Studies into Solvatochromic Nile Red as a Model Bioactive Agent: Removal by Chitin Compared to Chitosan

by

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Abstract

Syntheses of a Nile Red derivative were attempted by two different methods. Thin Layer Chromatography, Dry Column Chromatography and Fourier Transform Infrared Spectroscopy were used to determine whether the syntheses were successful. Pure purchased Nile Red was used to determine solvatochromism in various solvents using full wavelength scans on the Beckman Coulter DU720 General Purpose UV/Vis instrument. Test tube tests were conducted using chitin and chitosan separately by two methods, centrifuging and shaking, to determine if chitin or chitosan could sorb the Nile Red in 80:20 methanol: water solution. The shaking method involved four minutes shaking and two hours sitting, while the centrifuge protocol after a period of standing involved four minutes in the centrifuge and one hour standing. Once each test tube was done setting; the solution was extracted and was analysed by full wavelength scans on the Beckman Coulter DU720 General Purpose UV/Vis instrument. Results were analysed by comparing maximum absorbance and Freundlich Isotherm plots for each of the chitin/chitosan in 80:20 methanol: water Nile Red solution test tube tests for linearity and absorption capacity. The Nile Red binding to chitin and/or chitosan was used as a model for chitin/chitosan uptake of bioactive agents for potential wastewater treatment.

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Chapter 1. Introduction

1.1 Solvatochromic Dyes

A solvatochromic dye is an organic dye that changes color based on solvent polarity. Usually, an increase in polarity causes a bathochromic (red) shift, and a decrease in polarity causes a hypsochromic (blue) shift; the reverse order for polarity shifts would be called reverse solvatochromism.[1,2] Solvatochromic dyes have a high voltage sensitivity and may be used in color sensors, photosensitizers, photovoltaic devises, and monitoring neurological activity.[1,2]

1.1.1 Nile Red

Nile Red is a solvatochromic phenoxazinone dye[2,3] (Figure 1). Nile Red has a wavelength of 530 nm in apolar solvents and a wavelength of 640 nm in polar solvents.[4] The red shift from non-polar to polar solutions decreases the yield, or fluorescence of the solution, due to aggregation of the dye molecules.[3,4] The organic azo dye has poor solubility in some aqueous solutions, so it is difficult to obtain spectra.[4] The solubility of Nile Red is increased in acidic media; therefore, it should be observed in acidic conditions.[5] The color of Nile Red also degrades much faster in basic conditions compared to acidic; Nile Red may last months in acidic media, compared to days in basic.[5] The maximum absorbance is relatively stable, and should not shift significantly when subjected to dipole forces such as hydrogen bonding.[5] Since Nile Red is color sensitive to changes in the solvent, Nile Red can be used in comparing uptake or polarity changes in a sample.

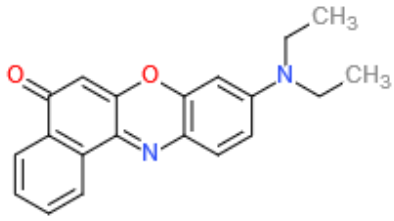

Figure 1 Chemical structure of Nile Red

1.2 Chitin/Chitosan

Chitin (Figure 2) is a component in shells of crustaceans, such as lobster, shrimp and crab, as well as in the exoskeletons of insects. Chitin is a long chain polysaccharide polymer with a structure similar to that of cellulose. Chitosan (Figure 3) is a derivative of chitin formed by deacetylation by baase,[6] which may be soluble in dilute aqueous solvents.[7] The structure of chitosan may be altered by processes such as cross-liking or modification of functional groups. Cross-linking increases stability as well as resistance.[7] Chitosan has reactive amino and hydroxyl groups, so it is easily used as a ligand for binding.[6] Reactions of chitosan may involve complexation, chelation, absorption, cross-linking, or combinations of these.[7] Chitosan can be used as a removal method for heavy metals, or other toxic substances in a water system.[6] and is a material of great potential to be used in wastewater treatment. Since crustaceans are plentiful, especially in coastal areas, or fisheries, chitin is a readily available substance. The abundance of chitin makes it and chitosan very economic materials to use.

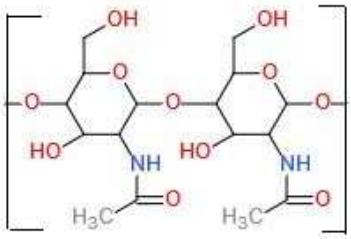

Figure 2 Chemical Structure of Chitin

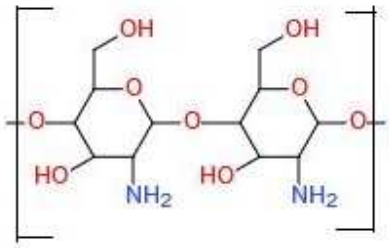

Figure 3 Chemical Structure of Chitosan

1.3 The Effect of Pharmaceuticals in Water

Pharmaceuticals and other bioactive agents are used extensively worldwide. The problem that arises with increased pharmaceutical use, is that only approximately 50% of the drug is used and kept in the body. Any remaining drug that is unused is excreted into wastewater as pure drugs or metabolites.[8] Currently, wastewater treatment plants do not have the capability to completely remove the pharmaceuticals, resulting in the diluted pharmaceuticals entering natural water sources.[8] Estrogen is one example of a drug of concern. As a result of increased use of estrogen, more of the hormone is being excreted into wastewater systems. Fang et al. reviewed the methods used to quantify the concentrations in water, however even the analytical techniques must be highly sensitive due to the complexity of the water systems, and the low activity of the estrogen molecules.[9] The accumulation is harming animal life by giving male organisms female characteristics. A more sensitive water treatment is needed to help combat the estrogen hormone accumulation in the environment.[9] Growing drug content in the water and subsequent possible bioaccumulation through trophic levels, possible changes in wildlife characteristics, and possible health effects for humans accounts for an equally growing need to investigate methods of removing pharmaceuticals from wastewater.

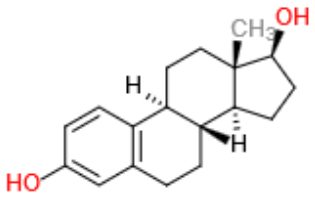

Figure 4 Chemical structure of estrogen

1.4 Scope of the project

The aim of the current work is to probe the uptake of Nile Red, a solvatochromic dye, by chitin and chitosan, two sorbents that would possibly be used in wastewater treatment to remove pharmaceuticals from the waste stream. Nile Red is used as a model for pharmaceuticals and other bioactive agents that may be present in wastewater. Further, since it is solvatochromic, it should give some indication of the polarity of the sorbent surface. Freundlich isotherm plots are used from UV-Vis absorbance spectra to determine the absorption coefficient, $K_{p,}$ and 1/n, representing linearity, for all chitin and chitosan treatments. The equation of the line is $\log[NR]_{chi} = logk_p + \frac{1}{n}\log[NR]_{solv}$; $\log[NR]_{chi}$ represents the log of the concentration of Nile Red sorbed by the chitin/chitosan, $\log[NR]_{sol}$ is the log of the concentration of Nile Red left in solution. A bigger absorption coefficient represents a larger binding affinity, while an n value of 1 is linear (discussed more in Chapter 4).

A second goal is to prepare a Nile Red derivative that would be more water soluble. Analysis of chitin and chitosan in a water-soluble Nile Red derivative would help to model the response of chitin and chitosan to water-soluble pharmaceuticals within a water system.

The specific mechanism of sorption or uptake of the Nile Red by chitin and chitosan is not discussed in this study, rather the focus is on whether or not sorption will occur. Absorption is when a substance is incorporated, or goes into the solid particle; adsorption is when the substance is retained on the surface through physical interaction.[10] Sorption is a term used when the mechanism of retention is unknown or unspecified. Sorption of Nile Red on chitin and chitosan as a sorbent will be discussed throughout the project.

Chapter 2. Experimental

2.1 Attempted Nile Red Derivative Synthesis

2.1.1 Method One

3-Aminophenol (2.0021 ± 0.0001 g; 0.018344 moles) was dissolved in a solution of deionized water (4.0 ± 0.05 mL) and 12 M hydrochloric acid (16.0 ± 0.35 mL) in a 50 mL 3 neck round bottom (RB) flask. The flask was cooled in an ice bath to approximately 0 ^{o}C. From a pressure equalized dropping funnel, a clear colorless solution of sodium nitrite ($NaNO_2$; 2.0121 ± 0.0001 g; 0.02916 moles) dissolved in water (15 ± 0.35 mL) was added dropwise over one hour. The solution was stirred for an extra 1.5 hours after the addition was completed. The mixture was then filtered through suction filtration using a 42.5 mm filter paper washed with small amounts of water and transferred to a 100 mL round bottom flask. The round bottom flask was placed on a Rotovap for 1 hour to reduce the total solution volume. The solution was dissolved in methanol and transferred to a 250 mL Erlenmeyer flask. Anhydrous magnesium sulfate powder was added to the flask to remove any remaining water present in the solution,

then filtered through fluted filter paper. The solution was placed on the Rotovap for 2 hours to remove the methanol. A dark red solution was the final product. The solution was distilled under reduced pressure using the rotary evaporator again for 4 hours to yeild a dark red slurry. The mass of the slurry was 13.7207 ± 0.0001 g. The slurry was then dissolved in acetonitrile in a 250 mL Erlenmeyer flask. Anhydrous magnesium sulfate was added to the solution and filtered out when the powder was free flowing in the solution. The solution was then transferred to a 100 mL RB flask and Rotovapped. The RB flask was dropped from the Rotovap and some of the acetonitrile solution was lost. The RB flask was placed back on the Rotovap until a solid weighing 4.9283 ± 0.0001 g was isolated.

An FTIR scan was conducted of the putative 5-amino-2-nitrosophenol (Appendix A). The dark red solid was dissolved in dichloromethane, forming a clear yellow solution. The solution was placed on a sodium chloride plate and analyzed using dichloromethane on a sodium chloride plate as the blank.

The crude 5-amino-2-nitrosophenol produced in step 1 was dissolved in acetonitrile (176 ± 1.4 mL), to form a clear dark red solution in a 250 mL round bottom flask and placed in a heating mantle. 1-Napthol crystals (5.1393 ± 0.0001 g; 0.035645 moles) and 12 M hydrochloric acid (6.0 ± 0.2 mL; 0.072 moles) was added to the round bottom flask. The solution was heated to reflux and stirred for 2 hours. The solution was transferred to a 500 mL round bottom flask and pressure reduced by the Rotovap for 5 hours resulting in an almost solid slurry.

2.1.1.1 TLC and DCC Testing

The red solid from method one was tested using Thin-Layer Chromatography, comparing the synthesized Nile Red derivative with 1-napthol and pure Nile Red, as a rough proxy for the intended Nile Red derivative. Each of the solids was dissolved in acetonitrile. The developing solutions included 60 dichloromethane: 40 ligroin, hexane, ethyl acetate, 50 dichloromethane: 50 ethyl acetate, and 70 dichloromethane: 30 ethyl acetate (Appendix B).

