction between the two cores thus



Scheme 5: Esterification of alcohol and carboxylic acid using EDC and sulfo-NHS

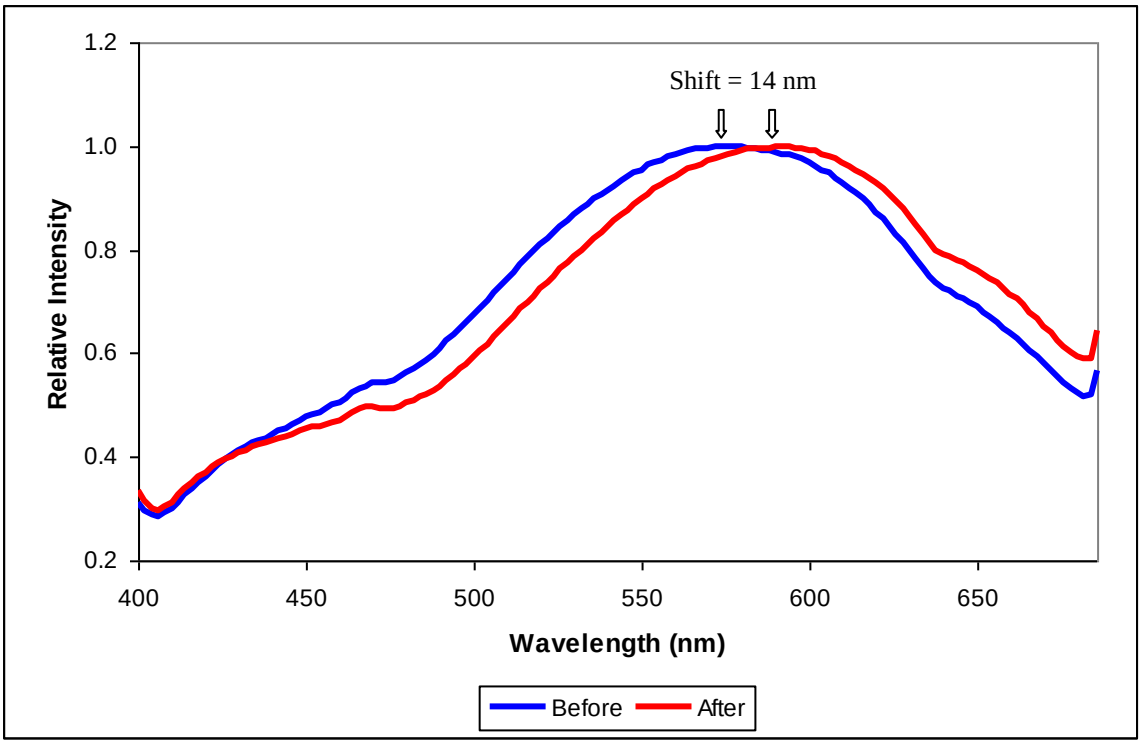


Figure 5: Effect of ester linkage between MSA- and TGL-QDs

increasing the red shift in the λ_{max}. This could be done by shortening the chain length of the linkage by using TGA and a short alcohol functionalized thiol, such as 2-mercapto-1-ethanol. Similarly a more permanent ester linkage could be formed using EDC and sulfo-NHS after multiple neutral pH dialysis chambers, reducing the reverse hydrolysis of the linkage.

There is a literature basis for the belief that the place-exchange reaction can occur on the surface of a QD, due to the lability of the sulfur-metal core bond. These reactions have been used to convert organic-soluble lead selenide (PbSe) QDs to water-soluble versions and place CE on the surface of gold (Au) nanoparticles.[25,26] However, there is little precedence for the place exchange reaction of two water-soluble thiols, such as the ones worked with thus far, so a determination had to be made of the ability of place

exchange to occur on the CdSe QDs in this research. This was attempted via a number of methods, primarily utilizing NMR and electrochemistry.

