The Use of Fluorescent Conjugated Polymer Nanoparticles to Detect Mercury in Solution

A thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Science degree in Physics from the College of William and Mary

by

Melissa Kay Dullum

Advisor: Elizabeth Harbron

Senior Research Coordinator: Henry Krakauer

Date: April 22, 2016

Abstract

Fluorescence spectroscopy is an excellent method of detection; highly sensitive and selective, fluorescent detectors can be used to detecting a wide variety of analytes at low concentrations. A fluorescent mercury detector was developed from conjugated polymer nanoparticles that had been doped with a fluorescent dye molecule. Upon contact with mercury, the dye molecule undergoes a ring-opening mechanism, becoming fluorescent. The fluorescence signal is further amplified through fluorescence resonance energy transfer (FRET) as energy from the conjugated polymer nanoparticles is transferred to the dye molecules. The amount of mercury in solution can be measured ratiometrically by comparing the fluorescence intensity of the dye to that of the conjugated nanoparticles. This allows for low levels of mercury detection, in the parts per billion range.

Introduction

Any mercury present in an ecosystem can have detrimental effects on the organisms living in that environment. Mercury concentration increases with trophic level as it travels up the food chain, in a process known as biomagnification. While it may initially not be present in harmful amounts, by the time it reaches the levels where humans come into contact with it, the concentration may have increased to as much as 20 mg/L, the level at which mercury starts to impair human muscle and memory function[1]. Studies show that between the planktivore and piscivore trophic levels (where humans are found), the typical increase in mercury concentration is tenfold[2]. Due to the great harm even a small amount of mercury can cause, the need exists for its detection at extremely low concentrations: the Environmental Protection Agency has set the maximum concentration of Hg^{2+} allowed in drinking water to 2 ppb[3]. One method by which mercury can be detected at these low concentrations is through the use of fluorescent nanoparticles.

Fluorescence occurs when a molecule absorbs light at one wavelength then reemits light of a different wavelength[4]. The molecule absorbs a photon, exciting an electron and causing it to jump from its ground state (S_0) to an excited state (S_1). Once a molecule is in its excited state, there are many paths by which it may relax. Fluorescence, the preferred relaxation pathway in this instance, occurs when the electron vibrationally relaxes to the lowest vibrational level of the excited state. From here, the molecule relaxes from the excited state to the ground state, releasing a photon of a different wavelength than that which was absorbed. The process by which fluorescence occurs, and any other process where a molecule absorbs and reemits light or energy, is

depicted in a Jablonski diagram, shown in Figure 1. The transitions between the ground and excited state are quantized, resulting in the absorption and emission of specific wavelengths of light. The relationship between the wavelength of light (υ) and the energy difference (ΔE) between electronic states is given by Equation 1. Planck's constant is represented by h.

Equation 1: $\Delta E = h\upsilon$

Energy can also be released radiatively by other mechanisms[4]. A photon could instantaneously be reemitted after absorption, producing light of the same wavelength. The excited electron could undergo intersystem crossing (ISC) and transition from a singlet state to a triplet state (T_1). Emission from a triplet state is referred to a phosphorescence. Because ISC is a forbidden transition between states, it will occur on a much slower time scale than the allowed fluorescence transition. The energy may also be lost through nonradiative means, such as heat dissipation.

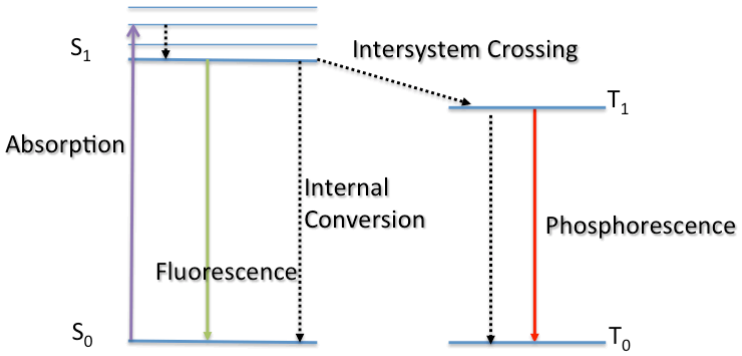

Figure 1: Jablonski Diagram

The photophysical properties of a molecule determine the fluorescent capabilities of a molecule. Aromatic or highly conjugated molecules typically make the best

fluorophores due to their numerous π bonds[4]. Better fluorophores have large extinction coefficients (ε), a measure of how strongly the fluorophores absorbs light. Good fluorophores also need to have a large fluorescent quantum yield (Φ_f), which can be calculated according to Equation 2. k represents the rate at which the process occurs, where k_f is the rate of fluorescence

Equation 2: $$\Phi_f = \frac{k_f}{\sum k}$$

Both the fluorescence quantum yield and the molecules extinction coefficient determine the intensity of fluorescence emission (I_f), as represented by Equation 3:

Equation 3: $$I_f \propto \Phi_f \cdot \varepsilon$$

Fluorescence is an excellent technique for detecting low concentrations of a molecule in solution. High spatiotemporal resolution can be obtained using fluorescence. It is also one of the more sensitive analytical techniques. As a result, many probes for mercury have been developed that make use of fluorescence.

