3-28-2016

Determining the Concentration of Nitric Oxide in Solvent

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J. N. Andrews Honors Program
Andrews University

HONS 497
Honors Thesis

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March 28, 2016

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Abstract

The body creates nitric oxide (NO) for signaling. One way that researchers study NO signaling is through the use of model complexes, or molecules that have the same metal ion and a similar molecular shape as biological molecules and are easy to synthesize. Currently researchers have limited ways to deliver precisely-known small quantities of NO to an experiment where they are trying to investigate NO-binding to a transition metal complex. A peak shift in the absorption spectrum at ~530 nm makes it possible for UV-Vis spectroscopy to observe NO binding to cobalt tetraphenylporphyrin (CoTPP). This makes it possible to measure the quantity of NO dissolved in an organic solvent. This project worked to obtain the UV-Vis spectra after varying amounts of NO-saturated solvent was added to CoTPP in a gas-tight cuvette. This spectral data was analyzed using existing computer software and a spectral analysis method developed here. The analysis of spectrophotometric titration data for two solvents yielded preliminary values that let researchers know the concentration of NO in two commonly used solvents: $[NO]_{DCM} = 2.29 \pm 0.04$ mM; $[NO]_{THF} = 0.9 \pm 0.1$ mM.

I. Introduction

Nitric Oxide (NO) is a gas that is present in the atmosphere in trace amounts, however the precise amount changes constantly. The primary source for atmospheric nitric oxide is lightening, and since lightening isn't constant, the concentration of NO in the atmosphere cannot be constant either. The body prepares this gas *in vivo*, and uses it for various purposes. Those purposes include intracellular communication where, for example, NO signals cells in the cardiovascular system and carrying signals between brain cells. NO is also used in the body as an antimicrobial and helps with immune response. (Hunt, **2015**). Due to its poor abilities as a reducing agent (E° = -800 mV), NO only reacts with metal ions such as Iron(II) (E° = 770 mV) which is found in hemoglobin. When NO binds with a metal ion in our bodies, it is involved with vasodilation, neurotransmission, and it assists with regulation of cell respiration. Researchers are able to learn about how the body communicates and what the chemical signals for that communication are through studies about NO. (Möller, **2005**)

Studying about the chemical function of NO in biological systems can be relatively difficult because it is necessary to extract and purify a complex compound (enzymatic protein) before characterizing it. Many chemists contribute to the understanding of biological molecules by working with model complexes. (Praneeth, **2006**) A model complex usually contains a metal ion and a "molecular shape" that may be similar to those found in the biological system. Model complexes are often relatively simple to synthesize so that the focus of the research can be on how the molecules react. The studies on model complexes can help explain how the molecule reacts in the body.

A common feature of model complexes is the organic portion of the molecule generally has a conjugated π bond system with alternating single and double carbon-carbon bonds.

Figure 1 shows an example of such a molecule. Molecules that are conjugated and contain metal ions can absorb light very efficiently at certain wavelengths, (Lim, **2007**) because the absorption of electromagnetic radiation (photons) causes electrons to move from occupied to unoccupied orbitals, which are at different energy levels in a molecule. By studying the changes in the absorption we are able to study NO. The goal of our research was to provide information for chemists who prepare model complexes that are reactive with NO.

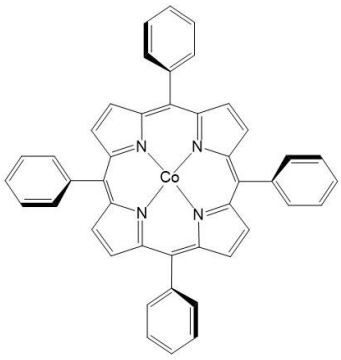


Figure 1: Cobalt(II) Tetraphenylporphyrin (CoTPP) Structure

Background: Spectroscopy

A spectrum, in the most general sense, is a graph that shows the response of the molecule to a range of wavelengths of light. In chemistry, this graph is created by an instrument that measures the difference in light between a sample and a blank. The spectrum shows the response of the sample as a function of the incident wavelength. A "peak" in the spectral line correlates with the absorption of more electromagnetic radiation at a specific wavelength, and the set of intensities as a function of a range of wavelengths creates a spectrum. That spectrum will be unique for each compound: each compound absorbs light in unique ways. When NO binds to cobalt in cobalt(II) tetraphenylprophyrin (CoTPP), a slightly different compound forms, and that new compound absorbs light at a different wavelength.

