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Detection of Iron (III) Using Agarose Beads Derivatized with Desterrioxamine B

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DETECTION OF IRON (III) USING AGAROSE BEADS DERIVATIZED WITH DESFERRIOXAMINE B

by

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ABSTRACT

The goal of our work is to provide marine scientists with a detection system which can be mounted on buoys and gliders for measuring the amount of iron (III) in sea water. Iron is the limiting nutrient for phytoplankton growth. Since phytoplankton play a key role in global carbon cycles and global warming, the iron in seawater does as well. This thesis is meant to be a proof-of-concept for a new approach to measuring iron in seawater. The essential elements of the research discussed herein include identifying particulate beads which are optically transparent in the visible region, modifying the surfaces of these beads to reversibly bind iron (III) from water, and measuring the detection limit directly on the transparent beads using UV-Visible (UV-Vis) spectroscopy.

Agarose beads were selected and shown to be semi-transparent in water. These beads were treated with polystyrene$_{50}$-b-poly(acrylic acid)$_{180}$; the acrylic acid portion of the polymer was then reacted with iron (III) chelator desferrioxamine B using EDC as a catalyst. Infrared spectroscopy was used to show that the block copolymer and DFB had attached to the beads. UV-Vis spectroscopy was used to study the iron uptake by these beads, using the red color produced when DFB chelates iron (III). The amount of time needed for the beads to take up iron (III) from a solution and the saturation point of the beads was also determined. It was shown that detection of low parts per trillion is possible and that at pH 7.5, the presence of oxalate would not affect iron (III) uptake.

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CHAPTER 1: Introduction

Motivation for Detecting Iron in Oceans

The increase in atmospheric carbon dioxide levels has been affecting the climate and shows no signs of slowing. One strategy which has been suggested to reduce the amount of carbon dioxide in the atmosphere is to increase the uptake of carbon dioxide by the world's oceans.

In as much as 40% of the world's oceans, the amount of carbon dioxide which can be sequestered by the oceans is limited by the amount of iron. A major source of carbon dioxide uptake by oceans is through the growth of phytoplankton. In certain areas of the ocean, known as "high-nitrate, low-chlorophyll" (HNLC) areas, there are excess levels of the major nutrients (nitrates, phosphates, and silicates) essential for phytoplankton growth. However, it has been demonstrated that seeding these areas of the ocean with iron results in increased phytoplankton growth, indicating not only that these organisms require iron to survive but that iron is the limiting nutrient. Because of this, it has been suggested that the level of atmospheric carbon dioxide could be reduced by seeding the oceans with iron (Boyd et al., 2007; Roy et al., 2008; Martin et al., 1994) . Seeding the oceans would produce more phytoplankton, allowing the ocean to draw more carbon dioxide from the atmosphere.

Before this strategy is implemented on a large scale, we need to understand the iron concentration distribution and profiles in oceanic waters and the complex mechanisms involved in iron uptake by organisms. The problem is that there is very little

data on iron levels in oceanic waters because of the difficulty of measuring iron at trace concentrations. Since iron is not very soluble in ocean water and any iron which is dissolved is in high demand by phytoplankton, concentrations can be very low (less than 1 nM). Also, much of the iron in sea water is complexed to organic molecules, rather than free in solution (Roy *et al.*, 2008).

Overview of Current Iron Detection Methods

The principal methods for measuring iron are lab-based. For example, one can use inductively-coupled plasma mass spectrometry (ICPMS) or graphite furnace atomic absorption (GF-AA). However, both of these methods require that the analyte (iron) be removed from sea water prior to analysis. This is because there are other ions present in sea water (often in much higher concentrations than iron) which can reduce the sensitivity of these analytical methods. Since these methods are laboratory-based methods, sampling procedures and transportation are time-consuming and tedious. Other methods which involve chemiluminescence and colorimetric analysis have been developed which can be used on a ship on the ocean (King et al., 1995; Lohan et al., 2006). It should be noted that these ship-based methods still require a high level of user involvement. Ship time is also very expensive and tedious sampling methods are required. What is needed to obtain the volume of data are methods that can be deployed on buoys and gliders to provide continuous and autonomous detection of iron levels.

A few methods for *in situ* deployment have also been developed. A couple of these methods can provide continuous and autonomous detection from buoys (Lam et al.,

2006; Barrero et al., 1995). However, none of their detection limits for iron were below 1 nM (Coale et al., 1991; Laes et al., 2005; Chapin et al., 2002). This makes it difficult to use these techniques for continuous, on-going, and on-site measurements (Roy et al., 2008; Lohan et al., 2005). Furthermore, these methods cannot be used on acidified samples, and this is required in order to compare to historical data (Roy *et al.*, 2008). Therefore, there remains the need to develop a sensor platform which could be automatized, have a detection limit below that of common oceanic iron concentrations, and be able to analyze acidified samples.

This provides the over-arching motivation for the research in this thesis. The work presented here involves the development of an essential element in an iron detection system that could be deployed on buoys and gliders.

Our Approach to Iron Detection – Desferrioxamine B

Our approach is based on the use of desferrioxamine B to detect iron. The structure of this molecule is shown in Figure 1.1.

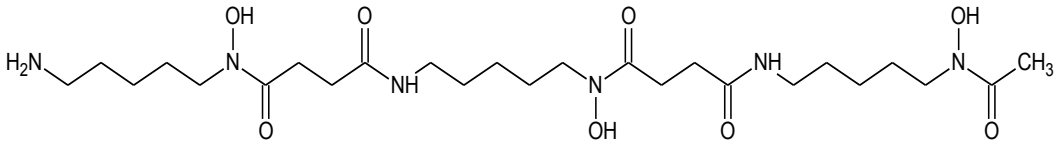

Figure 1.1: The structure of desferrioxamine B, fully extended.

Desferrioxamine B (DFB) is a siderophore, a molecule which chelates iron (III) ions. This particular molecule is naturally produced by prokaryotic organisms such as *Streptomyces pilosus* to acquire the iron they need to survive. DFB forms a hexa-coordinate structure with iron (III) (see Figure 1.2) with a very high affinity in sea water.

The stability constant for DFB binding with iron is typically on the order of 10^{32}. This is about 15 orders of magnitude higher than the stability constants of the next most strongly-bound ion, which is copper (II) (Roy et al., 2008; Hernlem et al., 1996; Martel and Smith, 1982; Witter et al., 2000). The high binding constant and selectivity is because iron (III) forms a hexacoordinate complex with DFB and the cavity size is ideally matched to the size of an iron (III) ion. These two properties make it an ideal molecule to form the basis of a detection system for iron. The high binding constant enables extraction of iron at low concentration levels, and its high selectivity means it is not susceptible to interference or error which may be caused by other transition metals present in the sample. In addition to being produced by prokaryotic organisms, it is also commercially available.

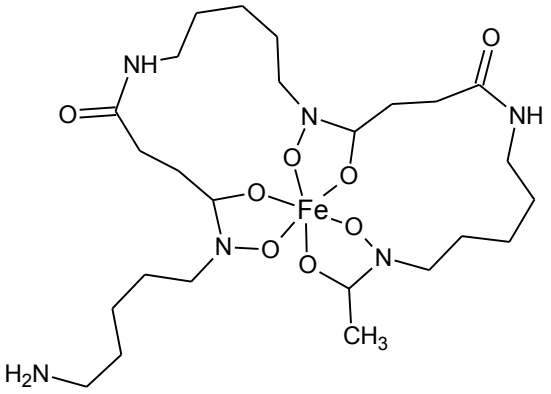

Figure 1.2: The structure of desferrioxamine B complexed with iron (III).

Detecting Iron with DFB and Infrared Spectroscopy

One method of DFB-based iron detection which has been explored is the use of infrared spectroscopy to detect the presence of iron chelated by DFB (Roy *et al.*, 2008). In this method, DFB was attached to a layer of mesoporous silica on a silicon wafer. The

silica layer was functionalized with a carboxylic-acid-containing alkoxy silane. DFB could then be attached to the surface via a reaction with the carboxylic acid groups, using *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) as a catalyst. Figure 1.3 shows an over-view of this process, while Figure 1.4 shows the mechanism by which the reaction with DFB occurs. Figure 1.4 shows that there are side-reactions which also produce other amides. Infrared spectroscopy may not distinguish between them. However, evidence that the treated surface was able to capture iron (III) shows that the reaction successfully produced the carboxylic acid-DFB linkage.

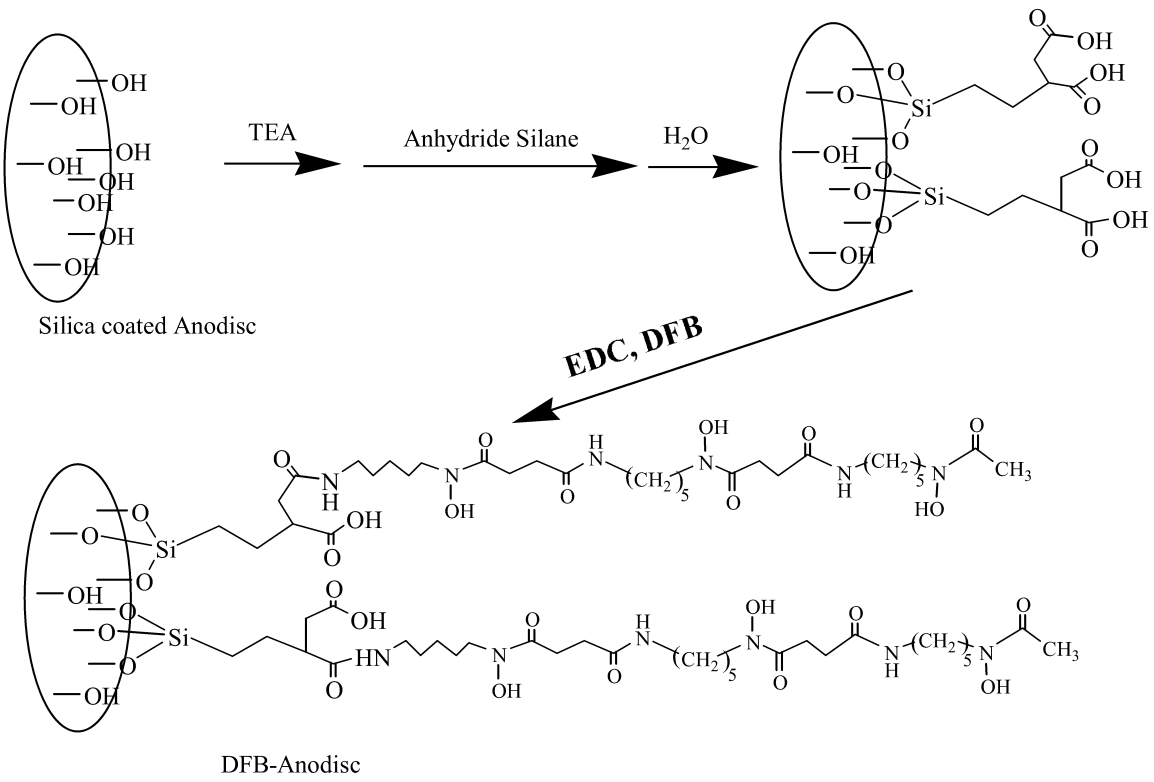

Figure 1.3: Reaction steps for attaching DFB to a mesoporous silica film. Figure by Eric Roy.