In Dry Column Chromatography (DCC), one end of a nylon tube was sealed and then filled with a layer of sand, with alumina powder filling the rest of the tube. The developing 70 dichloromethane: 30 ethyl acetate elutant was added to the column; the solution believed to contain the crude derivative of Nile Red was then poured onto the column until the solution reached the bottom. After the column dried, two cuts were made using the Rf values from the TLC test as a guide. The portions of tube were placed in beakers, washed with acetonitrile, and the solid was filtered off. The solutions were then placed on the Rotovap, and another TLC test was conducted using 70:20 Dichloromethane: ethyl acetate as the developing solutions, and compared to 1-naphthol and Nile Red. The Rf value of the test spot was only present in one portion of the DCC, which was equal to that of 1-naphthol; therefore, the Nile Red derivative synthesis was not successful (Table 11).

2.1.2 Method Two

2.1.2.1 Recrystallization

Tan-brown 3-aminophenol (10.0949 ± 0.0001 g; 0.092495 moles) was dissolved in ethanol and heated on a hot plate, forming a clear brown solution. Water was added to the solution until cloudy, and crystals formed. The mixture was warmed and activated charcoal was added, then filtered off through fluted filter paper. The resulting solution was clear light yellow. The light yellow solution was allowed to cool, and then placed in an ice bath to re-form the crystals. The crystals were then filtered off and isolated by vacuum filtration. The resulting amount of pure, clear pale grey 3-aminophenol crystals was 5.452 ± 0.001 g (0.04995 moles), with a percent yield of 54.0% by mole.

2.1.2.2 Synthesis via the Ammonium Salt of 3-aminophenol

Recrystallized 3-aminophenol (5.452 ± 0.001 g; 0.04995 moles) was transferred to a clean 250 ml Erlenmeyer flask. Diethyl ether (175 ± 1.4 mL) was added to dissolve the solid. Once in solution, of 12 M hydrochloric acid (4.2 ± 0.02 mL; 0.050 moles) was added by burette; a white precipitate formed upon addition. The solution was placed in an ice bath for 30 minutes, and filtered in a Buchner funnel by vacuum filtration. Grey rod-shaped ammonium salt formed in a yield of 4.387 ± 0.001 g (0.03013 moles). The percent yield of the ammonium salt was 60.3% on a mole to mole basis.

The salt (1.5056 ± 0.0001 g; 0.010341 moles) was placed in a 250 mL 3-neck round bottom flask with acetonitrile (100 ± 1.0 mL) and mixed using a stir bar in an ice bath. In a separate Erlenmeyer flask, 12 M hydrochloric acid (1.8 mL; 0.022 moles) was added to clear colorless sodium nitrite ($NaNO_2$; 0.7535 ± 0.0001 g; 0.01092 moles)

crystals, using a 2 mL syringe, forming a yellow paste. The yellow paste was then added dropwise to the apparatus containing the salt solution. The clear colorless solution turned slightly cloudy and yellow with the addition of the yellow paste. After an hour of stirring in the ice bath, a clear light yellow solution of 1-naphthol (1.4935 ± 0.0001 g; 0.010359) in acetonitrile (20 ± 0.3 mL) was added through a pressure equalized dropping funnel. As the 1-naphthol solution was added, the reaction mixture became darker and more orange in color; When the addition was complete (after 20 minutes) the reaction mixture was dark red-orange. The reaction mixture was refluxed overnight to yield the final dark red solution.

Multiple TLC tests were conducted on the final solution produced in method two of the Nile Red derivative synthesis, using 70 dichloromethane: 30 ethyl acetate solution, and 90 dichloromethane: 10 ethyl acetate solution for the eluant. The 90: 10 solution was prepared mixing dichloromethane (9 ± 0.2 mL) with ethyl acetate (1± 0.2 mL) in a 50 mL Erlenmeyer flask.

Next, the final reaction solution was placed on the rotovap for 53 minutes, until a dark red solid slurry remained. Dichloromethane (300 ± 1.1 mL) was added to the slurry, dissolving most of the solid. The dark red dichloromethane solution was placed in a 500 mL separatory funnel. 5.0 N sodium hydroxide was added 25 ± 0.3 mL at a time and the funnel was shaken and then allowed to separate. The aqueous (sodium hydroxide) and the organic (dichloromethane) layers were separated into two different Erlenmeyer flasks. The organic layer was added back into the funnel, and washed again with sodium hydroxide. The procedure was repeated four times, resulting in a clear red solution for the aqueous phase, and a clear light yellow solution for the organic phase. TLC tests were

conducted spotting each layer in 90:10 dichloromethane: ethyl acetate. Rf values were unable to be calculated, however, the aqueous layer was the only layer with visible spots. The spots in the aqueous phase were close in position to that of 1-naphthol when tested with 90:10 dichloromethane: ethyl acetate as the eluant.

2.2 Nile Red Analysis

2.2.1 Preparation of Stock Solution

Solid Nile Red (0.0050 ± 0.0001 g; $1.6 \cdot 10^{-5}$ moles) particles were placed in a 25.0 ± 0.03 mL volumetric flask. Acetonitrile was added to the volumetric flask to dissolve the solid. Once dissolved, acetonitrile was filled up to the line, a clear dark pink solution was formed (Figure 5).

2.2.2 UV-Vis of Nile Red in a Range of Solvents

Organic solvent (2.0 ± 0.006 mL) was added to a 1 cm quartz UV cell. Nile Red/Acetonitrile stock solution (0.050 mL) was injected into the cell. Acetonitrile (2.0 ± 0.006 mL) was added to a second cell and used as a blank. The samples were then analyzed using the Beckman Coulter DU720 General Purpose UV/Vis instrument. The procedure was repeated for the following media: 20:80 water: methanol, water, methanol, benzaldehyde, 1-propanol, 1-butanol, dichloromethane, acetonitrile, ethyl acetate, 1,4-dioxane, 2-chlorobutane, and diethyl ether. The solutions ranged in color from clear light pink (acetonitrile) to clear dark purple (methanol: water).

Figure 5 Nile Red in Acetonitrile

2.2.3 Calibration Curve

A solution was formed using methanol (10 ± 0.02 mL) and water in a 50 ± 0.05 mL volumetric flask. Nile Red stock solution (2.5 ± 0.021 mL) was added to a 25 ± 0.03 mL volumetric and filled to the mark with the 20:80 methanol: water solution. When mixed, red particles formed in a dark purple solution. A 1:25 dilution was tried using 1 ± 0.02 mL of Nile Red solution added to a separate 25 mL volumetric with methanol: water solution, and a 1:50 dilution of 1 ± 0.02 mL in a 50 ± 0.05 mL volumetric were also attempted. All solutions were insoluble.

An 80:20 methanol: water solution was prepared by adding water (20 ± 0.03 mL) into a 100 ± 0.08 mL volumetric flask, filled to the line with methanol. Nile Red solution (2.5 ± 0.021 mL) was added to a clean 25 mL volumetric flask and filled with 80:20 methanol: water solution. The resulting solution was clear dark purple (Figure 6).

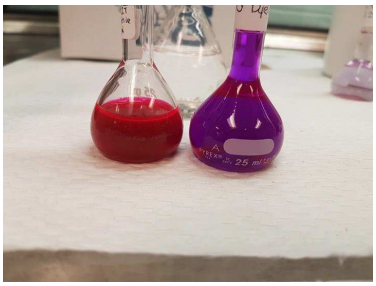

Figure 6 Nile Red in acetonitrile (left) and Nile Red in 80:20 Methanol: Water Solution (right).

Nile Red in 80:20 methanol: water solution (2.5 ± 0.021 mL) was added to a 5 mL volumetric flask and filled to the line with pure 80:20 methanol: water solution. A UV-Vis wavelength scan was taken for the 1:2 dilution. Nile Red (1 mL) stock solution was added to a clean 5 mL volumetric and filled with 80:20 methanol: water stock solution. The 1:5 diluted solution was

analyzed using the UV-Vis. Finally, a 1:10 diluted solution was formed by adding Nile Red (1 mL) stock solution to a 10 ± 0.02 mL volumetric flask filled with stock solution. A calibration curve (Figure 7; see Results) was plotted from the concentration and absorbance values.

2.2.4 Chitin Sorption Procedure

2.2.4.1 Method One: Shaking

For test 1, chitin (0.1013 ± 0.0001 g) was weighed and then placed in a clean glass test tube. The Nile Red in 80:20 methanol: water stock solution (2.0 ± 0.006 mL) was added to the test tube. The test tube was then corked and shaken for 4 minutes. After the 4 minutes, the test tube was placed on a test tube rack and allowed to sit for 2 hours. After 2 hours, the supernatant solution was extracted from the test tube and transferred to a quartz cuvette. A full wavelength scan from 400 to 750 nm was recorded by the Beckman Coulter DU720 General Purpose UV/Vis instrument. The procedure was replicated with 0.2003 ± 0.0001 g of chitosan, 0.4998 ± 0.0001 g, 0.6002 ± 0.0001 g, and 0.7987 ± 0.0001 g. The test using 0.7987 g of chitin did not produce a clear wavelength scan, so the results of said test were inconclusive.

2.2.4.2 Method Two: Centrifuge

In method two of the chitin analysis, chitin was measured on an analytical balance (±0.0001 g) at masses of approximately 0.1 g, 0.2 g, 0.5 g, and 0.6 g, in separate test tubes. Starting with the lowest mass, the stock Nile Red in 80:20 methanol: water solution (2.0 ± 0.006 mL) was added then to the test tube. The filled test tube was

weighed on a benchtop balance, and a second test tube was filled with deionized water until the mass of the water test tube matched that of the chitin with Nile Red. The test tubes were placed across from each other in a centrifuge after being allowed to sit for 5 minutes (ex. At positions 1 and 4). The centrifuge was turned on and operated for a total of 4 minutes. The samples were then removed, capped, and let to settle on the bench top for 1 hour. This procedure was repeated for the other chitin masses. The 0.5 g wavelength scan had a low absorbance curve, so it was re-done and averaged for the final graph values.

2.2.5 Chitosan Sorption Procedure

2.2.5.1 Method One: Shaking

Analysis was conducted with chitosan as the material in the same method as the chitin analysis in section 2.2.4.1. Chitosan was crushed using a mortar and pestle until the particles were close to uniform in size. The chitosan was then weighed out by analytical balance (±0.0001 g) into labeled test tubes at amounts of 0.1012 g, 0.2008 g, 0.5003 g, and 0.6002 g. Nile Red in 80:20 methanol: water solution (2.0 ± 0.006 mL) was added to the first test tube, and the test tube was capped. After capping, the test tube was shaken for 4 minutes, and let settle for 2 hours. After the hour was completed, the Nile Red solution was extracted from the test tube using a disposable pipet and placed into a quartz cuvette. The solution was analyzed by the Beckman Coulter DU720 General Purpose UV/Vis instrument, using 80:20 methanol: water as a blank. The procedure was repeated for the other mass samples.

2.2.5.2 Method Two: Centrifuge

For the centrifuged chitosan analysis, masses of 0.1026 ± 0.0001 g, and 0.6028 ± 0.0001 g were used, measured by analytical balance. When weighed out in the test tube, 2.0 ± 0.006 mL of Nile Red in 80:20 methanol: water solution was added and weighed using a general purpose bench balance. A different test tube was filled with water until it weighed equal to the chitosan and Nile Red filled test tube. Both the water and sample test tubes were placed in the centrifuge opposite to each other. The centrifuge ran for 4 minutes, and the sample was left setting on the benchtop for 1 hour. When the hour was completed, the solution was extracted using a disposable pipet and a full wavelength scan was obtained using the Beckman Coulter DU720 UV/Vis instrument.