Traditionally, mercury quantification relied on analytic techniques such as atomic absorption spectroscopy or liquid chromatography[5]. However, these methods, while sensitive, are not able to quickly detect mercury in the field or in the biological samples where mercury is being studied. Fluorescence was introduced as an attractive alternative to these other analytical methods, due to its speed and ease of measurement.

When they are being used as detectors, fluorescent molecules either function as "turn-on" or "turn-off" probes, where, respectively, a non-fluorescent molecule becomes fluorescent upon contact with mercury, due to a change in its structure, or the fluorescence of a molecule is quenched due to the heavy ion effect. There are relatively few examples of turn-off probes for mercury, but there are several turn-on probes with

fluorescent molecules such as anthracene, naphthalene, and rhodamine. Rhodamine, a fluorescent dye molecule (Figure 2), and its derivatives are excellent chemosensors due to their photophysical properties: a large extinction coefficient and a high fluorescence quantum yield[7].

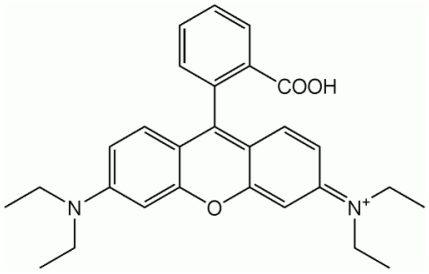

Figure 3: Rhodamine

One method by which the detection limit of a chemosensor may be improved is through the use of ratiometric fluorescence measurements[8]. Ratiometric fluorescence uses two molecules which fluoresce at different wavelengths. The intensity of fluorescent emissions is measured simultaneously at both wavelengths and the ratio then calculated. This ratio is then used to quantify the concentration of mercury present. Using a ratio of two intensities rather than a single intensity eliminates any intensity variations *not* due to the presence of mercury, such as environmental or instrumental fluctuations. This allows for much more sensitive measurements, as signals that would have been previously hidden by noise in the signal can now be revealed.

Conjugated polymer nanoparticles (CPNs) can act as excellent fluorescence sensors. The CPNs we developed in lab make use of ratiometric fluorescence measurements in order to work as sensitive mercury chemosensors. The two fluorophores present in the CPNs are a derivative of rhodamine, rhodamine-B disulfide (RB-S2) and a highly conjugated polymer poly[9,9-dioctylfluorenyl-2,7-diyl)-co-1,4-

benzo-{2,1′-3}-thiadiazole)] (PFBT). RB-S2 and PFBT are shown in Figures 3[9] and 4[8], respectively.

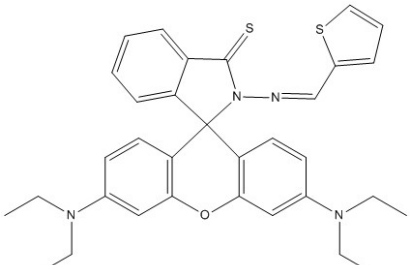

Figure 4: RB-S2 Dye

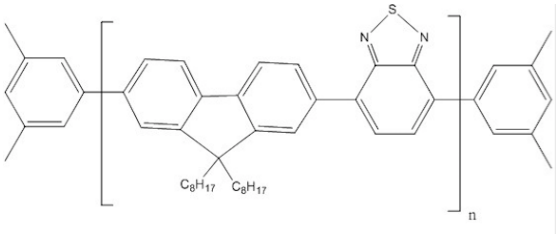

Figure 4: PFBT Polymer

The CPNs are composed of three different materials: the fluorophores RB-S2 and PFBT and a second, non-fluorescent coating polymer Poly(vinyl alcohol-co-vinyl acetate) (PV-CoCo) shown in Figure 5.