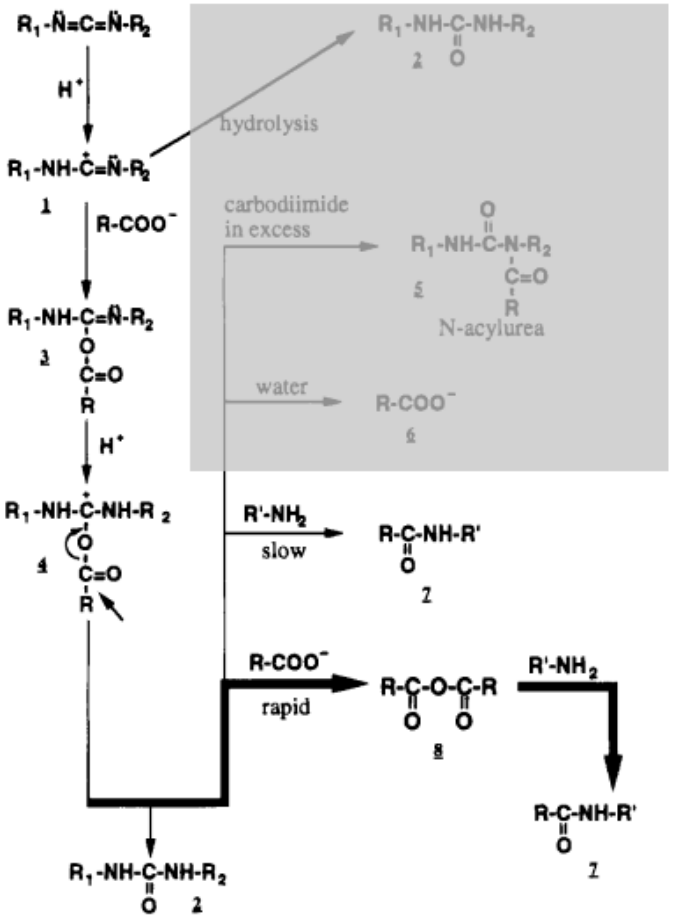

Figure 1.4: Mechanism for the formation of an amide from carboxylic acid and an amine, taken from Bioconjugate Chem. 1995, 6, 123-130 (Nakajima and Ikada, 1995). The gray box indicates side-reactions which can occur under various conditions.

The wafer was exposed to a 1 L sample and the iron would be extracted by the DFB anchored to the silica film. The wafer was then analyzed via infrared spectroscopy. A difference spectrum is produced by subtracting the spectrum of an untreated silica-covered silicon wafer (see Figure 1.5). When the DFB on the derivatized wafer has chelated iron, then the spectrum will contain a band at 560 cm^{-1} due to a Fe-O stretching mode. This band is not present in the infrared spectrum of a wafer without chelated iron. The intensity of this band is proportional to the amount of iron which has been captured,

so it can be used to quantify the amount of iron present (Roy *et al.*, 2008).

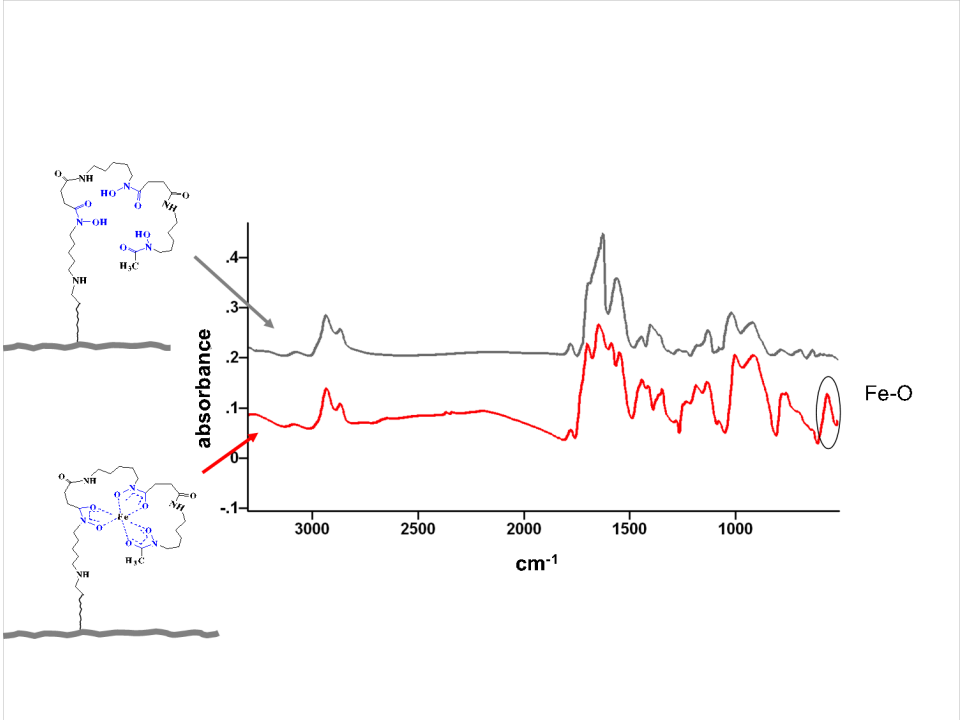

Figure 1.5: Infrared spectrum of a DFB-modified silica film/silicon wafer (gray curve) and after reaction with iron (red curve). A reference was recorded through the un-modified silica film/silicon wafer prior to reaction with DFB (Roy *et al.*, 2008).

It was determined that the presence of other metal cations did not interfere with the detection of iron in this method. Neither iron-containing colloids nor organic compounds containing iron interfered with the Fe-O peak. Furthermore, in field tests conducted off the coast of Alaska, the iron-profile analyses obtained by the IR detection method agreed with values obtained by flow-injection analysis with chemiluminescence detection. Moreover, this method can be done with samples which have been acidified to pH 1.8, which means measurements can be compared with previously-collected data and other standard methods (Roy *et al.*, 2008). However, this technique is still limited to use on a ship. Infrared spectroscopy requires dry samples because water absorbs very

strongly in the infrared spectrum. The presence of water can severely interfere with the ability to detect other bands. Therefore, this method would not be feasible for deployment on buoys and gliders. Additionally, the film was stirred in a beaker for 24 hours to ensure complete mass transport of iron to the wafer. Long stirring times are not practical for use on buoys and gliders.

Derivatization of a Hydrophobic Membrane Using Block Copolymers

In his MS thesis, Zachary Helm worked on an iron detection system which would not be limited by mass transport. Rather than treating the surface of a silicon wafer with DFB, a Teflon® membrane was used instead. It can accommodate a flow rate of up to 10 mL/min; thus, water samples can be flowed through the membrane. However, in order to accommodate these flow rates, the pores have to be large, and as such, the membranes have a relatively low surface area (Helm, 2013).

In order to increase the surface area on these membranes, they were treated with block copolymers of polystyrene and poly(acrylic acid) (PS-b-PAA). This was done by exposing the membranes to a solution of PS-b-PAA in water. The hydrophobic polystyrene portion of the copolymer will adsorb on the membrane simply because it is not water-soluble. The PAA block, which is intimately linked to the PS block, is water-soluble and thus dangles out from the surface into the water. During the initial stage of adsorption, the surface is bare and the blocks adopt a flat configuration on the surface. As time goes on, the surface becomes more crowded and the adsorbed polymer layer rearranges on the surface to accommodate the adsorption of more polymers arriving from

the solution phase. This leaves room for more of the block copolymers to attach to the surface. Eventually, the arrangement of the polymers will approach a brush-like configuration with the PAA extending out from the surface (Helm, 2013; Tripp and Hair, 1996).

DFB then reacts with each acrylic acid monomer unit on the block copolymer via a reaction between the carboxylic acid groups and the amine from the DFB, shown previously in Figure 1.4 (Nakajima and Ikada, 1995). Because the PAA blocks are long chains extending from the surface, this increases the number of binding sites for DFB on the membrane through a process called vertical amplification (Helm, 2013).

While the membranes are opaque in air, they are transparent when wetted by water. This is because the refractive index of the membranes is very close to that of water. When the water displaces the air and wets the surface, there is one continuous phase of similar refractive index and this is what leads to a transparent membrane. Therefore, one can analyze the surface via transmission Ultra-Violet-Visible Spectroscopy (UV-Vis). The DFB-Fe complex has a broad UV-Vis band around 470 nm which is not present when iron (III) is passed through a membrane that does not contain tethered DFB molecules. The peak intensity of this band is proportional to the amount of iron present on the membrane (Helm, 2013).

In the end, Helm found that the DFB-coated membranes were able to extract iron (III) from both fresh and sea water samples. This iron (III) uptake could then be measured by UV-Vis. The uptake efficiency increased at higher pH: between pH 9 and 9.3, the rate constant for DFB-iron (III) complexation increased from 1.65 to 6.87 $M^{-1}s^{-1}$.

Furthermore, decreasing the flow rate increased the percent of iron (III) captured. This indicated that increasing the contact time allowed for more time for DFB to capture the iron. At a pH of 2 and a flow rate of 0.1 mL/min, the detection limit for a 5 mL sample was 0.24 nM. However, it has been found that iron levels in the ocean can be as low as 30 pM. (Helm, 2013) Therefore, the detection limit of this method would have to be improved before it could be used to measure iron in the oceans.