Chapter 3. Results

3.1 UV-Vis of Nile Red in Organic Solvents

To determine solvatochromism of Nile Red, and how the maximum wavelength changed with polarity, UV-Vis spectra of Nile Red in various solvents were measured and recorded using the Beckman Coulter DU720 UV/Vis instrument (see section 2.2.2.). Examples of UV-Vis scans are shown in Figures 7, 8, and 9. The max wavelength and absorbance values from all scans are shown in Table 1.

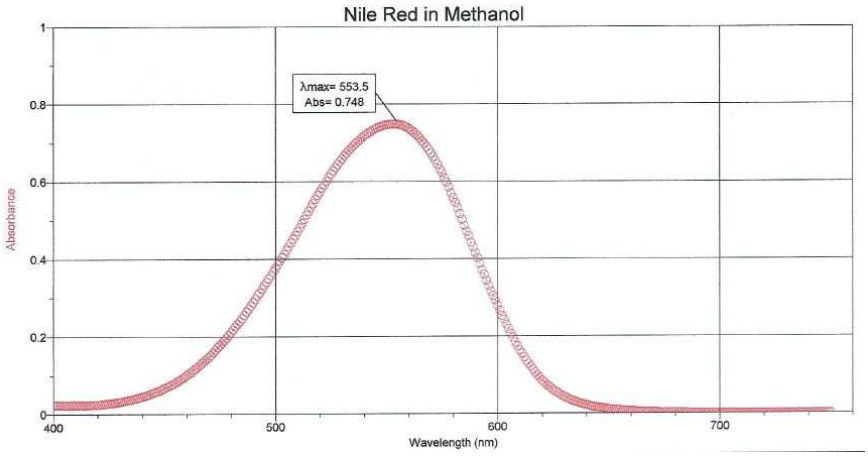

Figure 7UV-Vis full wavelength scan of Nile Red in methanol solution.

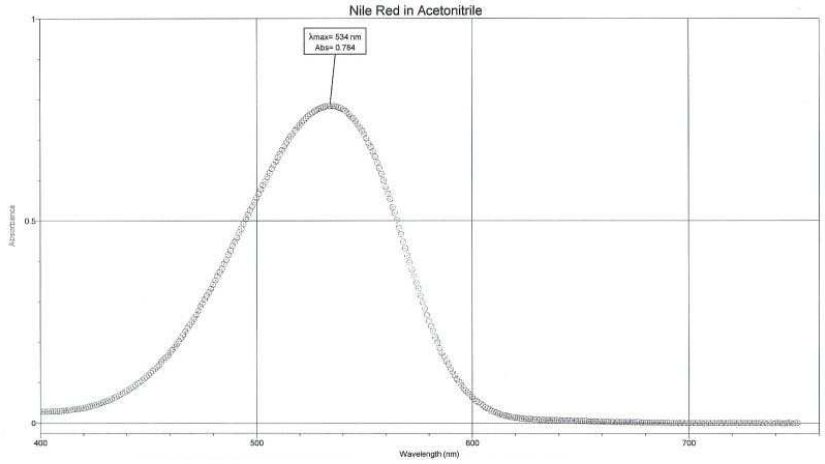

Figure 8 UV-Vis full wavelength scan for Nile Red in Acetonitrile.

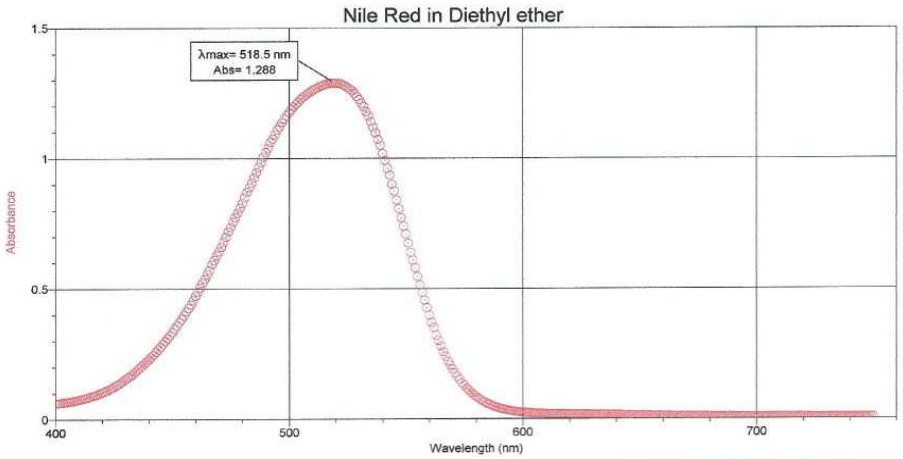

Figure 9 UV-Vis full wavelength scan for Nile Red in diethyl ether.

Table 1: Summary of λ_{max} and absorbance values for Nile Red solution in organic solvents.

Solvent	λ max (nm)	Absorbance
20:80 Water: methanol	588.5	0.299
Water	569.5	0.246
Methanol	553.5	0.748
Benzaldehyde	551.5	0.621
1-propanol	549	0.571
1-butanol	548.5	0.431
Methylene chloride	538	0.554
Acetonitrile	534	0.784
Acetone	532	0.464
Ethyl acetate	526	0.813
1,4-Dioxane	523	0.582
2-chlorobutane	523	0.867
Diethyl ether	518.5	1.288

3.2 Calibration Curve for Nile Red in 80:20 Methanol: Water Solution

A calibration curve (Beer-Lambert plot) was needed to determine molar absorptivity (ϵ) in the desired solvent (Figure 10). Due to low solubility in water, 80:20 methanol: water solution was used for analysis (see section 2.2.3). The molar absorptivity was later used to calculate sorption from UV-Vis values in the chitin and chitosan analyses (see sections 3.3 and 3.4).

Calculations were completed to determine the concentration of Nile Red in the stock solution, along with the diluted concentrations. The concentration data was summarized in Table 2, with absorbance and wavelength data from UV-Vis analysis, which was used to plot the Beer-Lambert calibration curve in Figure 10.

The equation of a Beer-Lambert calibration curve for UV-Vis analysis is $A = \in bc$.[10] With known concentration and absorbance values, ϵ (molar absorptivity) was calculated by $\in = \frac{A}{bc}$, or the slope of the linear regression line. A is absorbance, ϵ is molar absorptivity in $M^{-1}cm^{-1}$, c is concentration in mol/L, and b is path length (1cm).

$$conc.\,stock\ Nile\ Red\ Solution$$

$$\frac{0.0050\ g}{318.37\frac{g}{mol}} = 1.57\ \cdot 10^{-7} mol$$

$$\frac{1.57\ \cdot 10^{-7} mol}{0.025\ L} = 6.28 \cdot 10^{-4} M$$

80:20 methanol: water 1/10 dilution

$$conc. = 6.28 \cdot 10^{-4} M \times \frac{1}{10} = 6.28 \cdot 10^{-5} M$$

Table 2: Summary of concentration, absorbance, and λ_{max} for diluted 80: 20 Methanol: Water Nile Red solutions.

Dilution	Concentration (M)	Absorption	λ max (nm)
80:20 Methanol Water	6.28×10^{-5}	2.731	563
½	3.14×10^{-5}	1.581	562
1/5	1.26×10^{-5}	0.638	565.5
1/10	6.28×10^{-6}	0.326	565

Beer-Lambert concentration calculations:

Nile Red in 80:20 methanol: water solution

$$Conc.\,80{:}\,20\ solution = 6.28\cdot10^{-4}\times\frac{1}{10} = 6.28\cdot10^{-5}\ M$$

Stock solution diluted by 1/2

$$Conc.\frac{1}{2} = 6.28\cdot10^{-5}\times\frac{2.5\ mL}{5.0\ mL} = 3.14\cdot10^{-5}\ M$$

Stock solution diluted by 1/5

$$Conc.\frac{1}{5} = 6.28\cdot10^{-5}\times\frac{1.0\ mL}{5.0\ mL} = 1.26\cdot10^{-5}\ M$$

Stock solution diluted by 1/10

$$Conc.\frac{1}{10} = 6.28\cdot10^{-5}\times\frac{1.0\ mL}{10.0\ mL} = 6.28\cdot10^{-6}\ M$$

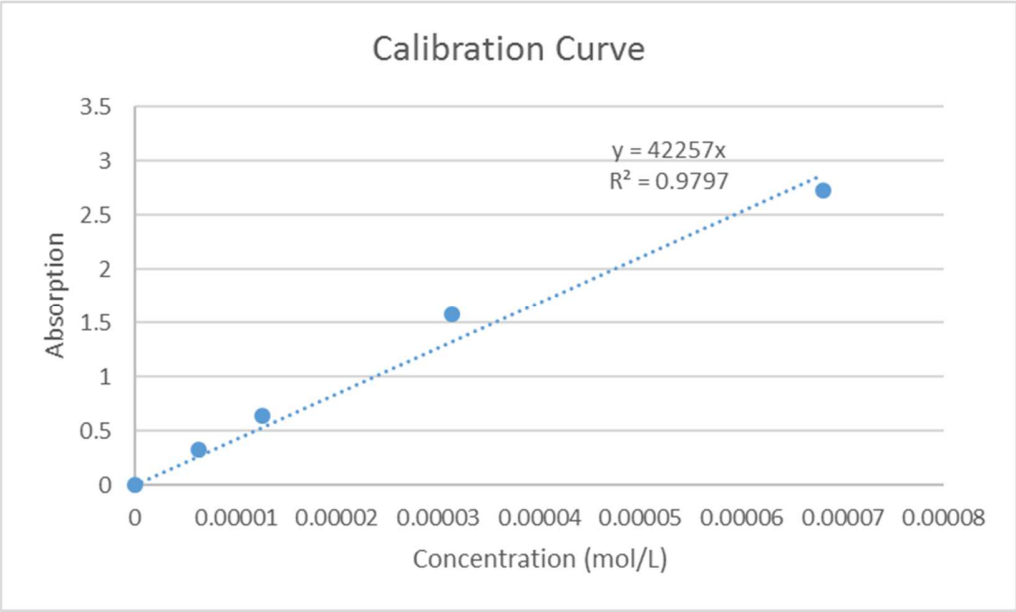

Figure 10 Calibration curve of Nile Red in 80: 20 Methanol: Water solution.

The Beer-Lambert plot shown in Figure 10 is reasonably linear (R^2= 0.9797). The slope of the line ($\epsilon = \frac{A}{lc}$) yields molar absorptivity (ϵ) in the 80:20 Methanol: water solvent: $42257\ M^{-1}cm^{-1}$. Corrected for significant figures, the molar absorptivity $(\epsilon)\ is\ 42300\ M^{-1}cm^{-1}$.

3.3 Chitin Sorption Analysis

Absorbance and maximum wavelength data were recorded for Nile Red in 80:20 methanol: water solutions after a sorption procedure with chitin to determine changes with increased chitin mass. The data collected was used to calculate Nile Red concentration in solution ($[NR]_{solv}$), and Nile Red concentration sorbed by chitin ($[NR]_{chi}$). The concentrations, provided in Tables 3 and 5 were used to plot Freundlich isotherms. Freundlich isotherm plots are used in sorption of ion exchange analyses to

determine how binding the species is to a sorbent with various sorption sites, such as chitin, chitosan, or bulk sediments.[11]

Table 3: Summary of values from Nile Red in Chitin analysis.