The method using ^{13}C NMR relied heavily on the idea that the MSA thiol was predicted to have shifts values from 20-40 ppm and 200-215 ppm, while the 15-crown-5 ether is predicted to have the ether carbons shifted to the 70-85 ppm range. This large window created by the MSA should allow for an easy determination of the presence of the CE peaks. This concept however yielded no results, after being attempted in D_2O, acetone-d_6, and even H_2O (to avoid complete drying by rotary evaporation, a technique that causes Ostwald ripening due to the necessary heat to remove water). The resulting spectra yield no conclusive peaks for MSA or CE, even with 64 thousand transients. This lack of information is due to the dilute nature of the QDs. Even in the neat solution of MSA made to be concentrated and large addition of CE (250 μL), followed by subsequent dialysis, there are no definitive peaks to conclude that the place exchange was successful.

This failed method lead to the use of place exchange of TGL, an alcohol functionalized thiol onto a MPA or carboxylic acid functionalized QD. Following this addition, dialysis was done to remove excess thiol and ferrocenecarboxylic acid was added (along with EDC and sulfo-NHS) to form an ester linkage between the TGL and ferrocene, and followed by dialysis to remove free ferrocene. This reaction should lead to an addition of ferrocene only to the QDs that contain the alcohol-functionalized TGL-QDs. Once this reaction was performed, UV-visible spectroscopy was preformed to observe if ferrocene was present in each of the sample, and differential pulse voltammetry was done to assess the amount of ferrocene present. The current observed in

the voltammetry experiment is based on the concentration of ferrocene and should be greater when higher amount of ferrocene are present. The results of this experiment show that not only does place exchange occur, but that there is a competitive reaction occurring, where high concentrations of TGL added yield a greater amount or percentage of MSA replaced by the TGL. This result can be seen in the linear relationship of resulting current in those trials with greater quantities of TGL (Figure 6).