Madhira Gammana, in her Ph.D studies (Gammana, 2014), followed up this work and investigated the underlying mechanism of iron (III) capture on DFB-coated membranes. In her thesis work, PS-b-PAA-coated membranes were reacted fully with DFB and at 50% coverage. She found that a membrane coated with half the amount of DFB possible was able to capture more iron and at a faster rate than a membrane which was fully-coated with DFB. Thus, the density of DFB on the membrane affected the ability of the membrane to take up iron (III) (Gammana, 2014). It was reported that at higher DFB densities, steric hindrance affected the amount of DFB that was active to capture iron (III), as well as impeding the rate of capture, as it hindered movement of the DFB to surround an iron (III) ion (see Figure 1.2).

Detection Limits

In order to get a better detection limit, a method is needed wherein as much iron as possible is placed within the path of the detection beam. Before taking any measurements, one can predict what is necessary to be able to measure iron at sub-nanomolar concentrations. The extinction coefficient of the DFB-Fe complex in solution,

ε, is $2.5\times10^{6}\ {}^{cm^2}\!/_{mol}$. Using this value in a Beer's Law relationship, one can determine how much iron would need to be absorbed in order to get an absorbance of 0.02. This absorbance was selected as a practical detection limit based on the work done in this thesis. For these example calculations, let the measurements take place in a 1 cm cuvette with a UV-Vis beam which has a diameter of 3 mm. In this case, the instrument would be measuring a cylinder the length of the cuvette's width and with a radius of 1.5 mm. Thus, the volume of the cuvette measured will be:

$$Volume = \pi r^2 h = \pi(0.15\ cm)^2(1\ cm) = 0.07065\ cm^3$$

Knowing this value allows one to calculate the amount of iron needed in the sample beam to get an absorbance of 0.02. The calculation is shown below.

$$A = \varepsilon c \mathrm{l}$$

$$c = \frac{A}{\varepsilon \mathrm{l}} = \frac{0.02}{(2.5\times10^{6}\ {}^{cm^2}\!/_{mol})(1\ cm)} = 8.0\times10^{-9}\ {}^{mol}\!/_{cm^3}$$

$$8.0\times10^{-9}\ {}^{mol}\!/_{cm^3} \times \frac{55.845\ g\ Fe(III)}{mol} = 4.5\times10^{-7}\ {}^{g}\!/_{cm^3}$$

$$\left(4.5\times10^{-7}\ {}^{g}\!/_{cm^3}\right)\left(0.07065\ cm^3\right) = 3.6\times10^{-8}\ g = 36\ ng$$

These calculations lead to the conclusion that in order to get an absorbance of 0.02 from the DFB-Fe band, we would need 36 ng of iron to be bound to DFB in the beam. That means that if one wanted to be able to measure an iron concentration of 36 parts per trillion (one ppt = 1 ng/L), one would need to collect the iron from 1 L of 36 ppt water.

Given that the work by Helm showed that flow rates of 0.1 ml/min are needed to

obtain capture rates greater than 90%, it is impractical to pass an entire liter of water through a DFB-derivatized membrane. However, work done by the King group at Colby has shown that it is possible to preconcentrate the iron in the sample by first passing 1 L of test water through a Toyapearl column derivatized with DFB (Helm, 2013). The iron can then be eluted using a reverse pulse through the column of 0.1 M oxalate at pH 1.5. Oxalate is another iron chelator. When at a pH of 1.5, oxalate has a stronger affinity for iron than DFB, so 1.5 pH oxalate will remove iron from DFB. Using this method, the iron could be eluted into a smaller volume, such as 1 mL (Helm, 2013). This is a much more practical volume to pass through a column. The spectrum of this column will tell us how much iron is present in the measured sample. Since the amount of water from which the iron came will be known, the original concentration can be calculated.

By preconcentrating the iron, not only will a measurement take less time, but one can also control the matrix in which the final measurements are performed. A sample of sea water contains different salts, any of which could potentially interfere with measurement. In preconcentrating, one could not only increase the concentration of iron for measurement but ensure that the detection medium is free of contaminants which would interfere with accurate detection.

Iron Detection Using a Transparent Column

Still, preconcentration is an extra step in the detection process. It may be preferable to develop a process in which elution into a small volume to pass through the membrane is not needed. This could potentially be done if, instead of using a membrane

to capture the iron, one uses a column similar to the Toyapearl but which is transparent in the visible region. As with the Toyapearl column, this column would be able to achieve 100 % capture of iron (III) at flow rates of 10 mL/min or higher. However, elution from the column into a small volume to be passed through a membrane is not needed because detection could be performed directly on the transparent column.

In order to make measurements directly on a column, the column needs to be transparent to allow signal through. As part of her thesis work, Gammana tested a couple of different potential materials for the column (Gammana, 2014). The first attempts were with Teflon® and silica beads; however, unlike the membranes, they were not sufficiently transparent in the UV-Vis signal range, so they could not be used. The second attempt was made with polyacrylamide hydrogel beads. When placed in water, these hydrogel beads swell and appear transparent. However, when attempting to derivatize these beads with DFB, Gammana found that they formed a white precipitate. Again, because they would not be transparent once derivatized, they could not be used in UV-Vis analysis.

This thesis presents a proof of concept of principles for iron detection using a transparent column. Here, agarose beads have been selected as the column material. Agarose gel has a refractive index just above that of water (between 1.333 and 1.337) (Byron and Variano, 2013; Gupta et al., 2013) and thus it is semi-transparent in water. Agarose beads (known under the brand name Sepharose) can be purchased at a size range of 45 – 165 μm, which should be large enough to allow for the flow of water. Moreover, they are hydrophilic, so they can be wetted by water.

The research detailed herein was done to study the possibility of using agarose

beads as the basis of a column in an iron detection system. First, we determined that the beads can be treated with PS-b-PAA in the same way we can with the membranes (and thus, they can be derivatized with DFB as well). Then, we set out to determine the transparency of agarose beads in the UV-Vis. Finally, we determined the kinetics of iron uptake by the DFB-derivatized agarose, the saturation point (i.e. the maximum amount of iron which can be captured) and the detection limit.

CHAPTER 2: Experimental Section

Equipment and Materials

Sepharose 4-B (agarose) was obtained from Sigma-Aldrich. Agarose was not used as received because the beads came suspended in a 30% ethanol solution. The bead suspension was centrifuged for 10 minutes to separate the beads from the 30% ethanol solution. The liquid was then decanted and DI water was added. The centrifuge process was repeated 3 times to produce a bead suspension in water.

Deferoxamine mesylate salt (DFB), iron (III) chloride ($FeCl_3$), hydrochloric acid, sodium hydroxide, ammonium hydroxide, potassium bromide (KBr), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were obtained from Sigma Aldrich. DFB, EDC, KBr, and ammonium hydroxide were used as received. EDC was kept frozen when not in use. Hydrochloric acid and sodium hydroxide were diluted as necessary in order to make any pH adjustments required. Polystyrene$_{50}$-b-poly(acrylic acid)$_{180}$ was purchased from ATRP solutions and used as received.

$FeCl_3$ was obtained in powder form. To make a solution of $FeCl_3$, the appropriate amount of $FeCl_3$ was weighed. It was then promptly added to the appropriate amount of DI water. (For example: to make 100 mL of a 100 ppm iron (III) solution, one would weigh out 29 mg of $FeCl_3$ and add it to 100 mL of DI water.) The pH of the mixture was then adjusted to a pH of between 2.5 and 2.8 to slow the precipitation of $Fe(OH)_3$.

A DU 640 Beckman Coulter Spectrophotometer was used to record UV-Vis spectra for the experiments described in the next section. This machine had a resolution

of 2 nm. All other UV-Vis spectra were recorded with an Ocean Optics-USB4000 Fiber Optic Spectrometer with SpectraSuite software. All infrared spectra were recorded using an ABB-Bomem FTLA 2000 spectrometer at 8 cm^{-1} resolutions. For a typical spectrum, 100 scans were used, requiring a total time of 2 minutes. To centrifuge samples, a 3400 RPM Cole Parmer centrifuge was used. Mass was recorded on a Mettler Toledo AG245 Dual Range Analytical Balance.

UV-Vis Spectra of Agarose Suspensions

First, the cuvette was weighed. The cuvette was then filled with water and a reference spectrum recorded. Measurements of transparency of the beads at different concentrations were performed with the suspension added to the cuvette. All dilutions were performed directly in the cuvette. To measure the "concentrated" sample, the cuvette was filled with the suspension after replacing the 30% ethanol with water. The agarose was then allowed to settle and a transmission spectrum was recorded through the settled agarose. The water was decanted off of the agarose and the agarose-filled cuvette was weighed. An equivalent weight of water was added and mixed thoroughly by inverting a stoppered cuvette. A spectrum of the suspension in the cuvette was taken immediately after mixing. For subsequent dilutions, the agarose was allowed to settle, and the water was decanted off. The cuvette was weighed again. Using the weight of agarose left, the amount of water for the required percentage of water was calculated and added. The contents of the cuvette were mixed by inversion and a spectrum was immediately taken.

General Process to Adsorb Polystyrene$_{50}$-b-Poly(acrylic acid)$_{180}$ and then React Desferrioxamine B with Acrylic Acid to Derivatize Agarose Beads

The exact amounts of agarose, block copolymer concentration and DFB/EDC used varied with each sample. The values for the samples whose data will be displayed are recorded in Table 2.1.

Table 2.1: Quantities Used in Preparation of Surface-Modified Agarose Beads

	Initial Agarose Beads	**Copolymer Concentration**	**DFB**	**Initial EDC**
Sample A	~0.033 mol	8.3 x 10^{-9} mol /mL	0.000107 mol	0.000263 mol
Sample B*	~0.33 mol	4.5 x 10^{-8} mol /mL	0.000190 mol	0.000627 mol

*Note: For Sample B, only about 2 g of the agarose was used in attaching DFB.

A solution of the block copolymer was made. The desired amount of DI water was adjusted to a pH around 8 with NaOH. The block copolymer was added to the DI water. The mixture was stirred and heated to a temperature between 50 and 70 °C. This was continued until the block copolymers were dissolved (note: in most cases, the solution was still cloudy, indicating that the polymers probably did not dissolve completely).