Mass Chitin (g)	λ max (nm)	Absorbance	$[NR]_{solv}$ (M)	$[NR]_{chi}$ (M)	$Log[NR]_{solv}$	$Log[NR]_{chi}$
0.0000	563	2.727	$6.8 \cdot 10^{-5}$	0	-4.17	---
0.1013	562	2.728	$6.4 \cdot 10^{-5}$	$4.0 \cdot 10^{-6}$	-4.19	-5.40
0.2003	562.5	2.630	$6.2 \cdot 10^{-5}$	$6.0 \cdot 10^{-6}$	-4.21	-5.22
0.4998	583	1.980	$4.7 \cdot 10^{-5}$	$2.1 \cdot 10^{-5}$	-4.33	-4.68
0.6002	595.5	*0.838	$2.0 \cdot 10^{-5}$	$4.8 \cdot 10^{-5}$	-4.70	-4.32

*Two readings were conducted for the 0.6 g sample; the values were averaged and placed in the table.

To calculate Nile Red concentration in solution, the absorbance reading (Table 3) is divided by the path length (1 cm) multiplied by molar absorptivity from Figure 10 (42300 $M^{-1}cm^{-1}$). The Nile Red concentration sorbed by chitin, was calculated by subtracting the Nile Red concentration in solution from the initial Nile Red concentration before chitin was added.

$$[NR]_{solv} = \frac{A}{\epsilon b} = \frac{2.728}{42300\ M^{-1}cm^{-1}\times 1cm} = 6.4 \cdot 10^{-5}\ M$$

$$[NR]_{Chi} = [Stock] - [NR]_{solv} = 6.8 \cdot 10^{-5} - 6.4 \cdot 10^{-5} = 4.0 \cdot 10^{-6}\ M$$

$$Abs\ 0.6\ avg = \frac{0.938 + 0.737}{2} = 0.8375 = 0.838$$

$$\log[NR]_{chi} = logk_p + \frac{1}{n}\log[NR]_{solv}$$

From the Freunlich Isotherm Plot (Figure 11), the slope is -1.93 and the intercept is -13.3. These results will be discussed in Chapter 4.

$$\log[NR]_{chi} = -1.93\log[NR]_{solv} - 13.3$$

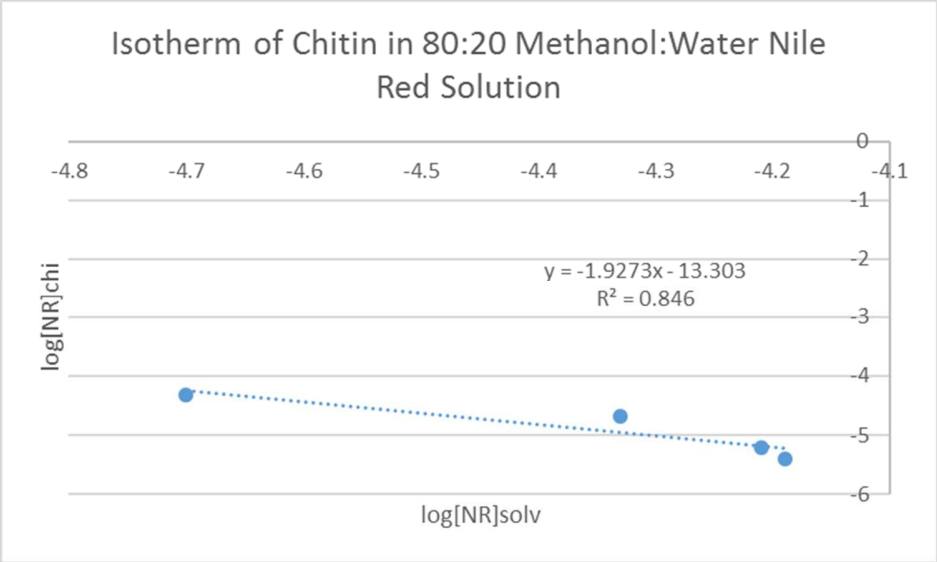

Figure 11 Freundlich Isotherm, equation log[NR]chi=logKp+ 1/nlog[NR]solv. Data from Table 3.

$$\frac{1}{n} = 10^{-1.93} = 1.17 \cdot 10^{-2}$$

$$K_p = 10^{-13.3} = 5.01 \cdot 10^{-14}\ M$$

The plot (Figure 11) has an R^2 value of 0.846, which is a moderate correlation coefficient. The Kp value represents how binding a sorbent is to determine the effectiveness (see Section 4.2).

Figure 12 is a plot showing the relationship between mass of chitin and absorbance from the data summarized in Table 3.

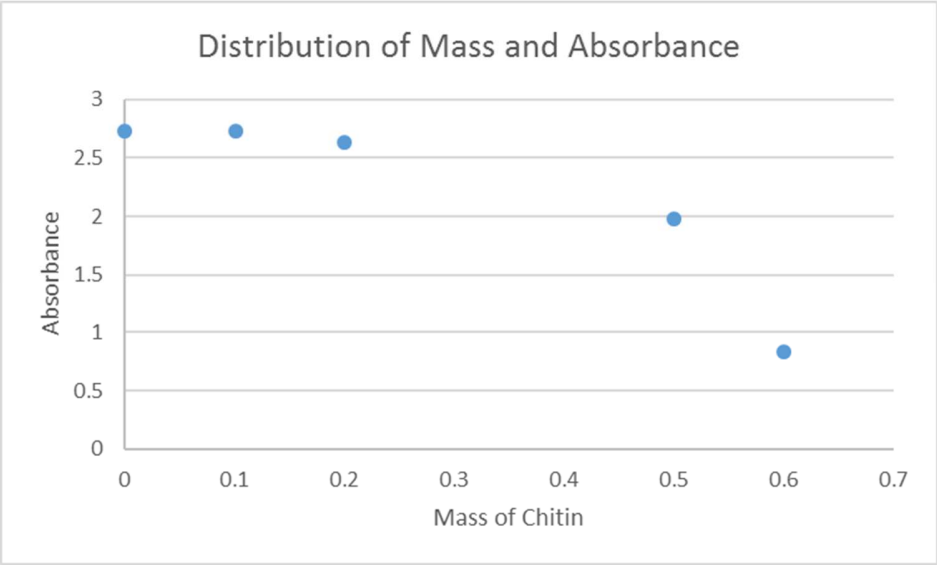

Figure 12 Mass of Chitin used vs. solution absorbance.

Like Table 3, Table 4, 5, and 6 provide summary data from UV-Vis analysis of solutions after sorption with chitin. The later tables differ from Table 3 because they are from method two of the chitin sorption analysis, where centrifuging is used rather than shaking (see Section 2.2.4). Table 4 provides the rough data directly from the UV-Vis graphs, Table 5 shows the averaged values and concentration data.

Table 4: Table of λ max, absorbance, and averages taken from at least two UV-Vis scans for each mass of chitin when centrifuged.

Mass Chitin (g)	λ max (nm)				Average λ max	Absorbance				Average Abs.
0.1013	563		561		562	2.735		2.731		2.733
0.2009	559		561		560	2.736		2.728		2.732
0.4996	591	589.5	587	562	582.4	0.878	1.170	1.709	1.689	1.362
0.5001	559.5		559.5		559.5	2.855		2.861		2.858
0.6004	565		565		565	2.666		2.679		2.673

Table 5: Summary table of results from UV-Vis analyses for centrifuged chitin samples and concentration data.

Mass Chitin (g)	λ max (nm)	Absorbance	$[NR]_{solv}$ (M)	$[NR]_{chi}$ (M)	$Log[NR]_{solv}$	$Log[NR]_{chi}$
0.0000	563	2.727	$6.8 \cdot 10^{-5}$	0	-4.17	---
0.1013	562	2.733	$6.5 \cdot 10^{-5}$	$3.0 \cdot 10^{-6}$	-4.19	-5.52
0.2009	560	2.732	$6.5 \cdot 10^{-5}$	$3.0 \cdot 10^{-6}$	-4.19	-5.52
0.4996	582.4	1.362	$3.2 \cdot 10^{-5}$	$3.6 \cdot 10^{-6}$	-5.49	-5.44
0.5001	559.5	2.858	$6.8 \cdot 10^{-5}$	0	-4.17	---
0.6004	565	2.673	$6.3 \cdot 10^{-5}$	$5.0 \cdot 10^{-6}$	-4.20	-5.30

$$[NR]_{solv} = \frac{A}{\epsilon b} = \frac{2.733}{42300\ M^{-1}cm^{-1} \times 1cm} = 6.5 \cdot 10^{-5}\ M$$

$$[NR]_{Chi} = [Stock] - [NR]_{solv} = 6.8 \cdot 10^{-5} - 6.5 \cdot 10^{-5} = 3.0 \cdot 10^{-6}\ M$$

Figure 13 shows the relationship between absorbance and chitosan mass provided in Table 5.

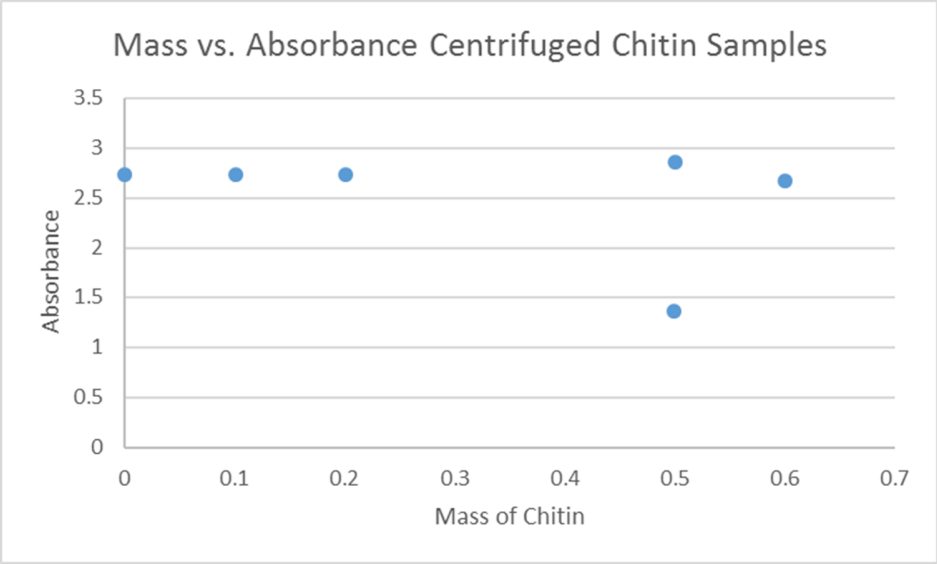

Figure 13 Mass vs. absorbance for centrifuged chitin samples.

Due to the dual value for the 0.5 g absorbance shown in both Figure 13 and Table 5, Table 6 was formed averaging the two values. Concentration of Nile Red in solution and Nile Red on chitin was re-calculated for the averaged 0.5 g value. The data was then used in the Freundlich Isotherm plot (Figure 14), and the mass absorbance distribution (Figure 15).

Table 6: Average UV-Vis results for centrifuged chitin samples, with 0.4999 g and 0.5001 g data averaged.