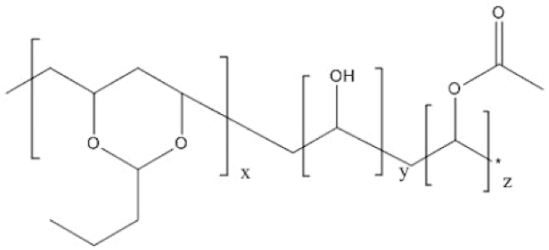

Figure 5: The coating polymer PV-CoCo

Each of these three materials is dissolved in the organic solvent tetrahydrofuran before being combined to create a "pre-nanoparticle" solution. To form the nanoparticles, a small amount of the solution is injected into water. Due to its hydrophobic nature, the conjugated polymer curls up on itself forming spherical nanoparticles, with the dye molecules randomly distributed throughout[8].

The stability of the CPNs in an aqueous environment is another advantage the CPNs offer as a mercury sensor. To be useful in determining the amount of mercury present in a sample, the chemosensor will need to be functional in the environments where mercury is being tested: usually aqueous or biological[2]. Unlike some sensors which are based in organic solutions, no changes will need to be made to our sensor as it is already exists in an aqueous suspension.

Besides being a more sensitive chemosensor due to the ratiometric fluorescence measurements, the use of two fluorescent molecules in the CPNs also increases the sensitivity due to fluorescence resonance energy transfer (FRET). In the CPN system, FRET works to increase the extinction coefficient and quantum yield and therefore the fluorescence intensity. Because fluorescence intensity is directly related to sensitivity, any process which increases intensity will also increase the sensitivity of the sensor.

FRET is a mechanism by which the energy of a donor molecule is transferred to an acceptor molecule[4]. FRET occurs between two molecules with good spectral overlap that are close in proximity to each other. Spectral overlap is necessary for FRET to occur because the energy transferred from the donor molecule needs to be at a wavelength the acceptor molecule can absorb. The FRET efficiency (E) is dependent on the distance (r) between the donor and acceptor[10]. The efficiency can be calculated using Equation 4, where R_0 is the Förster radius: the distance at which 50% FRET occurs.

Equation 4: $$E=\left[1+\left(\frac{r}{R_o}\right)^6\right]^{-1}$$

In this case, the donor molecule is the PFBT polymer and the acceptor molecule is the RB-S2 dye. PFBT was chose as the polymer because its absorption and emission spectra overlapped well with that of RB-S2[8].

The PFBT polymer is always in a fluorescent state, absorbing light at a wavelength of 460 nm and reemitting it at 537 nm. Initially non-fluorescent, RB-S2 begins to fluoresce upon binding with Hg^{2+}. As the RB-S2 begins to fluoresce, it absorbs the energy being emitted by the PFBT polymer at 537 nm and uses this energy to increase its own fluorescent signal at 593 nm. The emission from the PFBT is no longer seen, as all of the excited state energy is transferred to RB-S2. This process is depicted in Figure 5[8].

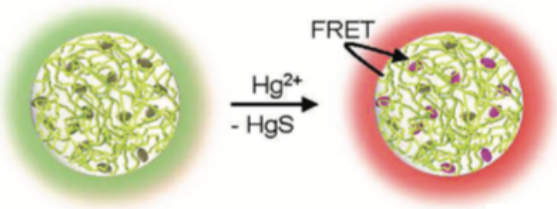

Figure 5: With the addition of mercury, the CPNs switch from fluorescing in the green range to the red range through FRET.

In addition to occurring between the polymer and the dye, FRET also occurs within the conjugated polymer itself. Composed of multiple fluorescent monomer units, conjugated polymers are multichromophoric and make excellent fluorophores[9]. The individual monomers in the molecule within close enough proximity to each other will undergo FRET amongst themselves, transferring energy from higher energy chromophores to lower energy ones, enabling the energy to be distributed in the most efficient way possible. It is in part due to this phenomena that conjugated polymers have such high extinction coefficients, making them such excellent fluorophores.

RB-S2 by itself is not an excellent chromophore; its extinction coefficient is around 1×10^5 M^{-1} cm^{-1}, while that of PFBT, 1×10^7 M^{-1} cm^{-1} is huge in comparison [9,12]. The difference in extinction coefficients and FRET enable the CPN to act as an antennae, amplifying the fluorescence intensity signal of the RB-S2 and making it a much more sensitive mercury sensor than it would have been on its own[12].

Experimental

RB-S2 Synthesis

The synthesis of RB-S2 is three steps long and begins with rhodamine B (RB), which can be commercially obtained. The first step in the synthesis converts RB to rhodamine B hydrazide (RB-H), following literature procedures[13]. RB (1 equivalent) is dissolved in ethanol and hydrazine (25 equivalents) are added. The solution refluxes at 85˚C until a color change is observed. The ethanol is then removed and the product filtered.