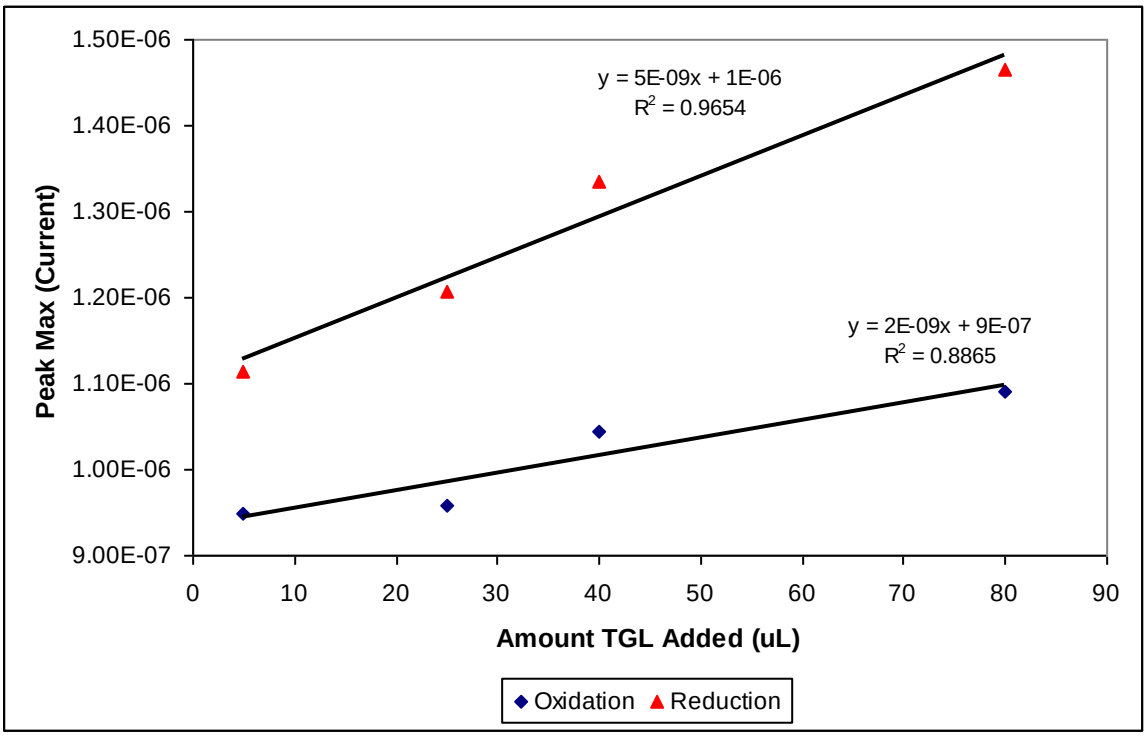


Figure 6: DPV current from ferrocene addition to TGL exchanged MPA QDs

Further work in this area of the effectiveness of the place exchange reaction should revolve around attempting to quantify the bound and free ferrocene in solution with each QD species by a shift in the reduction and oxidation voltage which has been seen to shift from 0.32 V to 0.415 V in the oxidation scans. This shift is due to the effect of the binding of the ferrocene and the effect of the QDs on the redox potential of the ferrocene, due to this fact see in an esterification of ferrocenecarboxylic acid to TGL-

QDs. A similar effect can be seen in the reduction scan shifting from 0.365 to 0.48 in the reduction scans (Figure 7).