Mass Chitin (g)	λ max (nm)	Absorbance	$[NR]_{solv}$ (M)	$[NR]_{chi}$ (M)	$Log[NR]_{solv}$	$Log[NR]_{chi}$
0.0000	563	2.727	$6.8 \cdot 10^{-5}$	0	-4.17	---
0.1013	562	2.733	$6.5 \cdot 10^{-5}$	$3.0 \cdot 10^{-6}$	-4.19	-5.52
0.2009	560	2.732	$6.5 \cdot 10^{-5}$	$3.0 \cdot 10^{-6}$	-4.19	-5.52
*0.4999	571	2.110	$5.0 \cdot 10^{-5}$	$1.8 \cdot 10^{-5}$	-4.30	-4.74
0.6004	565	2.673	$6.3 \cdot 10^{-5}$	$5.0 \cdot 10^{-6}$	-4.20	-5.30

*values averaged from 0.4996 and 0.5001 values for λ and absorbance from table 5

$$[NR]_{solv} = \frac{A}{\epsilon b} = \frac{2.110}{42300 \times 1cm} = 5.0 \cdot 10^{-5}\ M$$

$$[NR]_{Chi} = [Stock] - [NR]_{solv} = 6.8 \cdot 10^{-5} - 5.0 \cdot 10^{-5} = 1.8 \cdot 10^{-5}\ M$$

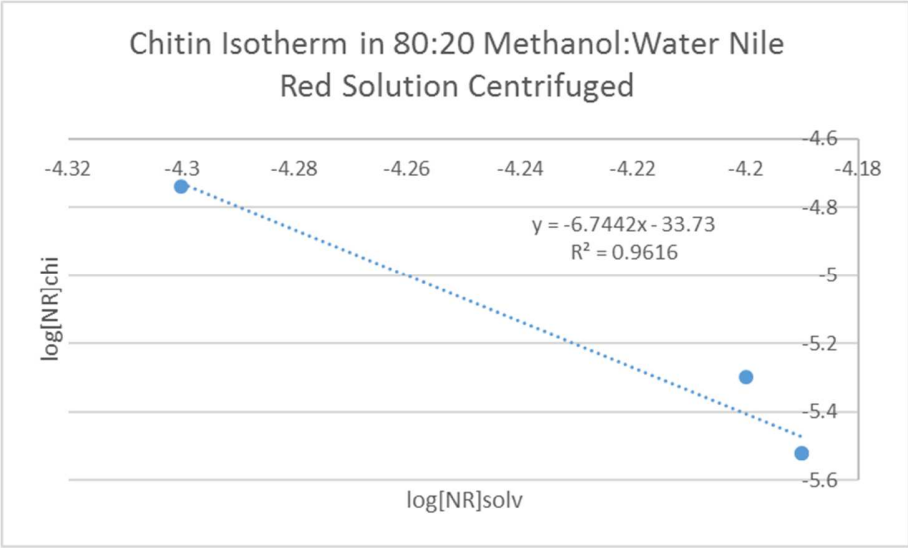

Figure 14 Freundlich Isotherm for centrifuged chitin analysis.

$$\frac{1}{n} = 10^{-6.74} = 1.82 \cdot 10^{-7}$$

$$K_p = 10^{-33.7} = 2.00 \cdot 10^{-34}\ M$$

The R^2 value of 0.9616 is a strong correlation coefficient, meaning the data fits well to the linear regression line. The slope of the linear regression line is -6.7442, and the intercept is -33.73. The calculated Kp value is $2.00 \cdot 10^{-34}$ M, which will be discussed in the next chapter. As seen in Table 6, two of the $\log[NR]_{solv}$ values are the same (0.1013 g and 0.2009 g), which leads to the appearance of only three data points in Figure 14.

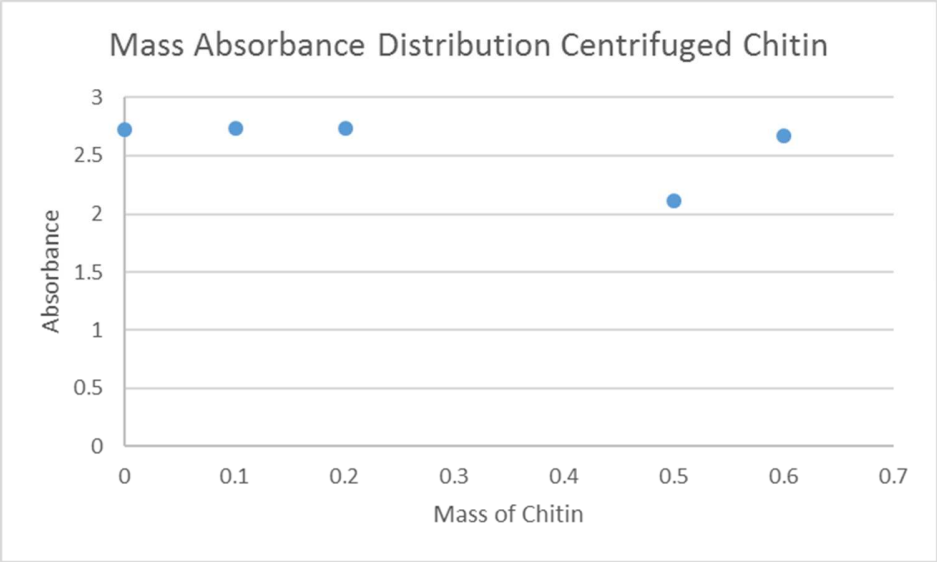

Figure 15 Averaged mass vs. absorbance for centrifuged chitin samples. Data from Table 6.

3.4 Chitosan Sorption Analysis

As with the chitin analysis, Nile Red in 80:20 methanol: water solution was analyzed using chitosan as a sorbent. Table 7 contains the rough data taken from the UV-

Vis wavelength scans, while Table 8 is the averaged values and calculated concentrations of Nile Red in solution and Nile Red sorbed by chitosan. The values from Table 8 were used in the construction of the Freundlich Isotherm plot (Figure 16), as well as the distribution of absorbance and mass of chitosan (Figure 17) used to show any trend in absorbance changes with amount of sorbent added.

Table 7: Chitosan absorbance and max wavelength data.

Mass Chitosan (g)	λ max (nm)		Absorbance	
0.1012	562	563	2.693	2.704
0.2008	563		2.691	
0.5003	562		2.714	
0.6002	564		2.734	

Table 8: Averaged results from chitosan analysis.

Mass Chitosan (g)	λ max (nm)	Absorbance	$[NR]_{solv}$ (M)	$[NR]_{chito}$ (M)	$Log[NR]_{solv}$	$Log[NR]_{chito}$
0.0000	563	2.727	$6.8 \cdot 10^{-5}$	0	-4.17	---
0.1012	*562.5	*2.699	$6.4 \cdot 10^{-5}$	$4.0 \cdot 10^{-6}$	-4.19	-5.40
0.2008	563	2.691	$6.4 \cdot 10^{-5}$	$4.0 \cdot 10^{-6}$	-4.19	-5.40
0.5003	562	2.714	$6.4 \cdot 10^{-5}$	$4.0 \cdot 10^{-6}$	-4.19	-5.40
0.6002	564	2.734	$6.5 \cdot 10^{-5}$	$3.0 \cdot 10^{-6}$	-4.19	-5.52

* Averaged from 2 runs λ max 562 and 563 nm, absorbance 2.693 and 2.704.

$$[NR]_{solv} = \frac{A}{\epsilon b} = \frac{2.691}{42300 \times 1cm} = 6.4 \cdot 10^{-5}\ M$$

$$[NR]_{Chi} = [Stock] - [NR]_{solv} = 6.8 \cdot 10^{-5} - 6.4 \cdot 10^{-5} = 4.0 \cdot 10^{-6}\ M$$

As seen in Table 8, the $\log[NR]_{solv}$ values are the same for all test chitosan masses, and all of the $\log[NR]_{chito}$ values with the exception of the 0.6002 g mass test are the same. Due to the similarity between all data points, a trendline was unable to be plotted on the Freundlich Isotherm graph (Figure 16). Without a trendline, the R^2 value and the line equation used to calculate Kp were unable to be formulated.

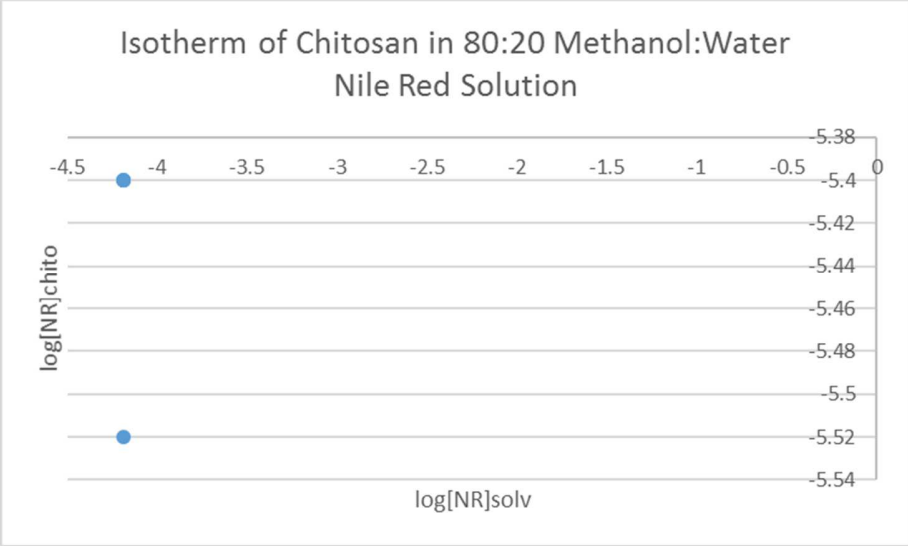

Figure 16 Freundlich Isotherm for chitosan analysis. Data from Table 8. Unable to make trendline.

Figure 17 shows very little change in absorbance with respect to increased mass of chitosan. As discussed in the next chapter, the small change and the inability to make a

trendline in Figure 16 leads to the conclusion that chitosan is not a very effective sorbent for the Nile Red in 80:20 methanol: water solution.

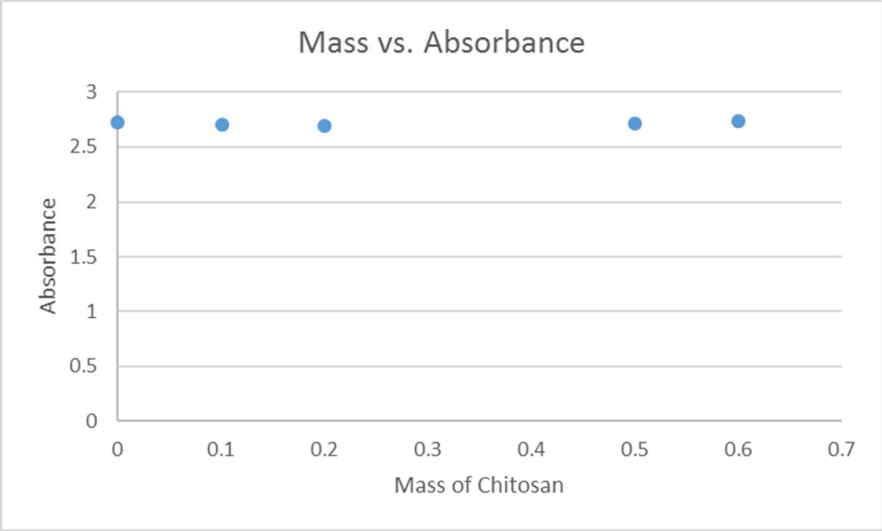

Figure 17 Mass of chitosan used vs. absorbance.