The solution was allowed to cool and the agarose was added. The pH of the suspension was adjusted with HCl to pH 3. The beaker containing the mixture was covered in parafilm and the mixture was left to stir at room temperature for 2 to 4 weeks. The infrared spectrum was recorded following the procedures outlined in the next section.

Once the infrared spectra indicated that the block copolymers had adsorbed on the agarose beads, the reaction of DFB with the adsorbed polymers was performed. The agarose beads were separated from the copolymer solution (using the same centrifuge method used to remove the ethanol from the stock agarose). Then, DFB and EDC were added to the agarose-block copolymer in DI water. The mixture was stirred for about two days; in this time, new EDC would be periodically added (usually about 3 – 5 times) because EDC can degrade over time. After two days, a small fraction of the suspension was removed, washed, and dried, and then an infrared spectrum was recorded using the procedure outlined in the next section. Once it was confirmed that DFB had fully reacted with the block copolymer, the DFB/EDC solution was removed using the same washing technique using to remove ethanol from the stock agarose. The end product was called derivatized agarose or ABD (agarose-block copolymer-DFB).

Infrared Spectroscopic Procedure

To obtain an infrared spectrum, the sample was first removed from the solution it had been stored in, unless it was already stored in DI water. To wash a sample, the mixture was centrifuged for 10 minutes to separate beads from liquid. The liquid was then decanted and DI water was added. The centrifuge/decant/add water cycle was repeated 3 times.

A background spectrum was taken with a pressed KBr disk in the beam. In taking the spectrum of plain (underivatized) agarose, the sample was spread onto a premade KBr disk. However, upon drying, the sample became stuck to the disk and the disk had to be

polished to remove the sample. Therefore, for subsequent infrared measurements, disposable KBr disks were made using KBr powder and a hydraulic press.

A small portion of the washed sample was spread onto the KBr disk and allowed to dry for 2 hours. At that point an infrared spectrum was taken and the disk was discarded.

Determining Amount of Time Necessary for Complete Iron Capture by Stirring in a Beaker

This experiment was done to ensure that in subsequent experiments, the derivatized agarose (ABD) was exposed for enough time to capture all iron. First, the pH of the ABD/DI mixture was adjusted to 2.62 (the same as the pH of the iron solution). The UV-Vis spectrometer was then turned on and a dark spectrum background was taken. Then, a cuvette was filled with 2 mL of the ABD/water mixture. The cuvette was placed in the spectrometer beam and the settings on the spectrometer were adjusted until the maximum signal could be obtained through the sample without saturating the detector. At this point, a background spectrum was taken of the ABD/water mixture directly after mixing.

The contents of the cuvette were poured into a small beaker. Then, 100 μL of 100 ppm iron (III) solution were added to the sample (thus adding 10 μg of iron). The sample was stirred in two-minute increments and a spectrum of the sample was taken after each two-minute stirring period. This was continued until the spectra no longer showed significant change in the DFB-iron(III) peak. At the completion of the experiment, the suspension had been stirred for a total of 16 minutes.

Measuring Saturation Point of Iron (III) on a Sample of ABD

First, the pH of ABD/DI mixture to be used was adjusted to 2.62 (to be close to the pH of the iron solution). The UV-Vis spectrometer was then turned on and a dark spectrum was taken by blocking the beam. Then, a cuvette was filled with 2 mL of the ABD/water mixture and a background spectrum was taken through water. A transmission spectrum was taken of the ABD to ensure that the optimum amount of beads was in the cuvette. The optimum amount of beads is the maximum concentration of beads which is still transparent. In order to be considered transparent, this transmission spectrum had to show a typical light scattering curve (as described in the section in Chapter 3 titled "Transparency of Agarose Beads"). A transmission curve which is flat indicates that a sample is opaque, even if the curve is at higher than 0% transmission. Thus, this test was done to determine the maximum concentration of beads which would show a typical light scattering curve.

Once the optimum amount of agarose was determined, a reference was recorded through the suspension. The cuvette was placed in the spectrometer beam and the settings on the spectrometer were adjusted until the maximum signal could be obtained through the sample without saturating the detector. At this point, a background spectrum was taken of the ABD/water mixture directly after mixing.

The sample was transferred to a 10 mL beaker and 10 μL of 100 ppm iron (III) solution was added to it. This mixture was stirred for 15 minutes before it was transferred to the cuvette and an absorbance spectrum was recorded. This process was repeated several times – for Sample B, a total of 12 10-μL additions were made, and for Sample A,

a total of 9 10-μL additions were made.

Measuring Iron Uptake in an Oxalate Environment

This experiment was done using Sample A. First, approximately 100 mL of 0.1 M oxalate solution was prepared. The pH was adjusted to 1.51 with HCl; this required 69 drops of HCl, so a total of 3.45 mL more liquid was added. To this solution 36.7 mg of $FeCl_3$ was added. The pH of the solution was then slowly brought up to pH 7.79 with ammonium hydroxide. The final volume was 119.5 mL, giving an iron (III) concentration of 106 ppm.

The UV-Vis spectrometer was then turned on and a dark spectrum background was recorded. Then, a cuvette was filled with 2 mL of the ABD/water mixture. A background spectrum was recorded with water followed by a transmission spectrum of the ABD suspension. This procedure was performed to ensure that the particles were partially transparent at 460 nm. Next, a new background was recorded through the ABD suspension. The cuvette was placed in the spectrometer beam and the settings on the spectrometer were adjusted until the maximum signal could be obtained through the sample without saturating the detector. At this point, a background spectrum was taken of the ABD/water mixture directly after mixing.

Then, the ABD was transferred to a 10 mL beaker and mixed with 35 μL of the iron (III)/oxalate solution and stirred for 30 minutes. The beads in the suspension turned red in color. After 30 minutes of stirring, the oxalate was washed from the sample using the same method described to wash agarose beads in the first section in this chapter. The

oxalate had to be removed prior to taking the spectrum because oxalate exposed to UV-light forms free radicals, which would destroy the DFB (Helm, 2013). However, the sample was only centrifuged twice because it was found that some beads were lost when decanting the fluid phase. In the interest of keeping the beads, the wash was stopped. The liquid which had been decanted was replaced with DI water and a spectrum was taken of the ABD.

Next the eluent removed during the wash (containing much of the oxalate/iron solution removed) was added back to the sample. This mixture was stirred for an additional 30 minutes. The oxalate was then washed from the sample; once again, the sample was only centrifuged twice to prevent sample loss. The liquid which had been decanted was replaced with DI water and another spectrum was taken of the ABD suspension. This was done to see if exposing the suspension to the iron/oxalate solution for longer would cause an increase in iron uptake.

Extinction Coefficient of DFB-Iron (III) at pH ~ 7.5 – With and Without Oxalate

A 3.44 mM iron (III) solution was prepared by adding 55.8 g $FeCl_3$ to 100 mL of DI water. A drop of HCl was added to the solution to lower the pH to 2.51, preventing the precipitation of iron (III) hydroxide. Using a 1000-µL pipette, the amount listed in Table 2.2 was measured into a 100 mL volumetric flask and then diluted with DI water. Ultimately, four different dilutions of the $FeCl_3$ solution were made and the pH of each was adjusted to around 2.5 with HCl. The concentration of each solution is also recorded in Table 2.2.

Table 2.2: Prepared Solutions of $FeCl_3$ in DI Water

Amount 3.44 mM Iron (III) Solution Added	Solution Concentration
500 µL	0.0172 mM
1000 µL	0.0344 mM
2000 µL	0.0688 mM
5000 µL	0.172 mM

For each dilution, 1000 µL each (as measured by the 1000-µL pipette) of $FeCl_3$ and 2 mM DFB were mixed together. The pH of this mixture was adjusted to around 7.5 with NaOH. Table 2.3 notes the pH of each concentration and the final concentration after mixing with DFB and pH adjustment. An absorbance UV-Vis spectrum was taken of each mixture with DI water as a background.

Table 2.3: pH and Final Concentration of $FeCl_3$/DFB Mixtures

Concentration of Iron (III) Before Mixing	pH	Drops Added for pH Adjustment	Final Concentration of Iron (III)
0.0172 mM	7.52	7	0.00732 mM
0.0344 mM	7.37	11	0.0135 mM
0.0688 mM	7.46	10	0.0275 mM
0.172 mM	7.39	10	0.0688 mM

Note: the drops added were taken into account for the final volume when calculating the final concentration. Each drop was assumed to be 0.05 mL.

A similar procedure was done wherein the original iron solution was made in 100 mL 0.1 M oxalate at pH 1.5 (rather than DI water). In this case, 53.8 mg $FeCl_3$ was used, making a 3.32 mM $FeCl_3$ solution. The following solutions were made:

Table 2.4: Prepared Solutions of $FeCl_3$ in Oxalate

Amount 3.32 mM Iron (III) Solution Added	Solution Concentration
500 µL	0.0166 mM

1000 µL	0.0332 mM
2000 µL	0.0664 mM
5000 µL	0.166 mM

In this case, 1000 µL of each dilution was taken and the pH adjusted to between 2.4 and 2.8 to ensure no iron hydroxide would precipitate. Then, 1000 µL of a 2 mM DFB solution was added to each sample and the pH was again adjusted to 7.5 with NaOH. Table 2.5 notes the pH of each concentration and the final concentration after mixing with DFB and pH adjustment. An absorbance UV-Vis spectrum was taken of each dilution with water as the background spectrum.

Table 2.5: pH and Final Concentration of $FeCl_3$/Oxalate/DFB Mixtures

Concentration of Iron (III) Before Mixing	pH	Drops Added for pH Adjustment	Final Concentration of Iron (III)
0.0172 mM	7.52	7	0.00732 mM
0.0344 mM	7.37	11	0.0135 mM
0.0688 mM	7.46	10	0.0275 mM
0.172 mM	7.39	10	0.0688 mM

In both cases, the absorbance of the DFB-Fe peak was plotted against the final iron (III) concentration. The slope of the best-fit line for each set of data points was then used to calculate the extinction coefficient. This plot is shown in Figure 3.7.