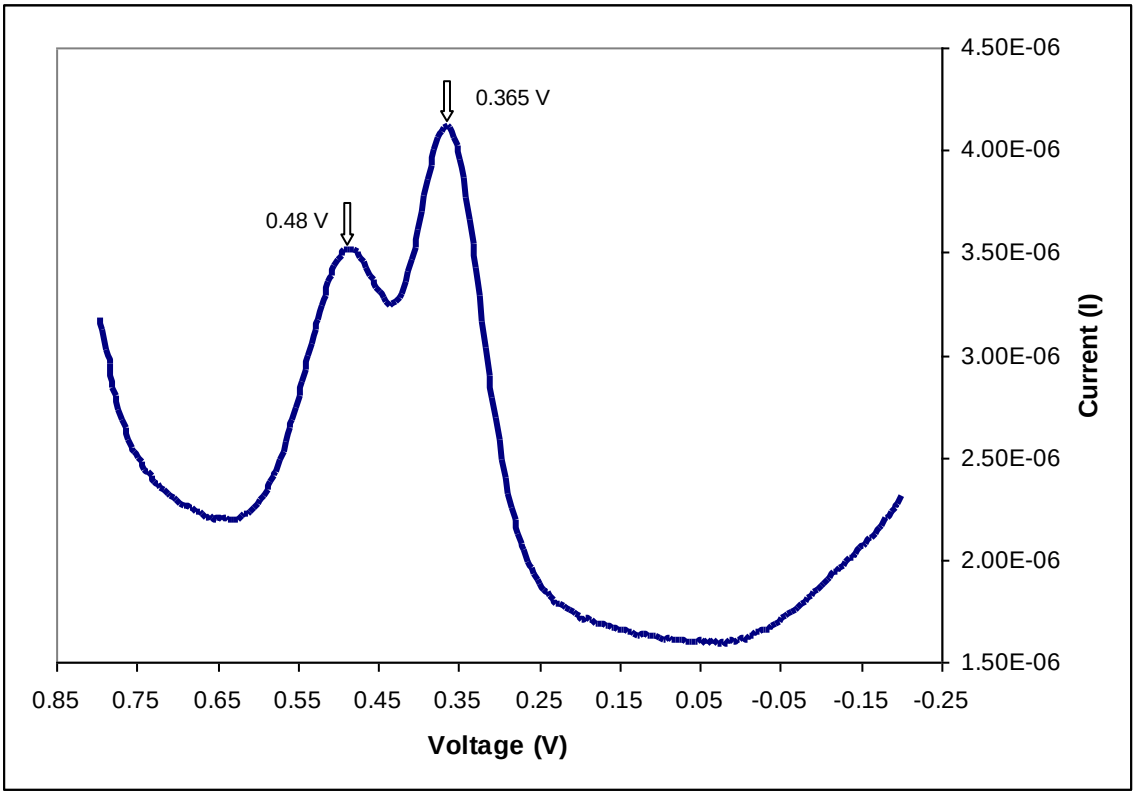


Figure 7: Reduction scan of TGL QDs containing esterified ferrocene

The synthesis of CdSe QDs with MSA and GLU is a simple reaction with good yields and excess for further studies. MSA and GLU were chosen for their activity over a wide range of potential pHs, relative to that of the other thiols that can be used, so these were judged as a good starting point. This proved to be beneficially because all addition of CE solution and EDTA tend to yield a solution with a lower pH, which caused the precipitation of the QDs. Thus, the resiliency of the MSA-CdSe to more acidic pH values proved to be useful, and by readjusting the pH, the QDs could be redissolved back into solution. Upon heating, the QDs were determined to increase in size (from Ostwald ripening) observed in the fluorescence shift from yellow to red wavelengths. This shift

shows a decrease in energy and a longer wavelength corresponding to a shorter band gap width between the valence electron shell and the higher energy conducting band, as predicted.

While the synthesis using NaOH as a base proved to be reliable method, the subsequent extraction of Na^+ was extremely troublesome and should be avoided. The EDTA interacts with the QD forming a pink precipitate in some quantity for all of the reactions and methods. The QDs interact with the EDTA and consequently lose their colloidal properties by aggregation, thus removing them from solution. This makes them inactive in fluorescence spectroscopy, removing a key property in biological systems and sensing. Simply, the EDTA proved to diminish the fluorescence of the QDs preventing further reactions.

The use of NH_4OH as a base for the synthesis of the monolayer-protected CdSe QDs did prove to yield QDs with very similar emission spectra. Similarly, this option does not appear to interfere with the colloid nature of the QDs and allows for further reaction with the CE. NH_4OH does not interfere with the complexation of K^+ following place exchange with the CE and the 15-crown-5 ethers have been shown to have low affinity for the bulky NH_4^+ ion. This is most likely due to the hindrance from the hydrogen encompassing the positive nitrogen from the CE's oxygen, so that the presence of ammonium ions should not affect further experimentation.[22]

Addition of K^+ has shown mixed preliminary results, but does appear to have an effect on the fluorescent emission spectrum of the QDs. These results have shown an intensity increase in the λ_{max} observed prior to addition, in some instances, twice the intensity with a narrow fwhm. This is an interesting occurrence, as from proof of concept

work; one would expect an increase in the λ_{max}. Intensity does appear to be a function of reaction time. The reactions were all carried out with at least one minute elapsing between the addition of K^+ and the acquisition of the fluorescence spectrum. When only one minute was allowed to elapse, the peak intensity shift can be achieved relatively consistently (Figure 8).