The summary of analysis when chitosan was centrifuged with the Nile Red in 80:20 methanol: water solution is provided in Table 9, along with the calculated concentrations of Nile Red in solution and Nile Red sorbed by chitosan. The centrifuge analysis was only conducted on two different masses of chitosan: 0.1026 g, and 0.6028 g. The discrepancy between the amount of data for the centrifuged chitosan tests with respect to the other sorption analyses was due mostly to time constraints. The data in Table 9 was used to plot the Freundlich Isotherm in Figure 18 and the mass/absorbance distribution shown in Figure 19.

Table 9: Centrifuged chitosan UV-Vis data.

Mass Chitosan (g)	λ max (nm)	Absorbance	$[NR]_{solv}$ (M)	$[NR]_{chito}$ (M)	$Log[NR]_{solv}$	$Log[NR]_{chito}$
0.0000	563	2.727	$6.8 \cdot 10^{-5}$	0	-4.17	---
0.1026	560	2.731	$6.5 \cdot 10^{-5}$	$3.0 \cdot 10^{-6}$	-4.19	-5.52
0.6028	563	2.499	$5.9 \cdot 10^{-5}$	$9.0 \cdot 10^{-6}$	-4.23	-5.05

$$[NR]_{solv} = \frac{A}{\epsilon b} = \frac{2.731}{42300 \times 1cm} = 6.5 \cdot 10^{-5}\ M$$

$$[NR]_{Chi} = [Stock] - [NR]_{solv} = 6.8 \cdot 10^{-5} - 6.5 \cdot 10^{-5} = 3.0 \cdot 10^{-6}\ M$$

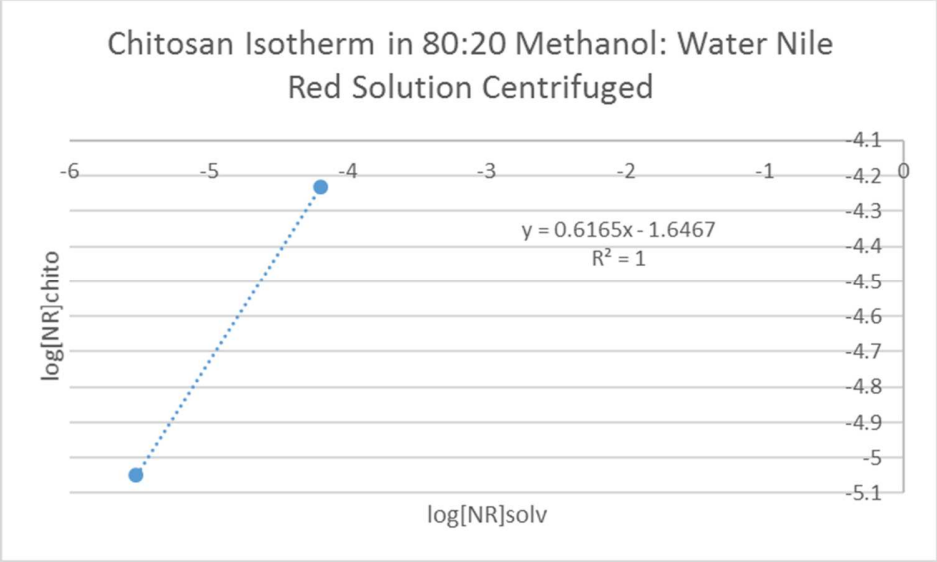

Figure 18 Freundlich Isotherm for centrifuged chitosan analysis. Data from Table 9.

$$\frac{1}{n} = 10^{0.617} = 4.14$$

$$K_p = 10^{-1.65} = 2.24 \cdot 10^{-2}\ M$$

The correlation coefficient was 1, meaning the Freundlich Isotherm plot was perfectly linear. The slope of the line was 0.6165, and the intercept was -1.6467. The intercept converted into Kp is $2.24 \cdot 10^{-2}$ M, discussed more in Section 4.3.

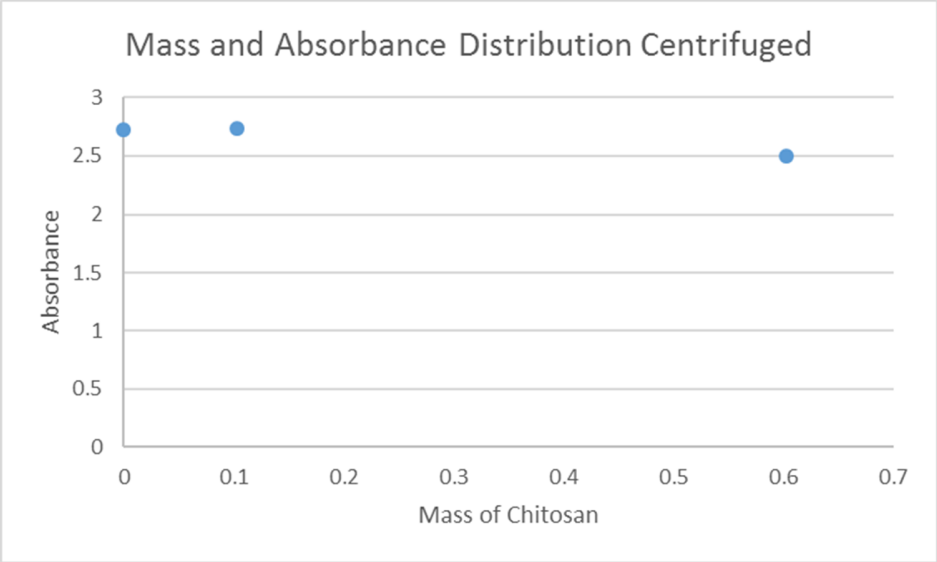

Figure 19 Mass and absorbance distribution for centrifuged chitosan samples.

3.5 Thin Layer Chromatography

To determine whether the water-soluble Nile Red derivative syntheses were successful or not, Thin Layer Chromatography tests were conducted comparing the product to the starting products, as well as Nile Red. The calculated Rf values were used to determine what the product consisted of. Table 10 consists of a summary of Rf values from TLC tests on the product formed in method one using various developing eluents (see Section 2.1.1).

$$^{12}Rf = \frac{distance\ travelled\ by\ the\ spot}{distance\ travelled\ by\ solvent}$$

Table 10: Summary of Rf values from testing the Nile Red derivative product from method 1 in various developing solutions.

	Rf		
	1-naphthol	Product	Nile Red
60:40 dichloromethane: ligroin	0.20	0.25	0.91
Hexane	0.23	0.25	0.08
Ethyl acetate	0.79	0.81	0.68
60: 40 dichloromethane: ligroin	0.17	0.25	0.06
50:50 dichloromethane: ethyl acetate	0.80	0.80	0.69
70:30 dichloromethane: ethyl acetate	0.81	0.83	0.67
50:50 dichloromethane: ethyl acetate	0.76	0.77	0.70

Table 11 is a summary of Rf values measured from TLC tests after Dry Column Chromatography provided more distance to separate the points. The TLC test in Table 11 was conducted using 70:30 dichloromethane: ethyl acetate as the eluent. The product from method one is clearly closest to 1-naphthol, meaning synthesis was unsuccessful.

Table 11: Rf values from each portion cut after during dry column chromatography from Nile Red derivative product produced by method one

	Rf		
	Nile Red	1-naphthol	Test
1-naphthol portion	0.736	0.858	0
Nile Red portion	0.685	0.833	0.815

The Rf values displayed in Table 12 are of the product from synthesis method two (see Section 2.1.2). The derivative showed two points, each corresponding to the starting products, discussed more in Chapter 4.

Table 12: Rf values from the Nile Red derivative prepared by method 2 in different developing solutions.

	Rf				
	Nile Red	1-naphthol	3-aminophenol	Derivative	
70:30 dichloromethane: ethyl acetate	0.795	0.926	0.484	0.910	0.433
90:10 dichloromethane: ethyl acetate	0.370	0.685	0.139	0.694	0.120

Chapter 4. Discussion

4.1 Solvatochromism of Nile Red and the Beer-Lambert Calibration Curve

The purchased Nile Red was tested to determine is it was indeed a solvatochromic dye. The UV-Vis spectra obtained and the data in Table 1 suggest that Nile Red does have solvatochromic properties. Nile Red in 20:80 water: methanol had the highest max wavelength (588.5 nm), and was darker and purple in color. Diethyl ether Nile Red solution had the lowest max wavelength (518.5 nm), with a clear, light pink color. Acetonitrile solution was around the middle of the 13 solvent wavelength range with a λ max of 534 nm, dark pink in color (Figure 5). Therefore, there was a 70 nm difference between the highest and lowest wavelength values for Nile Red solution.

As seen in the preparation of the original calibration curve, the Nile Red solution was largely insoluble in water. The UV-Vis wavelengths scans were dilute, so to the naked eye, the solution was uniform, however the low absorbance and possible double peak seen in the scan could be attributed to solid particles in solution or agglomeration.

4.2 Chitin Sorption with Nile Red

The experiment of sorption of Nile Red with chitin was successful, in that, with each different mass of chitin, there was a further decrease in solvent absorbance measured by UV-Vis. The chitin particles that were placed in the test tube also slightly changed color form white-orange to light pink depending on how much chitin was in the test tube compared to solution. A difference was also observed in the amount of solution recovered. Although there was 2 mL of Nile Red pipetted into the test tube for each test, the amount or solution extracted was lower with increased chitin mass. For example, in

the experiment with 0.8 g of chitin, there was an insufficient amount of solution extracted to obtain a proper spectrum. The change in the volume of extracted solution clearly demonstrates that chitin not only sorbed some dye, but also absorbed some of the 80:20 methanol: water solvent, likely causing the chitin to swell.

Errors that may have been present in the UV-Vis chitin test would be if any chitin was transferred to the quartz cell. The chitin particles were small, so there could have been possibly uptake in the disposable pipet. If any chitin was present in solution, light diffraction may have occurred, skewing the absorbance value. The method of the sorption experiment was not yet optimized to obtain ideal results.

The Freundlich Isotherm for chitin (Figure 11) has a K_p $5.01 \cdot 10^{-14}$ M and a 1/n value of $1.17 \cdot 10^{-2}$. The 1/n value measured is surprisingly low; most common values range from 0.3 to 1.7.[13] Since a Freundlich Isotherm is in terms of log, n close to 1 would be linear.[14,15] K_p is an absorption coefficient, so the greater the value, the higher the absorption capacity or binding affinity of the solute to the sorbent surface.[15] In the case of Figure 11, the absorption capacity seems to be low for chitin.

For the centrifuged chitin analysis, the original 0.4996 g UV-Vis scan had a low absorbance, without a clear maximum wavelength. A UV-Vis wavelength scan was completed for a different sample of 0.5001 g. Data from each of the masses were placed in Table 4 and averaged in Table 5. A Freundlich Isotherm was unable to be plotted because the chitin concentration of the 0.5001 g sample was 0. The two 0.5 g sample data were averaged in Table 6, and plotted in Table 11. The K_p value for the centrifuged chitin Freundlich Isotherm was $2.00 \cdot 10^{-34}$ M, and the 1/n value was $1.82 \cdot 10^{-7}$. The K_p value is very small, suggesting a lower absorption capacity. The 1/n value is also far from 1,

meaning the plot is far from linear. The centrifuged chitin analysis seemed lower in absorption capacity and linearity than the non-centrifuged samples. More of the solution was able to be extracted from the centrifuged sample as compared to the shaken chitin samples, and the UV-Vis distribution was bell-shaped with a clear peak rather than slumped in the centrifuged method.