CHAPTER 3: Results and Discussion

Initial Screening of Materials for Transparency in the UV-Vis Spectral Region

The first step was to identify particles suitable for use as a packed column. The criteria for selection were that the material 1) had a refractive index similar to that of water and 2) was commercially available in particulate form with a particle diameter size between 35 and 200 μm. The refractive index needed to be matched to that of water to reduce the scattering of light, causing the beads to be transparent in the UV-Vis spectral region. The particles needed to be larger than 35 μm to ensure that water could flow through the particles at a reasonable rate, e.g. 10 mL/min. At the same time, the particles needed to be smaller than 200 μm because larger particles have a lower surface area, which would reduce the density of DFB in the UV-Vis beam area. As discussed in the Introduction, this reduction in surface area can be off-set by the application of block copolymers to the surface, which amplify the surface area. Still, if the particles are too large, the block copolymers will not amplify the surface area sufficiently.

The materials listed in Table 3.1 were chosen for initial tests based on the criteria described.

Table 3.1: Properties of Potential Column Materials

Material	Particle Size (μm)	Refractive Index
Teflon®	35	1.34 – 1.35
Glass Beads	Up to 20	1.43 – 1.46
Polyacrylamide Hydrogel	45 – 90 (wet)	1.332
Agarose	45 – 165	1.333 – 1.337

It was found that the silica and Teflon® beads were opaque in water, both as received and after adsorption of the block copolymers. The polyacrylamide beads were transparent in water in the UV-Vis region as received and after block copolymer adsorption. However, they decomposed after the reaction between the adsorbed copolymers and DFB (Gammana, 2014). As discussed in the next section, agarose beads were found to be transparent in the UV-Vis region. Furthermore, after adsorption of the block copolymers and derivatization of DFB, the particles turned red, indicating that the beads successfully formed the DFB-Fe complex. Thus, the best candidate for these experiments was found to be agarose.

Transparency of Agarose Beads

Figures 3.1 and 3.2 are the recorded transmission spectra of agarose suspensions in a 1 cm cuvette as a function of concentration. The term "concentrated" refers to beads which were allowed to settle to the bottom of the cuvette. This in effect mimicked a packed column of 1 cm pathlength. These curves show that agarose is more transparent at longer wavelengths than at shorter wavelengths. At all concentrations, the transmission is greater than 20% at 1000 nm and decreases at shorter wavelengths. These are characteristic curves due to light scattering; thus, the decrease in transparency is not due to light absorption but scattering.

Over all, Figure 3.1 and 3.2 also show that agarose transmits light at relatively high particle densities in the wavelength region of relevance. In the region in which the DFB-Fe peak appears (λ_{max} = 470 nm), the concentrated agarose had around 4-7%

transmission (depending on the exact wavelength). As the amount of agarose with respect to water is decreased, the transmission increased. The percent transmission increases to around 16-20%, 25-32%, 31-37%, 48-54%, and 68-73% for 50%, 40%, 30%, 20%, and 10% agarose respectively.

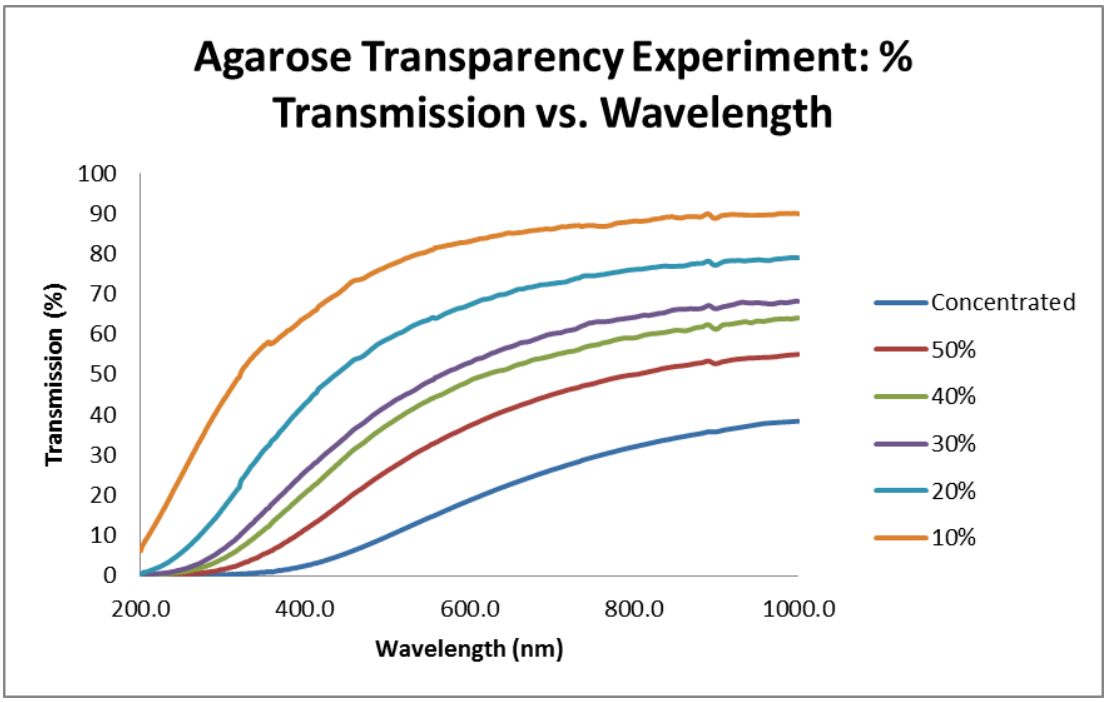

Figure 3.1: Transmission (%) vs. wavelength curves for different concentrations of agarose in water.

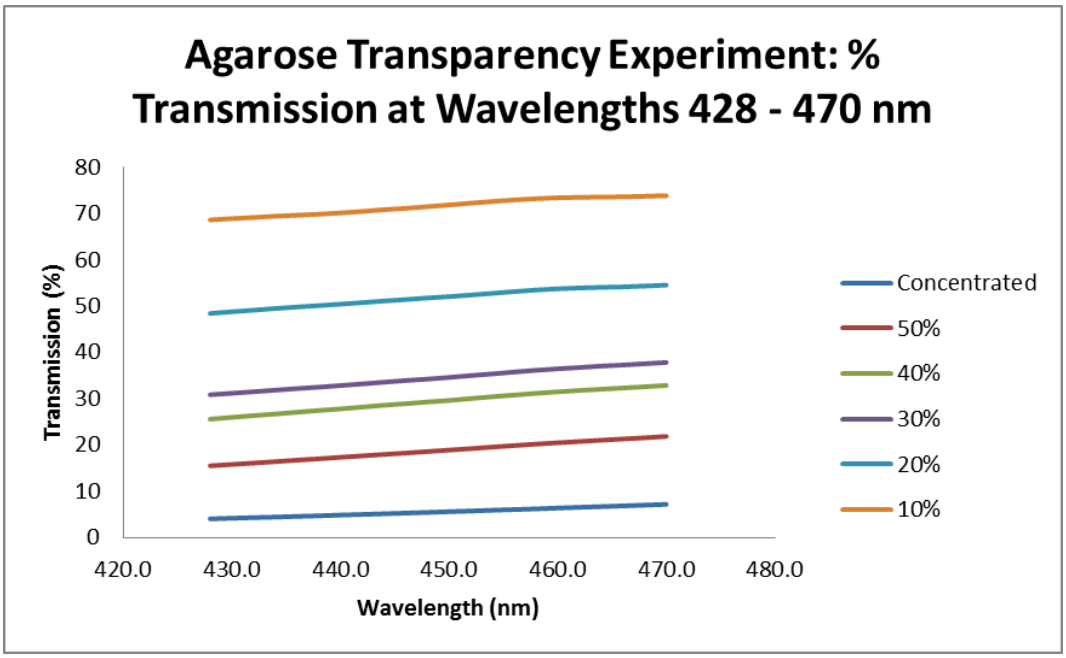

Figure 3.2: This figure shows the section of the above figure in which the DFB-Fe peak should appear.

Even 4% transmission is sufficient to obtain spectra with sufficient signal-to-noise for detection of iron. In the Introduction, it was shown that 1 L of a 36 ppt iron (III) solution would produce a band at 470 nm of 0.02 absorbance. In recording a 100% baseline spectrum through the concentrated agarose in a 1 cm cuvette (in other words, recording a reference spectrum through the agarose immediately followed by a transmission spectrum through the same agarose), peak-to-peak noise levels of 0.001 absorbance were obtained with 1 minute scan times. Thus even at 4% transmission a signal-to-noise of 10:1 would be obtained for a band intensity at 470 nm of 0.02 absorbance.

There were additional hurdles identified in recording transmission spectra through agarose suspensions. At higher concentrations, the beads settled to the bottom of the

cuvette in a matter of minutes. Because of this, the amount of beads probed by the beam varied with time, causing a variation in signal intensity. We attempted to solve this problem by using a stirrer in the cuvette. However, the stirrer only stirred beads near the bottom of the cuvette and it would often get stuck to the side of the cuvette, further inhibiting stirring. Thus, stirring did little to solve this issue.

Adsorption of the block copolymers on the surface of the beads resulted in more stable suspensions which took longer to settle. Therefore, optimization of the particle concentration to minimize settling was only done after adsorption of block copolymers on the surface followed by derivatization with DFB. This is discussed in a later section. Note that any issues with particles settling are only relevant to our cuvette-based measurements. These issues would not apply to a packed column.

Adsorption of Polystyrene-b-Poly(acrylic acid) and Derivatization with DFB

The use of block copolymers to provide vertical amplification of Teflon® membranes has been demonstrated (Gammana, 2014); (Helm, 2013). In this earlier work, the amount of block copolymer adsorbed and the subsequent reaction of carboxylic acid groups with DFB was monitored with infrared spectroscopy. However, using this approach to monitor the derivatization process on agarose beads was somewhat difficult. This was because there were several strong agarose bands throughout the infrared region and removing water from the agarose also proved to be very difficult.

As seen in Figure 3.3, the changes in the spectra of agarose as it is derivatized are just barely visible above the bulk modes. The fact that they are small is to be expected

because the surface area of the beads is relatively low. When determining whether the block copolymers have attached to the surface, one looks for the presence of a small peak in the spectrum, around 1730 cm^{-1}, due to the carbonyl stretching from the poly(carboxylic acid) portion of the polymer. In the work done on the Teflon® membranes, there was another peak at 700 cm^{-1} due to the styrene groups; however, this band was not visible in the spectra of the ABD. Even after drying, there is some water in the beads, so there is a larger peak around 1640 cm^{-1} which overlaps with the carbonyl stretching region. Nonetheless, a small peak in the infrared spectrum has been circled and provides some evidence that the block copolymers have been adsorbed.