The second step of the synthesis begins with RB-H and yields the product thiooxorhodamine B hydrazine (T-RB-H) and proceeds according to the literature[9]. RB-H (1 equivalent) and Lawesson's reagent (1 equivalent) are dissolved in dry toluene. The solution refluxes for 24 hours at 90˚C. The solvent is removed and the product dissolved in potassium carbonate and extracted with methylene chloride. The product is then purified through column chromatography using an eluent of 40:60 hexanes to ethyl acetate.

The third and final step of the synthesis converts T-RB-H to RB-S2 and follows literature procedures[9]. T-RB-H (1 equivalent) and 2-thiophenecarboxaldehyde (1.5 equivalents) are dissolved in boiling methanol. The solution refluxes for 12 hours at 60˚C under argon gas. The product is recrystallized with ethanol and should be stored in a -80˚C freezer in a light-block vial.

Nanoparticle Synthesis

The polymer PFBT is dissolved in tetrahydrofuran (THF) in a 1 mg : 1 mL ratio, resulting in a concentration of 1 mg/mL. The PFBT solution is stirred for 2 hours under argon gas before being filtered though a 0.7 μm filter. The polymer PV-CoCo is dissolved in THF in a 1 mg : 1 mL ratio, resulting in a concentration of 1 mg/mL. The PVCoCo solution is stirred for 20 minutes. The RB-S2 dye is dissolved in THF is a 1 mg : 10 mL ratio, resulting in a concentration of 0.1 mg/mL. The RB-S2 solution is stirred for 20 minutes.

The three prepared solutions are then combined with THF to form a pre-nanoparticles solution. The final nanoparticles solution should have a formula of 18 ppm PFBT, 10 wt% RB-S2, 6:1 PFBT : PV-CoCo. The amounts of each solution needed are calculated accordingly:

18 ppm PFBT in solution = .018 mg PFBT/mL × 25 mL total solution = 0.45 mL PFBT

.45 mL mL PFBT ÷ 6 = 0.075 mL PVCoCo

0.1 x 0.45 mL PFBT = 0.045 mL RB-S2 × 10 mg/mL = 0.45 mL RB-S2

25 mL – 0.45 mL PFBT – 0.075 mL PVCoCo – 0.45 mL RB-S2 = 24.025 mL THF

The pre-nanoparticles solution is sonicated for 30 seconds, along with 8 mL of ultrapure water. A 1 mL aliquot of the solution is then injected into 8 mL of sonicating ultrapure water. The nanoparticles sonicate for 2 more minutes before removing the THF through high vacuum pumping or degassing. The resulting solution is once more filtered through a 0.7 μm and 0.22 μm filter to remove any aggregates that might have formed.

Titrations with Mercury

Fluorescence measurements are performed on Varian Eclipse spectrophotometers, with the solutions in semimicro glass cuvettes. 2.3 mL of the prepared nanoparticles are added to the cuvette. All scans on the nanoparticles are performed with an excitation wavelength of 450 nm, and scanning occurs from 460 nm to 700 nm. The excitation slit is set 5 nm width and the emission slit is set to either 2.5 or 5 nm, depending on the intensity of fluorescence.

Stock solutions of Hg^{2+} have been prepared in a variety of concentrations, ranging from 10^{-4} to 10^{-6} molarity. Titrations are usually first tried with the 10^{-5} molar solution and the concentration can be adjusted either up or down according to how sensitive that solution of nanoparticles is. Hg^{2+} is added in 0.5 µL aliquots with a 10 µL micropipette until 5 µL total of Hg^{2+} solution has been added. The aliquot size is then increased to 1 µL and added until there are 10 µL of Hg^{2+} total. This is increased to 5 µL for the 10 µL to 20 µL range, and 10 µL for any aliquots added beyond that.

After each addition of Hg^{2+} solution, the nanoparticles solution is mixed thoroughly with a glass pipette and the fluorescence scan is not begun 1 minute after the addition, to allow time for the dye particles to fully react with all the mercury in solution.

Data and Results

Purification of RB-S2

It is sometimes necessary to perform the synthesis of RB-S2 several times in order to obtain the correct form of the product . While H-NMR spectra of the products are the definitive proof of whether or not the correct product has been synthesized and purified, the color of the product is also a good indicator of product purity. As discussed before, RB-S2 undergoes a ring opening mechanism upon contact with mercury and begins to fluoresce in the red light range, as depicted in Figure 6[9].