4.3 Chitosan Sorption with Nile Red

The chitosan analysis was very similar in terms of wavelength and absorbance for all mass samples (Table 8). The Freundlich Isotherm was unable to show a linear trendline because three out of four points were the exact same (Figure 16). The Mass vs. Absorbance graph (Figure 17) also confirms the minimal absorbance change with increased chitosan mass; The chitosan did not take up any of the dye.

Because the chitosan analysis was unsuccessful, and time was limited, only two samples were analyzed by the centrifuge method: 0.1026 and 0.6028 g. The absorbance of the 0.1 g sample was close to the absorbance without any chitosan, the absorbance was slightly lower for the 0.6 g sample. The Freundlich Isotherm had a K_p value of $2.24 \cdot 10^{-2}$ M, and a 1/n value of 4.14. The 1/n value is close to 1, so the Freundlich Isotherm is close to linear. The K_p is highest for centrifuged chitosan compared to all of the other methods, however this could be due to the lower number of tests. Chitin (Figure 12) had the largest distribution of absorbance, decreasing with increasing amount of chitin.

The only difference between the structure of chitin and chitosan is the acetyl group that is removed by base in the conversion from chitin to chitosan (see section 1.2). Therefore, it can be concluded that the acetyl group (CH_3CO) is essential for the sorbance

of Nile Red, as a model for pharmaceuticals in wastewater. It can be suggested that portions of the dye structure interact with the acetyl group through hydrogen-bonding or dipole-dipole interactions. The lack of the acetyl groups in chitosan reduces the sorption of the dye below the point of detection by the UV-Vis spectroscopic method used. Further work is required in this area in order to provide a definitive mechanistic answer (see Chapter 6).

The experimentation with Nile Red was conducted in 80:20 methanol: water solution rather than water, so it is unclear whether the results can be transferred to aqueous conditions in wastewater treatment plants. To decrease the possible discrepancies that could result from the differing conditions, attempts were made to prepare a more water soluble analogue of Nile Red that would be under conditions more representative of wastewater treatment. The attempted syntheses will be discussed next.

4.4 Attempted Water-Soluble Nile Red Derivative Syntheses

4.4.1 Method One

Method one synthesis involved two reactions: nitrosation and condensation. First, 3-aminophenol was dissolved in a solution of deionized water and hydrochloric acid and cooled in an ice bath. A solution of sodium nitrite and water was added dropwise through a pressure equalized dropping funnel, then stirred an extra 1.5 hours after completion. The solution was filtered by suction filtration, then rotovapped for 1 hour. The remaining mixture was dissolved in methanol; then anhydrous magnesium sulfate was added and filtered off. After more time on the Rotovap a red slurry was yielded. The slurry was dissolved in acetonitrile, anhydrous magnesium sulfate was added, and filtered off again

when free flowing. After the solution was evaporated using the Rotovap one last time, a red solid was isolated and tested using FT-IR spectroscopy. The condensation reaction involved dissolving the crude nitroso product in acetonitrile, then adding 1-naphthol and concentrated hydrochloric acid to the apparatus. The mixture was stirred and refluxed using a condenser for 2 hours, then the solution was placed in the Rotovap to yield a dark red solid.

4.4.1.1 Fourier-Transform Infrared Spectroscopy

The FT-IR spectra of the crude 5-amino-2-nitrosophenol prepared in method one of the Nile Red synthesis had most peaks concentrated in the fingerprint region. The C-N peak is usually found around 1110 cm^{-1}, a broad peak at 1119.01 cm^{-1} may have been the C-N stretch in the compound;[16] a clear O-H stretch is not observed in the spectra. No other bond stretches were prominent enough to be determined. By the FT-IR analysis, an amine was produced, however, it may not have been the desired 5-amino-2-nitrosophenol. The synthesis procedure was ended due to time constraints. If 5-amino-2-nitrosophenol was formed, it may have degraded by the time the next step of the reaction occurred.

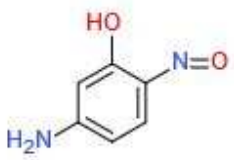

Figure 20 Structure of 5-amino-2-nitrosophenol

4.4.1.2 Thin Layer Chromatography

For the TLC test analysis from method one, 70:30 dichloromethane: ethyl acetate developing solution was most successful as separating each point, without any moving past the top (Table 10). The product from method one had the similar Rf values to 1-naphthol, suggesting unsuccessful synthesis. The dry column chromatography (DCC) was cut in two places, measured by multiplying the length of the tube to the Rf values of 1-naphthol and Nile Red from the previous TLC test. The TLC test from DCC suggest that the synthesized material was closer in properties to 1-naphthol than Nile Red, and there may have been an error in determining which section should contain what chemical; If the product was 1-naphthol it should have been observed in the 1-naphthol section rather than the Nile Red section (Table 11).

4.4.2 Method Two

The 3-aminophenol in method two of the synthesis was recrystallized to remove any impurities that were present, in attempts to increase the purity and yield of the derivative formed through method two. The starting 3-aminophenol was a tan-brown solid, after recrystallization the solid was light grey and rod-shaped. The percent recovery by a mol: mol basis was 54.0 %.

The recrystallized 3-aminophenol was dissolved in diethyl ether, and converted into the ammonium salt after addition of concentrated hydrochloric acid by burette. The aminophenol salt was transferred to a 3-neck RB flask with acetonitrile and stirred while cooled in an ice bath. A mixture of concentrated hydrochloric acid and sodium nitrite was added dropwise to the apparatus and stirred for an hour. After the hour of stirring, a

solution of 1-naphthol dissolved in acetonitrile was added by pressure equalized dropping funnel over a 20 minute period. Later, the solution was removed from the ice bath to come to room temperature, then heated to reflux overnight.

4.4.2.1 Thin Layer Chromatography

TLC tests were conducted for the product in method two using 70:30 dichloromethane: ethyl acetate then 90:10 dichloromethane: ethyl acetate developing solutions (Table 12). For both developing solutions, the product was tested against Nile Red, 1-naphthol, and 3-aminophenol. In both TLC tests, the product spotted twice, one for 3-aminophenol and another for 1-naphthol. Both 1-naphthol and 3-aminophenol were starting products of the procedure, so it appeared unsuccessful. To distinguish between the product having two different TLC spots, the solution was separated into an aqueous and organic layer. Visually, all of the color contained in the dye solution was extracted into the aqueous layer. Another TLC test was conducted using 90:10 dichloromethane: ethyl acetate developing solution, spotting both the layers. The TLC test was fairly inconclusive because of the spearing nature of the aqueous layer; however, the organic layer did not appear to contain anything similar when compared to the previous TLC tests. Both method one and method two TLC testing results, including Dry Column Chromatography and separation, point towards unsuccessful syntheses.

Method two was the most promising method of attempted synthesis because it was a one-pot synthesis, where loss of integrity by continual transfer of the reaction mixture does not occur. Also, isolation of the ammonium salt of 3-aminophenol was preferred because it is much more stable than the nitroso product that was isolated in

method one. One issue was the acetonitrile may not have completely dissolved the 3-aminophenol salt before the hydrochloric acid/sodium nitrite addition since the ice bath blocked the view of the RB flask. To combat this issue, the solution should be prepared outside of the apparatus and added when completely dissolved. Similarly, the hydrochloric acid and sodium nitrite solution was a slurry rather than a solution, which may have caused error or side-reactions.

Chapter 5. Conclusion

Nile Red is a known solvatochromic dye that is highly sensitive to changes in polarity, [2,17] which was found to have λ_{max} values ranging from 588 nm in 20:80 methanol: water to 518.5 nm in diethyl ether, thus giving a wide range of 70 nm, depending on the polarity of the solvent system.

5.1 Attempted Nile Red Derivative Syntheses

5.1.1 Method One

The synthesis of the water-soluble Nile Red derivative in method one was unsuccessful due to time spent in between steps, where the nitrosoaromatic intermediate likely decomposed, although the FT-IR spectrum was inconclusive. The TLC tests also show Rf values of the product more like 1-naphthol than the target Nile Red derivative (Table 10). The TLC tests completed after separation by Dry Column Chromatography (DCC) also show the product of the method one synthesis containing 1-naphthol.

5.1.2 Method Two

Method two of the synthesis was also unsuccessful. One issue may have been adding the salt and acetonitrile into the apparatus separately, instead the solution should have been made and then placed in the apparatus. The mixture of hydrochloric acid and sodium nitrite was a paste rather than a solution, which may have had an impact. The TLC tests had both 1-naphthol and 3-aminophenol in the product, however no Nile Red, and the when the dye was separated, the color was in the aqueous layer instead of the organic where it should have been if the product was the desired Nile Red derivative.

5.2 Sorption of Nile Red

5.2.1 Chitin

The sorption tests with shaking chitin and Nile Red solution were successful. The absorbance of the solution in the UV-Vis analysis lowered with increasing amount of chitin, the appearance of chitin changed from white-orange to slightly pink, and the amount of solvent available to extract decreased with increasing amount of chitin. The results of the sorption test suggest that chitin may be a promising medium for increasing the sensitivity of wastewater treatments in removing pharmaceuticals from the water.

Another set of sorption tests were conducted using the centrifuged method. The amount of solution extracted from the sample was greater than the amount extracted from the chitin without centrifuging. The UV-Vis graphs had a clearer peak, however, there was less difference between absorbance values and the Freundlich Isotherm equation showed low absorption capacity and linearity.

5.2.2 Chitosan

The chitosan analyses, both with and without centrifuging were unsuccessful for sorption of Nile Red. In the non-centrifuged samples, all except one absorbance was the same, showing no difference with added chitosan. For the centrifuged method, there was a slight absorbance decrease, however it was only a difference of 0.232. The Freundlich Isotherm equation cannot be sufficiently compared to that of the chitin due to the lower amount of data points used with the centrifuged chitosan. The appearance and color of the chitosan also changed very little after Nile Red addition, unlike that of chitin. The chitin and chitosan analyses were not completed under acidic conditions because the acid could cleave the chitin/chitosan chains.

The amount of data present in the chitosan sorption analysis was insufficient when centrifuged due to time constrains, however more testing must be conducted to compare each method and sorbent through the use of Freundlich isotherms. The Freundlich isotherm data may also be improved by analysis of more different masses of the sorbent material, of with a lower concentration of the Nile Red solution. Decreasing the concentration of the Nile Red solution would produce lower absorbance values that may be beneficial in comparing the methods. The high absorbance values measured in the above analyses were above the instrument screen limit, so some changes in absorbance may have been harder to see.

The difference in sorption between chitin and chitosan is attributed to the acetyl groups that are present in the structure of chitin, but removed when converted to chitosan. Although the exact mechanism cannot be determined concretely without further research,

the sorption of Nile Red on chitin may be due to hydrogen bonding, or dipole-dipole interaction between the two chemical species.

Chapter 6. Future Research

Future research on this project could be another attempt at the Nile Red derivative synthesis. Method two was promising, however, the solutions after the salt is extracted (salt in acetonitrile, and sodium nitrite in hydrochloric acid) should be prepared separately before being added to the apparatus, ensuring each is a complete solution with all components dissolved. A salt ice bath for all steps before refluxing may have also help with maintaining a constant temperature.

More analyses would have to be conducted with more different masses of chitin and chitosan by both methods. The same procedures should also be done with chitin/chitosan in a water soluble Nile Red derivative to get a model better representative to pharmaceuticals and other bioactive agents in water. Optimization of the chitin and chitosan analysis would also be beneficial for future research where sorption may be more effective with different timings of centrifuging or shaking and setting.