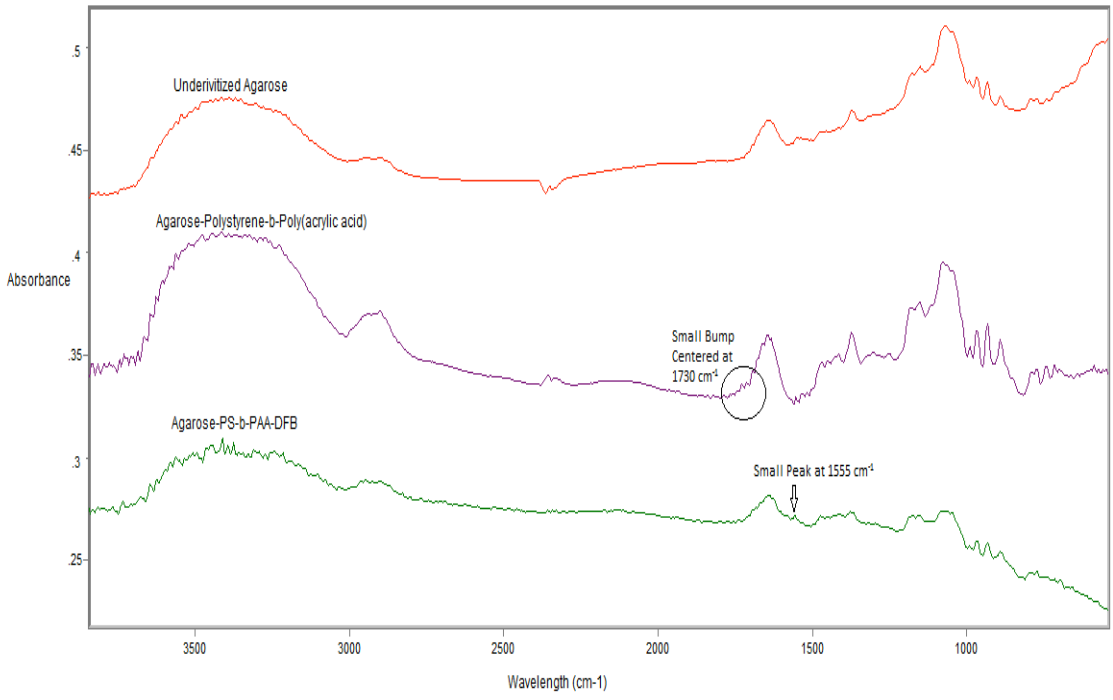

Figure 3.3: Infrared spectra of agarose prior to derivatization (the red curve), agarose which has been treated with polystyrene-block-poly(acrylic acid) (the purple curve), and agarose after the block copolymer has been reacted with DFB (the green curve).

A stronger indication that the block copolymer adsorbed on the agarose beads was the change in behavior of the agarose suspension. Block copolymers are often used to prevent flocculation and settling of particulate suspensions. When the block copolymer adsorbs, the charged poly(acrylic acid) block extends out from the surface and electrostatically repel other particles. This is a process known as steric stabilization. In our case, after stirring the agarose beads with the block copolymers for 2 to 4 weeks, the particles were more stable and did not settle as readily from the suspension.

The next step was the reaction of DFB with the carboxylic acid groups of the poly(acrylic acid) block. When DFB reacts with the carboxylic acid groups, one expects to see the carbonyl stretching peak disappear and amide peaks to appear. As shown in the bottom spectrum in Figure 3.3, there is no longer a peak at 1730 cm^{-1}. At the same time, a very small peak around 1550 cm^{-1} (in the case of this particular sample, it was 1555 cm^{-1}) appears, which is one of the expected amide peaks. In theory, there should also be a second amide peak around 1650 cm^{-1} but in this case it is obscured by the much stronger water peak at 1640 cm^{-1}.

These changes in the infrared spectrum suggest that the block copolymers adsorb on the surface of the agarose beads and that the copolymers, in turn, are derivatized by DFB. However, more direct proof is provided by other evidence. As mentioned above, the agarose suspension was more stable after adsorption of the block copolymer on the surface. The same can be said of the derivatization of the agarose to contain DFB anchored to the block copolymers on the surface. In this case, when the DFB derivatized particles were immersed in a solution containing iron (III) the particles turned red in

color. When iron (III) is chelated by DFB, the resulting complex is red, so the fact that the particles turned red is evidence that the surface was successfully derivatized. In a control experiment, the agarose particles containing only the adsorbed block copolymer did not turn red in color when exposed to a solution containing iron (III).

Minimizing the Effect of Settling

Although agarose particles are more stable after derivatization, they still do settle out to the bottom of the cuvette with time. In Figure 3.4, the UV-Vis transmission spectra for ABD are shown as a function of settling time.

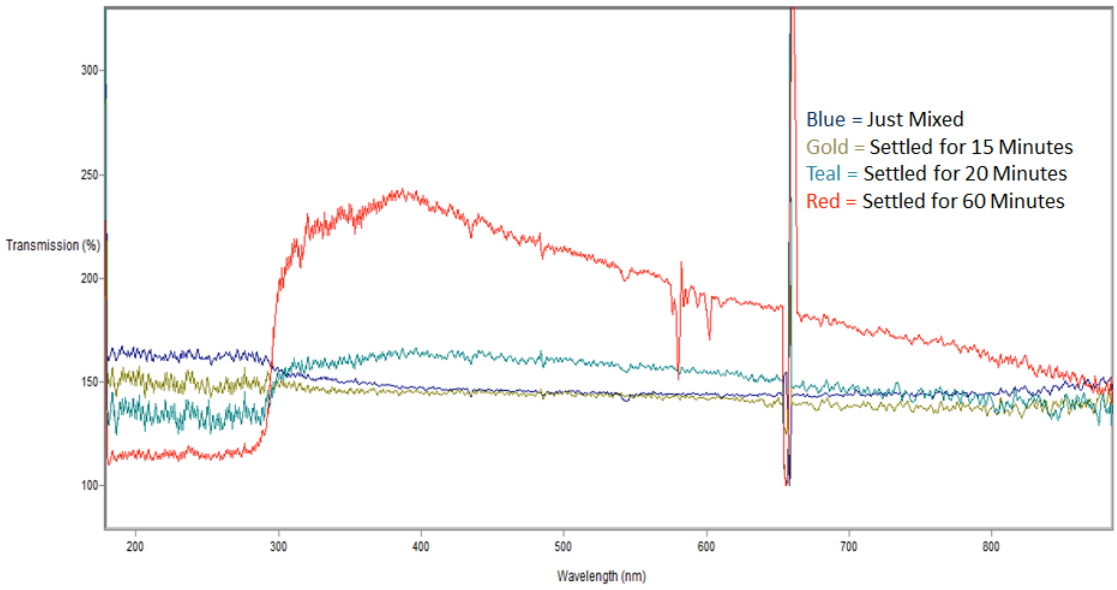

Figure 3.4: UV-Vis spectra of ABD in water. The blue curve is the spectrum recorded just after mixing the suspension in the cuvette. The gold, teal, and red curves show the UV-Vis spectrum after the agarose was allowed to settle for 15, 20, and 60 minutes, respectively.

These spectra show that transmission remains constant at 100% for the first 15 and 20 minutes. A constant value near 100% indicates no change in the amount of agarose probed by the beam. After about 50 minutes of settling (10 minutes before the

red curve was taken), it was observed that most of the agarose beads had settled to the bottom and the transmission through the cuvette increases above 100%. Therefore, as mentioned previously, it is not feasible to do experiments wherein the agarose remains in the cuvette longer than 20 minutes without stirring. Using a stir bar inside the cuvette was ineffective. Instead, the sample was stirred in a beaker and only added to a cuvette for the time required (no more than 1-2 minutes) to take a measurement. This did not allow the agarose to settle sufficiently to alter the baseline significantly.

Determining Amount of Time Necessary for Complete Iron Capture by Stirring in a Beaker

The experiment discussed in this section was done to determine the amount of time the agarose needed to be stirred in order to ensure a complete reaction with DFB. The baseline spectrum, shown in Figure 3.5, was taken prior to contact with an iron (III) solution and thus it does not have any DFB-Fe peak. When the agarose was exposed to a solution containing excess iron (III) (100 μL of a 100 ppm iron (III) solution), a broad band appeared which was centered at 470 nm. This peak is due to the complexation of iron (III) with DFB. The peak increases in intensity for the first six minutes (there is a difference between 2 minutes and 4 minutes as well as a difference between these two spectra and the spectra for longer contact times). However, the peak intensity reaches a plateau after 6 minutes. This shows that the iron is probably fully captured after about 6 minutes. To be on the safe side for subsequent experiments, it was decided that samples should be stirred for 15 minutes after each new iron addition.