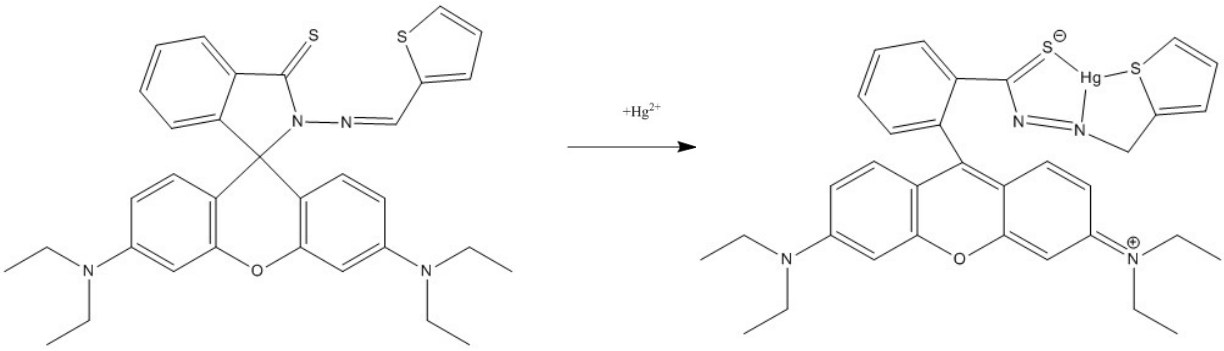

Figure 6: Ring Opening Mechanism

Unfortunately, this ring-opening mechanism will also occur due to degradation of the RB-S2 dye, causing it to become pink and fluorescent before coming into contact with mercury. This degradation occurs as result of exposure to heat and light over time.

As a result, color can be used as an indicator of whether or not the pure, unopened RB-S2 dye has been obtained in the final step of the synthesis. The final product should ideally be tan; however, if any open RB-S2 is present, or other undesired forms of

rhodamine dye, the product will be orange, or even pink, due to fluorescent molecules. In these instances, it is recommended that the product be repurified, and sometimes resynthesized.

Optimization of T-RB-H Column Chromatography

An important step in ensuring the purity of RB-S2 at the end of the synthesis is the purification of T-RB-H through column chromatography, performed during step two of the synthesis. The column will separate out into bands of different colors: yellow, orange, pink, red and dark purple, as shown in Figure 7.

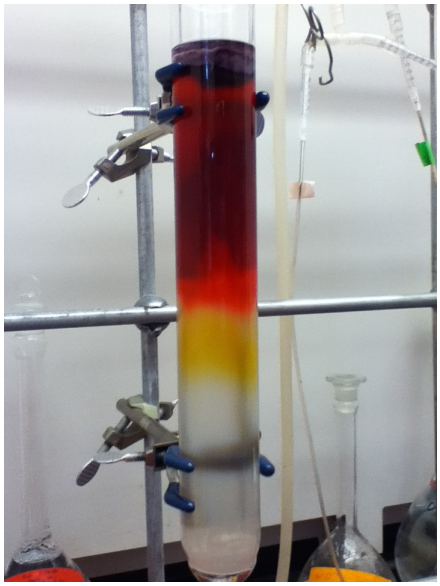

Figure 7: A photograph of a column run while purifying T-RB-H

The desired product, T-RB-H, is usually found entirely in the yellow band, determined using thin layer chromatography (TLC). This is also consistent with what we know about the color of the desired final product: the darker colors, like pink and purple, are indicative of undesired forms of rhodamine.

I found while performing the synthesis that the eluent recommended in the literature, 4:1 methylene chloride: petroleum ether[4], did not create sufficient separation between the product and impurities. In order to find a more effective eluent, I used TLC to determine which eluent achieved the greatest separation. Chloroform and methanol, methylene chloride and methanol, and hexanes and ethyl acetate were all tested against methylene chloride and petroleum ether to determine which would create the greatest separation. Hexanes and ethyl acetate brought about the most separation, so different ratios of hexanes to ethyl acetate were then tried, in the range of 30:70 to 70:30 hexanes to ethyl acetate. 40:60 hexanes to ethyl acetate proved to have the greatest separation, as determined by TLC, so it is now the preferred eluent for the column.

Determination of Optimal CPNs Formula

In order to function properly as a mercury sensor, the CPNs need to have proper ratios of polymer, dye and coating polymer. The ratio that continually yields the best results is given by the formula 18 ppm PFBT, 10 weight percent RB-S2 and 6:1 PFBT to PV-CoCo. This formula was empirically determined, by synthesizing a variety of pre-nanoparticle solutions with different amounts of PFBT and RB-S2 present. After nanoparticle synthesis, the nanoparticles were titrated with mercury. The spectra were then compared to see which yielded the best results. Several criteria were used to determine if the nanoparticle formula was an appropriate mercury sensor. First, the polymer must be able to fold properly into spherical nanoparticles. If there is too much dye compared with the amount of polymer present, instead of folding into spheres, the nanoparticles will aggregate. Aggregates are too large to pass through the microfilter in

the final step of the nanoparticle synthesis, so they will "filter out" leaving only an aqueous solution devoid of nanoparticles. The nanoparticles can also filter out if the ratio of coating polymer to PFBT is too large.