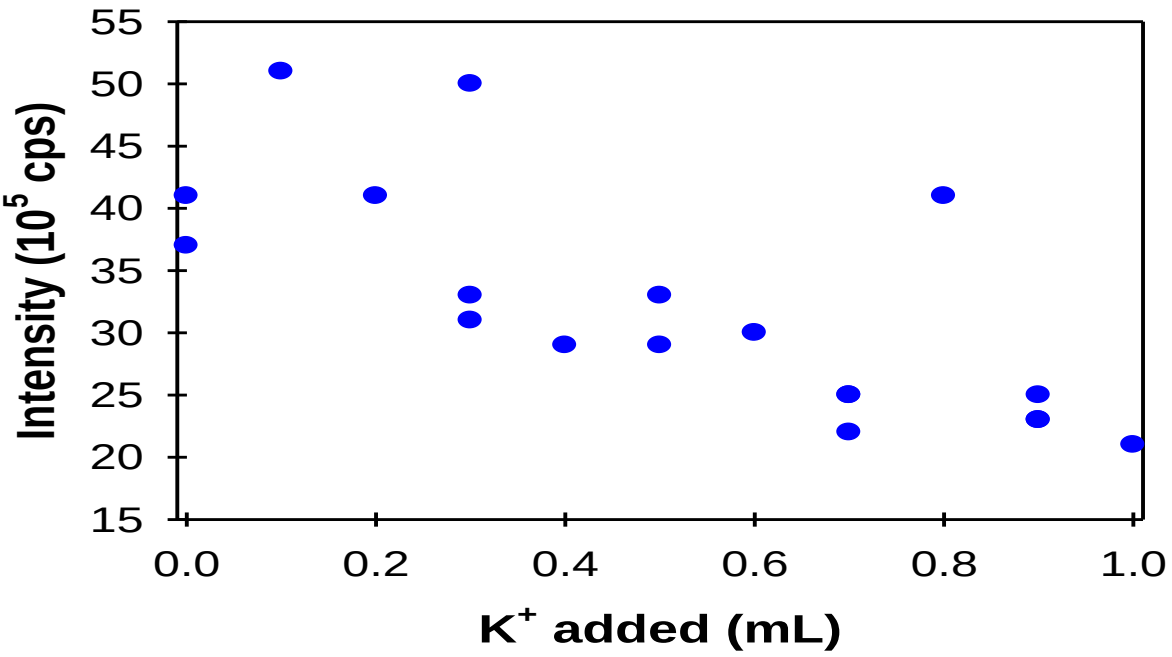


Figure 8: Intensity maxima as a function of K^+ added

However, when longer intervals are allowed between the addition and measurement, the intensity becomes variable and inconsistent. There are no instances of intensity increases for reading 5 minutes after the introduction of the K^+. Due to this intensity decrease as a function of time, it does provide problems at the longer fluoresced wavelengths due to the delay and reading time needed for the instrument to get to the longer wavelengths. This delay could hide or miss intensity shifts to longer wavelengths by small QDs because it scans from blue to red emissions. Thus far, the complexating of K^+ has not shown to have any lasting effects and may be due in part to a shorter chain

length increasing the steric resistance to complexation or lack of presence of CE to QDs in the reaction mixtures due to the diluteness of the CE solution.

This finding made it necessary to explore the effect of concentration on the relative intensity of the resulting fluorescence. This was done to ensure that the K^+ ions and formation of the complexes were responsible for the decrease in fluorescence and not the decreasing concentration of the QDs. MSA CdSe QDs that were made previously were determined to retain significant fluorescence. These QDs underwent a series of dilution from 100% to less than 5% of the original concentration of the neat or non-diluted reaction solution. The concentration of quantum dots after synthesis in water, considered initial concentration or a neat solution. The results of these experiments showed that as the concentration of QDs decreased, the intensity increased. This increase continued steadily until a concentration of about 7 % and remained constant until about 6 % for the major peak around 665 nm. However, the major peak (seen in other samples) of 550 nm is observed to peak at 4.7% (Figure 9). These findings are of significance because it shows that depending on the starting concentration of a QD solution there may be in increase or decrease in the intensity, thus affecting quantum yield measurements or similar experiments.

This effect may be attributed to the instrumentation in which a high concentration of quantum dots, while having a fairly standard relaxation time, as one of the dot fluoresces, the emitted photon is sequestered and excites a neighboring QD. This process, known as self-sequestering or self-absorbing effect, continues until the fluorimeter stops reading prior to all of the QDs reach relaxation. Thus, the intensity would be lower due to the presence of these still excited species within the sample. Similarly, it will be relevant