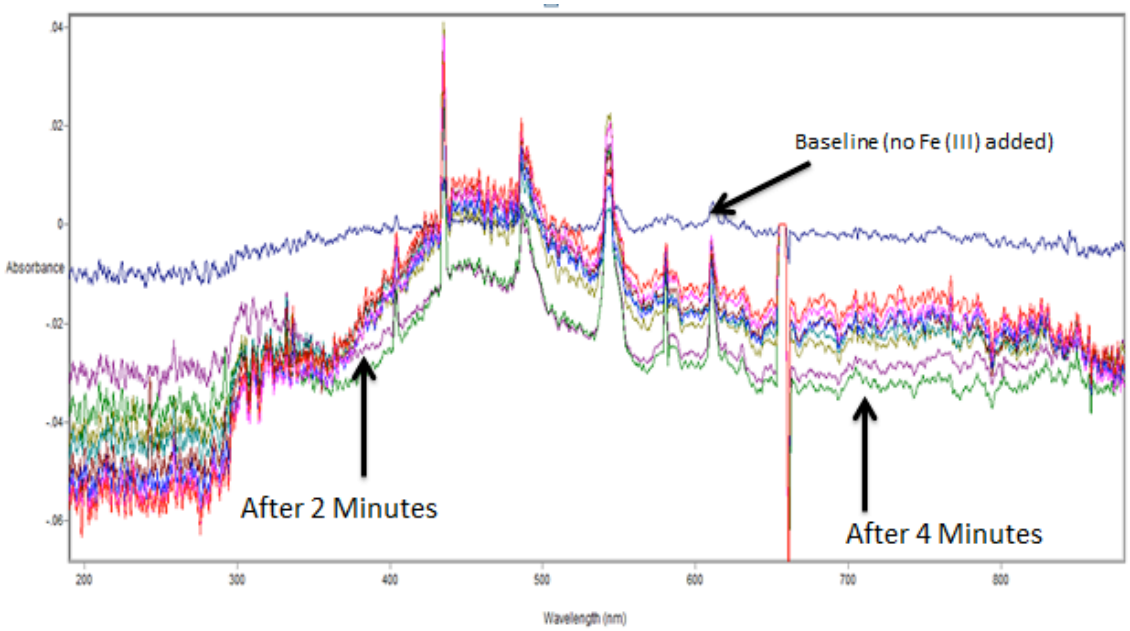

Figure 3.5: UV-Vis spectra of ABD which was mixed with 10 µg of iron (III). The blue curve which is pointed out was taken before the iron was added. Spectra were taken after several two-minute increments of stirring.

Measuring Saturation Point of Iron (III) on a Sample of ABD

In these experiments, a suspension of ABD was exposed to different amounts of iron (III) and stirred for 15 minutes. To select the bead concentration, transmission spectra were taken of ABD/water mixtures with water as a background. The maximum concentration which would give a scattering profile in the transmission spectrum was used. Unfortunately, the ABD beads were not as transparent as their underivatized counterparts, so a lower concentration (<10%) had to be used.

A UV-Vis spectrum was taken and the intensity of the peak centered at 470 nm was measured. Figure 3.6 is a plot of the intensity of this peak versus the amount of iron (III) added to the sample. The amount of iron (III) is shown in units of mass because the concentration was changed each time more iron (III) was added. The two curves

represent two different batches of ABD. Despite the fact that these two samples were not controlled to have the same amount of DFB, they both seem to plateau after 5 or 6 µg have been added. This shows some repeatability in the synthetic steps of block copolymer adsorption and DFB reaction. Furthermore, when they plateau, they each seem to plateau at an absorbance around 0.016. This indicates that these two samples probably have around the same amount of available DFB on them.

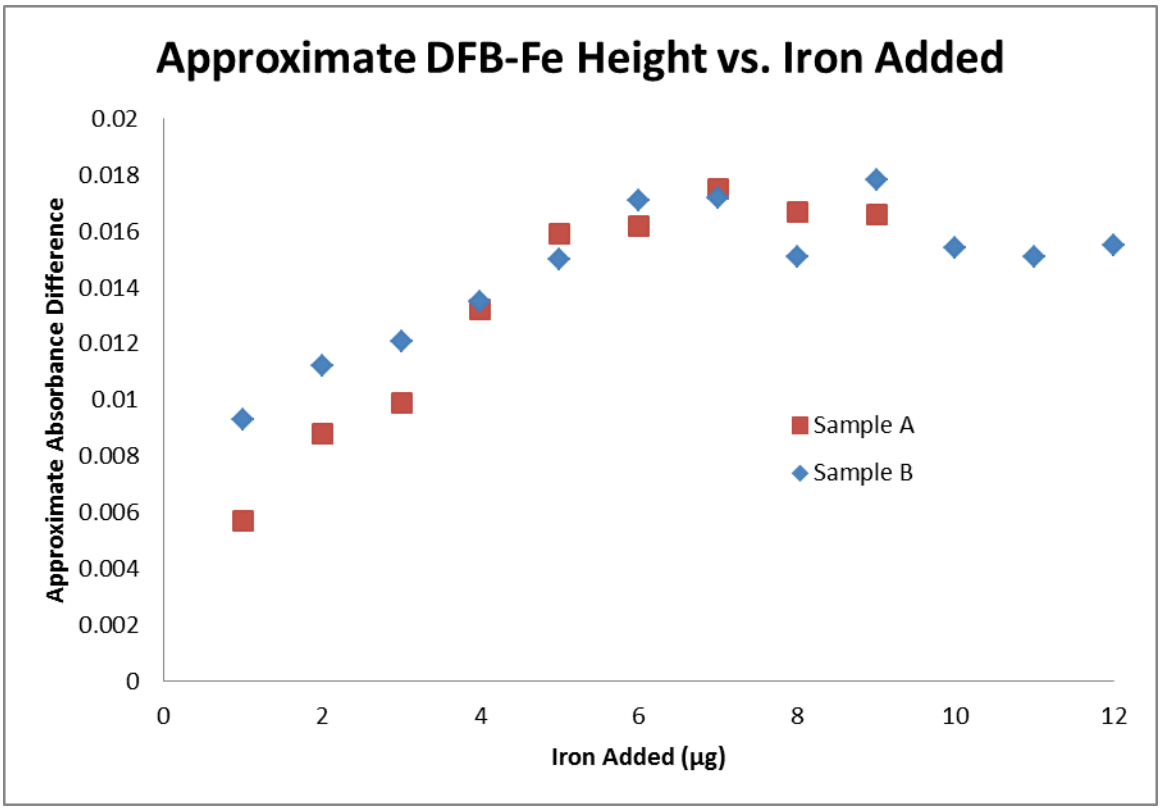

Figure 3.6: Intensity of 470 nm band versus amount of iron (III) added. Samples A and B refer to different batches of ABD.

For both samples, there is a linear portion of the graph which increases as the amount of DFB increases. As mentioned previously, the graph plateaus after 5 or 6 µg were added – this is because all available DFB was used up, so no more iron can be

chelated.

Knowing how much iron was added to saturate the DFB, we can determine how much DFB was in the sample beam. Sample A appears to plateau at 5 μg. We will assume that this became evenly-distributed throughout the sample. In the introduction, the volume which is probed by the sample beam was calculated to be 0.07065 cm^3. The total volume was 2.05 mL because the original agarose suspension volume was 2 mL and then five 10 μL-aliquots of 100 ppm iron (III) solution was added to it. This means that about 3.4% of the solution was analyzed by the UV-Vis beam. Thus, the amount of iron actually being analyzed is 0.17 μg, or 3.1 nmol. For every mol of iron captured, there must be 1 mol of DFB to capture it, so there were 3.1 nmol of DFB in the beam. Sample B appears to plateau around 6 μg, so by similar reasoning we can calculate the amount of DFB in the beam on that sample. In doing so, we conclude that there was 3.7 nmol of DFB probed by the beam in Sample B.

This experiment also seemed to indicate that it may be difficult to get 0.02 absorbance in a detector using ABD. After all, both samples seemed to plateau before even getting to that point. Thus, at the very least, the system will need to be adjusted to increase the density of DFB in the beam to improve the range of detectability – either the amount of DFB on the agarose or the concentration of agarose will need to be increased.

Measuring Iron Uptake in an Oxalate Environment

In this experiment, ABD in water was exposed to iron (III) in a solution of oxalate at a pH around 7.5. Oxalate is another iron chelating agent with a pK_a of 1.23 (Perrin,

1965). The oxalate-iron (III) complex absorbs light at 426 nm, which is near where the DFB-iron (III) peak is (Pitter, 2005). At a pH of 1.5, oxalate's binding constant for iron is higher than that of DFB. However, at a higher pH, DFB is the stronger iron chelate. This experiment was done because if a preconcentration step is required (as discussed in the introduction), the iron applied to the ABD column will be in an oxalate solution. Thus, it is important to know how the presence of oxalate affects the ability of this system to detect iron (III).

ABD was mixed with 3.7 μg of iron in oxalate for 30 minutes. An attempt was made to wash the oxalate off, but some agarose was lost in the process and thus the wash was stopped earlier than originally intended. Therefore, it was likely that some oxalate still remained on the sample. Then, the agarose was mixed with iron in oxalate for another 30 minutes and washed again. A UV-Vis spectrum was taken both times. Although some oxalate likely remained in the sample, a peak was still visible. After 30 minutes, the peak intensity was about 0.031 absorbance, while after another 30 minutes, the peak intensity increased to about 0.043 absorbance.

In comparing these values to those obtained in Figure 3.6 (where agarose was exposed to iron without oxalate), it appears that more iron was chelated by the agarose in this experiment than in the experiment described in the previous section. At first, this may seem surprising because only 3.7 μg of iron were added—this is below the saturation point measured for this batch of ABD (Sample A), yet the absorbance for the DFB-Fe peak is much higher than it was at any point in the experiment done without oxalate. Moreover, a similar concentration of agarose was used.

However, the experiment described in the previous section was done at pH 2.5 in order to prevent the precipitation of iron from solution as iron (III) hydroxide. The DFB chelation with iron (III) is much more effective at a higher pH of 6 or 7 because DFB has been deprotonated to form DFB^{-3}. Therefore, at higher pH, more of the DFB are available to bind iron (III). It is also possible that the agarose were not uniformly derivatized and the portion used for this experiment happened to have more DFB.

Extinction Coefficient of DFB-Iron (III) at pH ~ 7.5 – With and Without Oxalate

Figure 3.7 shows the DFB-Fe peak absorbances at varying concentrations of iron (III) in the presence of excess DFB. In one instance, the iron (III) solution was prepared in DI water while in another instance, the iron (III) solution was prepared in a 0.1 M oxalate solution at pH 1.5.