The dye molecule also must be accessibly located on the nanoparticle in order for it to come in contact and bind with mercury. Dye molecules must be present on the surface of the nanoparticle; they cannot all be folded into the interior of the sphere. Below a certain dye concentration, there are not enough dye molecules to guarantee that this occurs. The nanoparticles form correctly, but do not adequately bind to mercury. The concentration below which there was not sufficient mercury binding was found to be 5 weight percent of the nanoparticle solution.

As described before, the efficiency with which FRET occurs is dependent on the distance between the donor and acceptor (Equation 4). As FRET is the phenomenon responsible for the sensitivity and effectiveness of the CPNs as mercury sensors, it is necessary to maximize the efficiency at which it occurs. The distance between the polymer and dye needs to be chosen to optimize the FRET efficiency. One factor that controls the distance is the ratio between the amounts of dye and polymer. Through experimentation we can determine which ratios produce the most efficient FRET, which would produce the largest increase dye fluorescence.

While experimenting with different nanoparticle formulas, I would keep two out of the three formula variables constant, changing only one at a time, so I could be certain of which factor had influenced the CPN synthesis and behavior. I found it easiest to organize the formulas I had tested in a table, recording whether or not the CPNs

aggregated, reacted with mercury in solution, and experienced efficient FRET. Table 1 is an excerpt from the table right before I selected the optimal CPN formula.

Table 1: Experimental CPN Formulas with Results

Ppm PFBT	Weight % RB-S2	Polymer Ratio	Filtered Out	Reaction with Hg	FRET
18	5	5:1	No	Yes	No
18	10	5:1	No	Yes	Yes
18	15	5:1	Yes	-	-
18	10	5:1	No	Yes	Yes
18	10	6:1	No	Yes	Yes
18	10	7:1	No	Yes	Yes

I had previously decided that 18 ppm PFBT was the most effective polymer concentration, so I kept the PFBT concentration constant for the duration of the experiments. By keeping the polymer ratios constant, I was able to determine that 10 weight percent RB-S2 allowed FRET to occur the most efficiently without causing aggregation. The polymer ratios were then varied, and 6:1 PFBT to PV-CoCo yielded the best results: a large increase in the intensity of the dye peak at 593 nm, indicating the occurrence of FRET (shown in Figure 8). The optimal CPN formula was experimentally determined then to be 18 ppm PFBT, 10 wt% RB-S2 and 6:1 PFBT to PV-CoCo. The fluorescence scans taken of the titration of these CPNs with 10^{-5} M Hg^{2+} are shown in Figure 8.

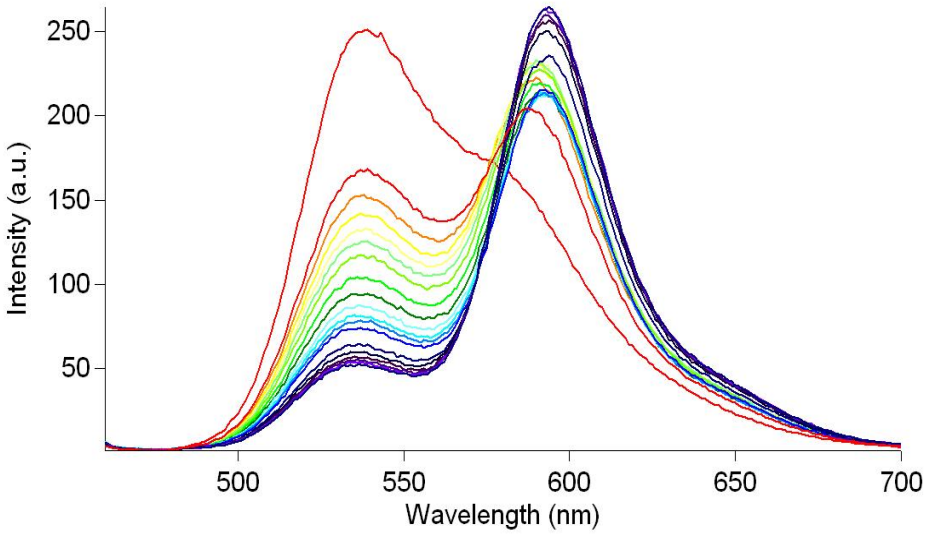

Table 2

Total Hg (µL)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
6.0
7.0
8.0
9.0
10.0
15.0
20.0