Tests for solvatochromism may also be replicated using a more sensitive syringe to obtain possible better results. The solvatochromism procedure would also have to be repeated for any Nile Red derivative successfully synthesized.

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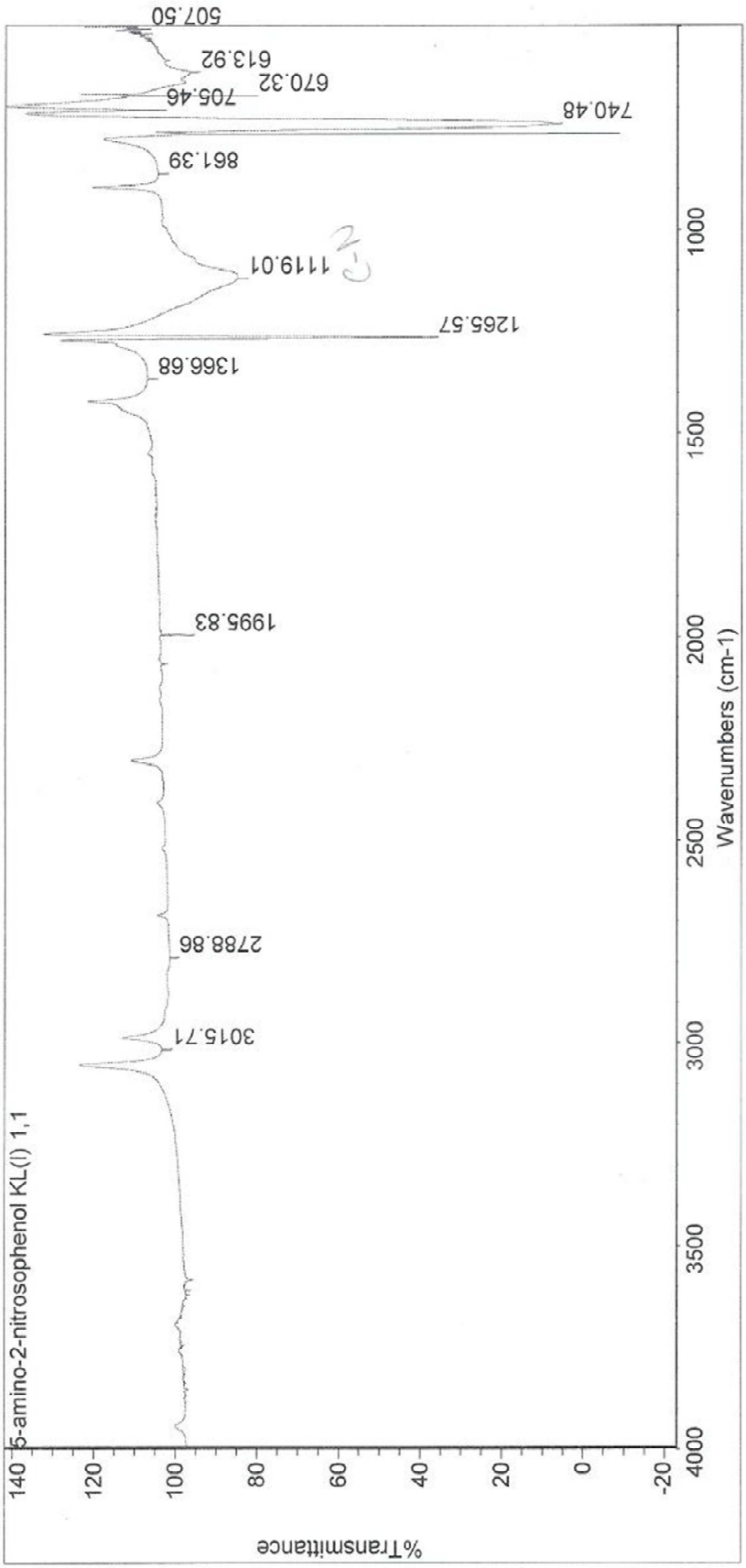
Appendix A

FT-IR Scan

Derivative Synthesis Method One

5-amino-2-nitrosophenol

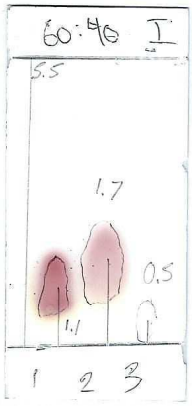

5-amino-2-nitrosophenol KL(I) 1,1
%Transmittance
Wavenumbers (cm-1)
140
120
100
80
60
40
20
0
-20
4000
3500
3000
2500
2000
1500
1000
3015.71
2788.86
1995.83
1366.68
1265.57
1119.01
861.39
740.48
705.46
670.32
613.92
507.50

Appendix B

TLC Analyses:

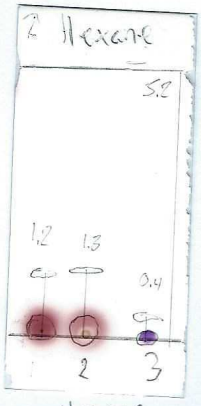
Synthesis Methods One and Two

Method 1 TLC analysis 1= 1-naphthol 2= Method 1 Product 3= Nile Red



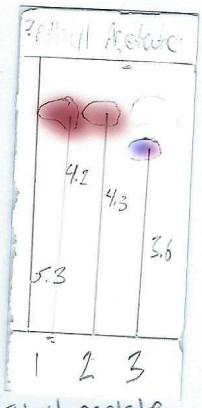
60:40 dichloromethene: ligroin

Rf 1 = $\frac{1.1}{5.5}$ = 0.20
2 = 0.25
3 = 0.91



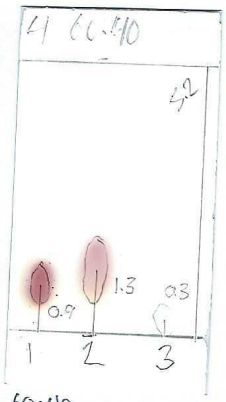
Hetane

Rf 1 = 0.23
2 = 0.25
3 = 0.08



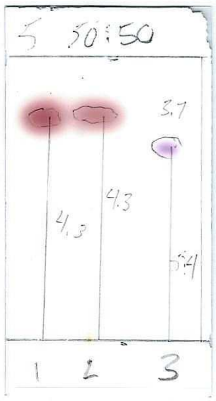
Ethyl acetate

Rf 1 = 0.79
2 = 0.81
3 = 0.68



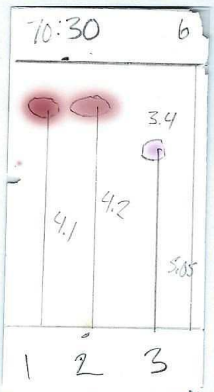
60:40 dichloromethane: ligroin

Rf 1 = 0.17
2 = 0.25
3 = 0.06



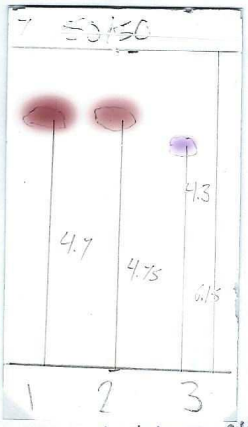
50:50 dichloromethene: ethyl acetate

Rf = 1 = 0.80
2 = 0.80
3 = 0.69



70:30 dichloromethene: ethyl acetate

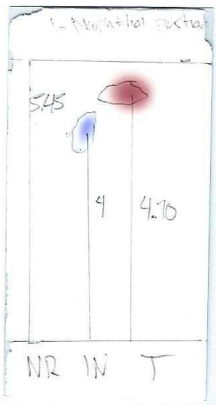
Rf: 1 = 0.81
2 = 0.83
3 = 0.67



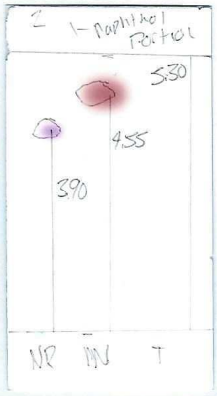
50:50 dichloromethene: ethyl acetate

Rf 1 = 0.76
2 = 0.77
3 = 0.70

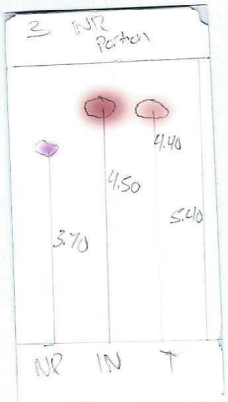
After Dry Column Cromatography (Method One)
NR= Nile Red IN= 1-naphthol T= Test or product from synthesis



1-naphthol portion (1)
Rf NR=
IN=
T=
Not lined up well
tried attempt 2



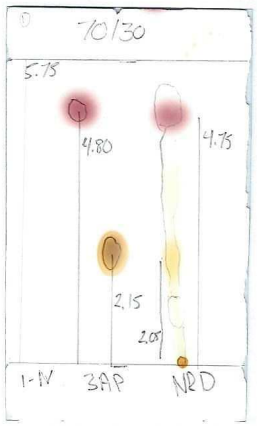
1-naphthol portion (2)
RF NR= 0.736
IN= 0.858
T= Ø
* used in analysis



Nile Red portion
Rf NR= 0.685
IN= 0.833
T= 0.815

Synthesis Method Two

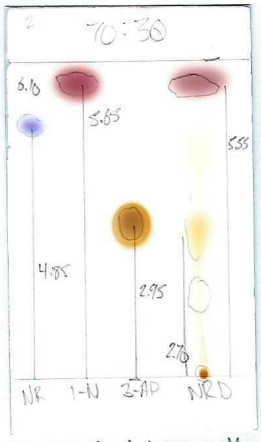
1N= 1-naphthol 3AP= 3-aminophenol NR= Nile Red NRD= Synthesis Product



70:30 dichloromethane: ethyl acetate

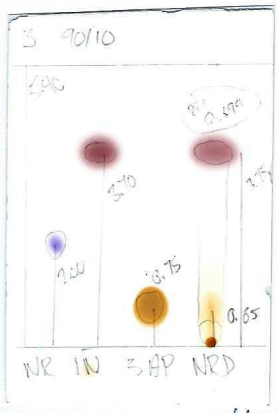
Rf= 1-N =
3AP=
NRD=

Not lined up well



70:30 dichloromethane: ethyl acetate

Rf= NR= 0.795
1N= 0.926
3AP= 0.484
NRD= 0.910 and 0.433

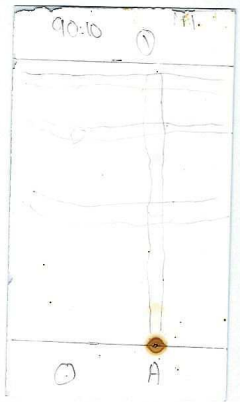


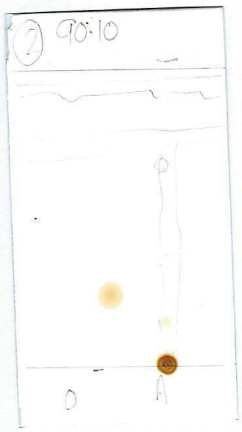
90:10 dichloromethane: ethyl acetate

* Rf NR= 0.370
1N= 0.685
3AP= 0.139
NRD= 0.694 and 0.120

After separation A= aqueous layer, O= organic layer

90:10 dichloromethane: ethyl acetate compared to #3 above*





Unable to get proper Rf values.