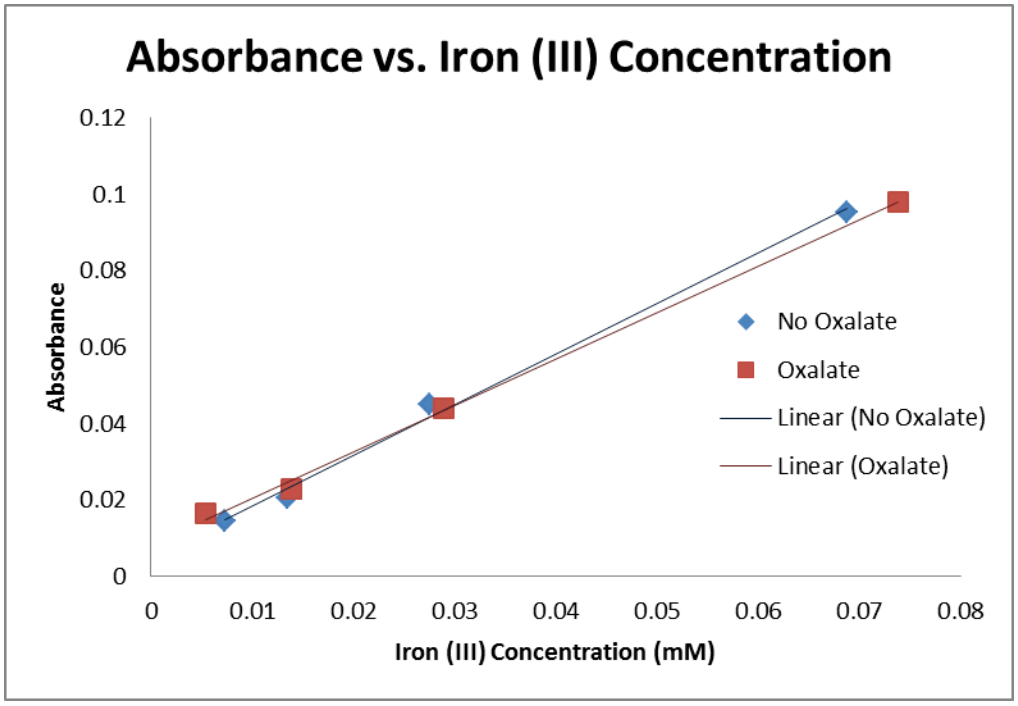

Figure 3.7: Intensity of the DFB-Fe UV-Vis peak at 470 nm as a function of the concentration of iron (III) at pH 7.5. The blue diamonds indicate values obtained with no oxalate present while the red squares

indicate values obtained with oxalate present. The best-fit lines for each set of data are also included.

For each case, the slope of a best-fit line for the data was used to calculate the extinction coefficient. To understand how this was done, note that using Beer's Law, the equation for this line can be written as:

$$A = \varepsilon l c$$

Thus, the slope represents εl and has units of mM^{-1}. To find the extinction coefficient, one merely needs to divide the slope by 1 cm and use dimensional analysis to convert this value to cm^2/mol. Without oxalate, the extinction coefficient was 1.3 x 10^6 cm^2/mol and with oxalate, it was 1.2 x 10^6 cm^2/mol.

These two extinction coefficients are very close in value, indicating that the presence of oxalate at pH 7.5 does not significantly interfere with formation of the DFB-Fe complex. This information indicates that one can take measurements of iron (III) in the presence of oxalate at pH 7.5. If one were to create a detection system in which preconcentration on Toyapearl was used in collecting the iron, there would likely be oxalate present when measurements took place. (Although the oxalate may be washed away, it may not be possible to get rid of it entirely.) Thus, it would be important to know if oxalate could interfere with iron (III) measurements.

Conclusions

The initial experiments where the absorbance of agarose compared to water showed that agarose beads are partially transparent in water. This showed that it may be possible to make UV-Vis spectroscopy measurements directly on the surface of agarose

beads. Infrared spectra showed that it is possible to attach polystyrene-b-poly(acrylic acid) to the surface of agarose beads. It is further possible to react DFB with the poly(acrylic acid), effectively attaching DFB to the agarose beads as well, creating DFB-derivatized agarose or ABD.

Based on calculations reported in the Introduction, it is likely that a preconcentration step will be necessary in order to measure iron (III) at the concentrations common to the world's oceans. The preconcentration step involves collecting the iron on a DFB-derivatized column of Toyapearl and then eluting off into a smaller volume with oxalate. This means that when the agarose column is exposed to this iron, oxalate will be present as well. However, at a pH of 7.5, the presence of oxalate does not appear to have a significant impact on the measurement of iron (III) in solution. Thus, though the oxalate would probably be washed prior to measuring (since oxalate can form radicals when exposed to UV light), measurements are unlikely to be affected if some trace oxalate remained.

When DFB is stirred with ABD, several experiments have shown that a broad peak around 430 – 470 nm does appear. This is the expected peak for DFB which has chelated iron (III), so it is clear that the DFB on the ABD is still able to capture the iron. However, the two samples of ABD tested so far were only able to take up about 5 or 6 μg of iron, producing absorbances around 0.016. In order to have a strong signal, it would be ideal to have a maximum absorbance which is higher than that, because peaks which are much smaller than that can be difficult to observe.

It has also been shown that the derivatized beads can detect iron (III) when the

iron is added in the presence of oxalate. At the same time, there is evidence that at a higher pH, more iron will be chelated than at a lower pH. However, in order to make a true comparison of iron uptake with and without oxalate present, more data points would be needed and the oxalate would need to be properly washed away in between scans. This could be one future experiment.

The main goal of any other future experiments would be to increase the maximum amount of iron (III) which can be measured with the ABD. This could be done either by increasing the concentration of ABD or by increasing the amount of free DFB on the agarose beads. The former is somewhat unlikely. For these experiments, the transparency of the beads was checked by measuring the transmission spectrum of the beads with water as a background. The highest possible concentration of ABD which would result in a scattering pattern (i.e. a slanted baseline) was used because this indicated that some light is actually getting through the beads (as opposed to merely transmitting between the beads). Thus, increasing the concentration of the ABD as-is is unlikely to succeed.

On the other hand, it may be possible to increase the amount of DFB on the beads, thus allowing them to capture more iron. Perhaps if a greater amount of block copolymer is used or the sample is allowed to stir for longer in the block copolymer solution, more DFB could be attached. Thus, potentially, the beads could capture more iron, allowing for a higher maximum uptake. On the other hand, if this results in very tightly-packed DFB, then this could hinder the ability of the DFB to chelate iron. This is because DFB must wrap around into a curl in order to chelate the DFB. If the DFB are too tightly-packed, then they may not be able to curl around the iron. Thus, even if more DFB is attached, if

adding more means tight packing, it may not increase the ability of the beads to acquire iron.

CHAPTER 4: Future Work

Although in this thesis it has been shown that it is possible to detect iron collected on derivatized agarose beads (ABD), it would be ideal to detect iron by measuring it in solution with DFB. The agarose beads, while somewhat transparent, still do scatter light. This can cause alterations in the baseline between measurements, ultimately leading to difficulty measuring the intensity of the DFB-Fe peak at 470 nm in the spectra. In order to put detection systems on buoys and gliders, the measurement process will need to be automatized. However, if the spectra have artifacts in the baseline, automatically calculating the peak height may not result in accurate and precise measurements.

The main reason one cannot simply add DFB to a sample of ocean water is that the concentrations are too low for a UV-Vis spectrometer to measure. However, it may be possible to preconcentrate the iron into a small volume, making the concentration high enough to measure. The signal could be further enhanced if the sensitivity of the instrument were improved.

Recently, Rushworth *et al*. were able to perform trace metal analysis of iron (II) and nitrite in water using cavity ringdown spectroscopy. In this type of analysis, the sample is placed in a flow cell which is situated between two mirrors. A laser is pulsed through the cell, and the beam bounces off the two mirrors, causing it to go through the sample many times. The signal eventually decays because the second mirror is not perfectly reflective, and light which leaks through the mirror is captured in the detector behind this second mirror. The decay in the signal is analyzed to determine how much

light was absorbed by the sample. The detection limits were 1.9 nM for nitrite and 3.8 nM for iron (II) (Rushworth *et al*., 2013).

Cavity ringdown spectroscopy can be used to enhance signals because the signal goes through the sample many times. Therefore, it can be used to measure trace amounts of materials which would otherwise be undetectable by a normal spectroscopy method. Cavity ringdown spectroscopy may make it possible to detect iron (III) in solution. To determine if this is a feasible way to improve our ability to detect iron in solution, we will measure an iron-DFB complex in solution via cavity ringdown and regular UV-Vis and compare the two to determine how much cavity ringdown can improve the signal from the sample.

We will also explore another method of capturing iron from the solution. It has been established that iron (III) can be collected with a Toyapearl column derivatized with DFB. Our work showed that iron (III) can be collected by stirring agarose beads in a beaker. The difficulty is in capturing the agarose beads afterwards for analysis by UV-Vis spectroscopy. However, this could be circumvented using magnetic particles. While normal iron beads would be a source of contamination, one can also buy carbon-coated magnetic cobalt beads known as TurboBeads. The TurboBeads are 50 nm diameter and would be surface treated with the block copolymer and derivatized with DFB following the protocols developed in this thesis. In theory, if stirred in a sample of water, these beads would collect all the iron in the water. Large initial volumes (1-10 L) could be used to increase the amount of iron(III) captured on the beads and hence improve the detection limits. Then, using a magnet, one could easily collect the beads and siphon off the water.

The beads could then be put into a small volume (1 mL) of oxalate solution at a pH of 1.5, taking the iron off of the beads. DFB would be added to the small volume of oxalate and raising the pH to 7 would cause the iron to chelate to the DFB instead, producing a red color which could be measured directly in solution. There would be no baseline issues as spectroscopic analysis would be performed in solution. This concept would not have been developed without the fundamental work performed in this thesis.

We plan to determine whether it is feasible to collect the iron in this manner. First, we need to determine what volume of water the beads can easily be collected from, as well as whether water can be eluted off easily without losing any of the beads. Second, we need to determine whether oxalate can successfully remove iron from the beads. This can be done by attempting to remove the iron with oxalate and then adding DFB to see if a signal appears. Then, to measure the kinetics of the beads' iron uptake, we would measure the DFB-Fe signal after the beads are exposed to an iron solution for varying amounts of time. Ultimately, we would need to determine how much of the iron in the original solution could be taken up by comparing a measurement of iron in the original solution to a measurement of iron after the iron has been collected and concentrated by the beads. This will form the basis of my M.Sc. work.

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Kaiya Hansen was born in Whitefish, Montana on November 14, 1991. She was mostly raised in South Portland, Maine and graduated from South Portland High School in 2010. She majored in chemistry and has a minor in mathematics. She also received the Top Scholar Award for the duration of her matriculation at the University of Maine.

Upon graduation, Kaiya is planning to stay at the University of Maine and continue to work on the project started in this thesis (as described in Chapter 4). In the process, she plans to earn a Master's Degree in Chemistry.