Figure 8: Fluorescence Spectra of 18 ppm PFBT, 10 weight % RB-S2, 6:1 PFBT:PVCoCo CPNs

Analysis of Fluorescence Spectra

The fluorescence spectra was analyzed ratiometrically, comparing the intensity of the peak at 537 nm (PFBT) to the peak at 593 nm (RB-S2). The initial peak intensity of the polymer peak was much greater than that of the dye. Upon addition of 0.5 µL of 10^{-5} M Hg^{2+} FRET occurs, causing the dye peak to increase in intensity as it gains energy from the polymer. At the end of the titration, the polymer peak was almost nonexistent and the dye peak's intensity was equal to the initial intensity of the polymer peak. These results were encouraging, indicating that FRET is occurring quite efficiently: nearly all of the polymer's energy is being transferred to the dye. The limit of detection for the CPNs can be determined from the initial addition of mercury: 0.5 µL of 10^{-5} M Hg^{2+}, corresponding to approximately 0.7 ppb, which is well below 2 ppb, the maximum allowed concentration of mercury in drinking water allowed by the EPA[3].

In order to determine how effective FRET was at increasing the intensity of fluorescence emission, a solution containing only RB-S2 was titrated mercury and the results compared to a titration performed with the CPNs. The maximum intensity of emission for each step in the titration was calculated and plotted against the concentration of mercury, resulting in the graph shown in Figure 9. When the maximum intensity of the CPNs and the dye were compared, the CPNs' intensity was found to be 40 times greater than that of the dye alone.

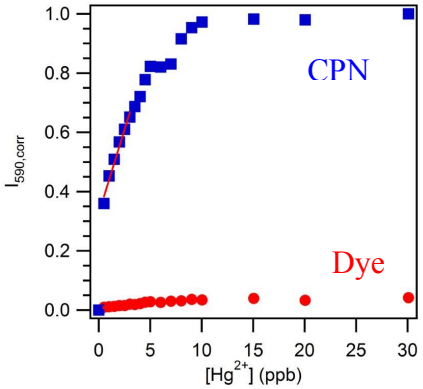

Figure 9: Graph showing intensity difference between CPN and dye alone

The average number of excited dye molecules per nanoparticle was also calculated from the absorption spectra of the CPNs at 570 nm, the wavelength at which RB-S2 absorbs light. After the first addition of mercury, it was calculated that, on average, 1 dye molecule was excited per CPN. This number increased to 3 dye molecules on average per CPN by the end of the titration. Three excited dye molecules is actually far less than we were expecting to be present on the nanoparticle. A similar experiment where the CPNs were also synthesized with PFBT polymer and a fluorescent dye reported 40 excited dye molecules per CPN[12], so there are improvement which could still be made to the CPNs. One method by which the number of excited dye molecules

per CPN could be increased is by adding the dye molecules after the CPNs have already formed. In this method, the dye molecules could only adhere to the surface of the CPNs, allowing even more to be accessible to mercury and increasing the sensitivity.

Reproducibility Challenges

Despite the success of the nanoparticles of the formula 18 ppm, 10 weight % and 6:1 PFBT to PV-CoCo, lately we have had challenges replicating these results. As mentioned previously, the dye molecule RB-S2 is unstable if exposed to heat or light and will degrade with time, undergoing a ring opening mechanism which makes it unusable as a mercury sensor.

This ring-opening mechanism has started to occur during the CPN synthesis as well. It appears to be occurring while the solvent tetrahydrfuran is being removed by the high pressure vacuum. If a fluorescence scan is taken before the CPNs are placed under vacuum, directly after sonication, the dye molecule is still closed and non-fluorescent, as shown by the red curve in Figure 10. After being exposed to the high-pressure vacuum for as little as 5 minutes, the dye molecule will be fluorescent, indicating the ring-opening mechanism occurred, shown by the blue curve in Figure 10. In this instance, a titration with mercury can no longer be performed, as there is little dye left with which mercury can bind.