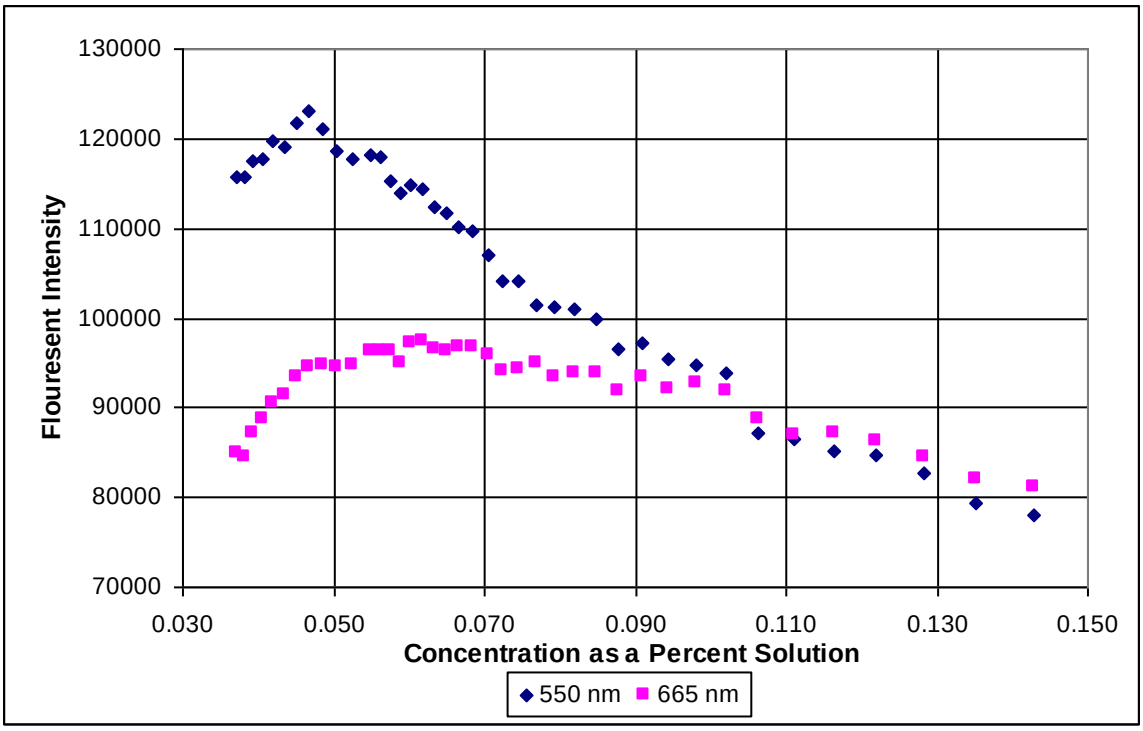


Figure 9: Fluorescence as a function of concentration

to ensure that all CE experiments remain near a 5% concentration to ensure maximum fluorescence and that the concentration is not leading to increases or decreases in intensity. The data presented earlier on the effect of K^+ is not suspected to have decreased to a concentration as low as 5%. However, some of this was purified by dialysis and could have lead to an unknown and decreased concentration, but it is unlikely that it reached 5%. Other trials were run on 20% and 40% samples, so that they are suspected to have had increasing fluorescence rather then decreasing, confirming the effect observed.

Conclusion

EDTA has been shown to decompose and precipitate QDs thus, removing two of their essential properties of the fluorescence and being a colloid solution. NH_4OH has proven to be a useful base for the manipulation of pH in QDs synthesis and has not

shown adverse effects on the CE addition or subsequent initial complexation with K^+. There does appear to be some legitimacy to the concept of an increasing λ_{max} through the linking of QDs through a non-labile linkage. However, preliminary data indicates that CE does affect the fluorescence of the QDs, but not a lasting effect. This interaction has also not yielded a shift in the fluorescent emission λ_{max}, but rather an intensification of the present λ_{max}. Lastly, the concentration experiments demonstrate that care must be exercised with regard to the effect of concentration on the fluorescent intensity of these nanoparticles.

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