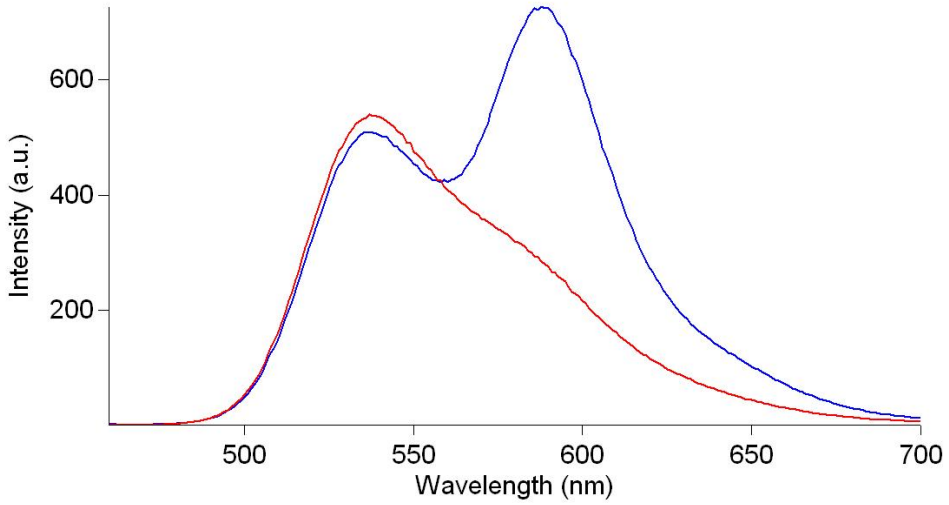

Figure 50: CPN scan before (red) and after (blue) the high-pressure vacuum

While we are still trying to determine the reason the dye is no longer stable under vacuum, I have also been experimenting with another method of THF removal: degassing. Degassing is the process of bubbling an inert gas through a solution, removing the most reactive element of the solution. In this case, the inert gas being used is argon and THF is the reactive element being removed. We have not yet been successful in removing the solvent by degassing yet, but we are hopeful that with some fine-tuning, such as the addition of gentle heat, this method could prove successful.

Conclusion

Through a series of trial and error experiments, a formula was developed for conjugated polymer nanoparticles, composed of a PFBT polymer doped with RB-S2 dye, coated with the polymer PV-CoCo that work as sensitive and selective Hg^{2+} detectors. The sensitivity of these CPNs arises from their ability to selectively bind mercury in solution and amplify their signal through FRET. The mercury detection signal amplification due to FRET is 40 times greater than if the dye molecules alone were used to create the signal. In addition to the amplification due to FRET, the fluorescence of the polymer also allows the concentration to be ratiometrically determined. The confluence of these two factors creates a highly sensitive mercury sensor, allowing for detection in the parts per billion range.

References

1. Zahir, F.; Rizwi, S. J.; Haq, S. K.; Khan, R. H. *Environ. Toxicol. Pharmacol.* **2005**, *20,* 351-360.

2. Bowles, K. C.; Apte, S. C.; Maher, W. A.; Kawei, M.; Smith, R. *Canadian Journal of Fisheries and Aquatic Sciences.* **2001**, *58*, 888-897.

3. Mercury Update: *Impact on Fish Advisories*, EPA Fact Sheet EPA-823-F-01-011; EPA, Office of Water: Washington, DC, **2001**.

4. Lakowicz, J. R. Introduction to Fluorescence: *Principles of Fluorescence Spectroscopy,* 2; Kluwer Academic/Plenum Publishers: New York, **1999**; 1-23.

5. Nolan, E. M.; Lippard, S. J. *Am. Chem. Soc.* **2008**. *108*, 3443-3480.

6. Wu, J.; Hwang, I.; Kwang, Kim, K. S.; Kim, J. S. *Organic Letters*. **2007**, *9*, 907-910.

7. Huang, W.; Song, C.; He, C.; Lv, G.; Hu, X.; Zhu, X.; Duan, C. *Inorg. Chem.* **2009**, *48*, 5061-5072.

8. Childress, E. S.; Roberts, C. A.; Sherwood, D. Y. Leguyader, C. L. M.; Harbron, E. J. *Anal. Chem.* **2012**, 84, 1235-1239.

9. Zhou, Yi.; You, X.; Fang, Y.; Li, J.; Liu, K.; Yao, C. *Org. Biomol. Chem*. **2010**, *8*, 4819-4822.

10. Kremers, G.; Goedhart, J.; van Munster, E. B.; Gadella, Jr., T.W.J. *Biochemistry*. **2006**, *45*, 6570-6580.

11. Harbron, E.J.; Davis, C.M.; Campbell, J.K.; Allred, R.M., Kovary, M.T.; Economou, N.J. *J. Phys. Chem. C* **2009**,113, 13707-13714.

12. Zhang, X.; Chamberlayne, C.F.; Kurimoto, A.; Frank, N.L.; Harbron, E.J. *Chem. Comm*. **2016**, *52*, 4144-4147.

13. Xiang, Y.; Tong, A.; Jin, P.; Ju, Y. *Org. Lett.* **2006**, *8*, 2863- 2866.