Background: NO in solutions

When working with solids and liquids, scientists have precise methods to measure the quantity of substance delivered over a wide range of masses and volumes. In contrast, a challenge of working with a gas such as NO is that it can be difficult to deliver precisely known quantities, or specific numbers of moles of gas, especially small quantities of gasses. This presents a challenge to chemists working with model complexes because they cannot do

reactions with similar NO levels as exist in the body. NO can be measured on the scale of liters, but the ability to measure the millimolar and smaller concentrations that are involved in cell signaling and other biological functions is a task that is currently not possible.

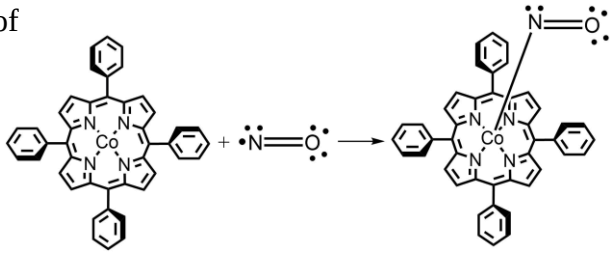
One way to address this is to make use of the fact that most gasses including NO dissolve in liquid solvents like water or organic solvents like DCM (dichloromethane or methylene chloride) or THF (tetrahydrofuran). Henry's Law describes this phenomenon. Accordingly, when scientists perform experiments that require the presence of a precisely known quantity of NO(g), the easiest way to add a precise quantity is to add a known volume of liquid organic solvent saturated with NO. However, the exact number of moles of NO that is added is difficult to know. For many years researchers have dissolved NO in solvents. (Shaw, **1977**) However, the list of concentrations of NO in solvents is incomplete and does not reflect the solvents that are commonly used in more modern model chemistry: specifically missing from this work are DCM and THF. Thus, researchers in this field have no way to know the precise amount of NO that they are adding to a reaction.

Background: My contribution

The basis of this research project is the quantitative chemical reaction between cobalt(II) tetraphenylporphyrin (CoTPP, figure 1) and nitric oxide (NO, nitrogen monoxide, figure 2) where one molecule of CoTPP can react with only one molecule of NO and one molecule of NO can react with only one molecule of CoTPP. This one-to-one reaction can be used to calculate the concentration of NO in organic solvents used by synthetic scientists as they try to study NO reactivity. CoTPP behaves as an "NO-detector" because when CoTPP reacts with NO, the absorption spectrum of CoTPP changes. As shown in reaction scheme 1 CoTPP binds with NO to form CoTPP·NO, where the NO is bound to cobalt through the nitrogen. This project

Figure 2: Lewis structure of nitric oxide (NO)

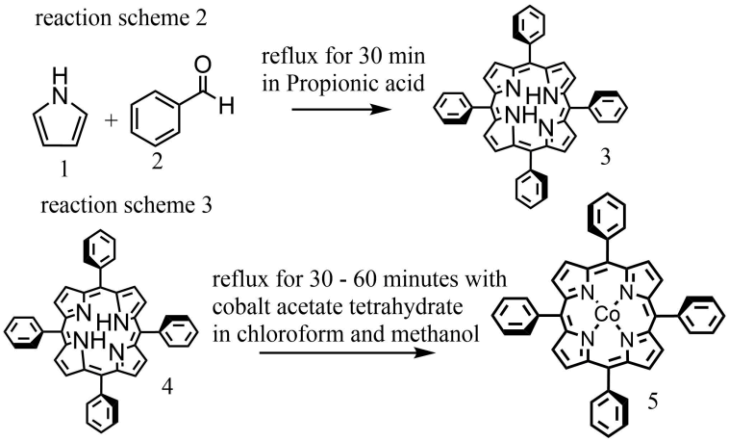
provided important information to add to a table of values for researchers who want to be able to add a specific amount of NO to a reaction. I synthesized the CoTPP, performed spectrophotometric titrations, and developed a numerical analysis method to analyze the data.



Reaction Scheme 1: This figure shows the reaction between CoTPP and NO.

II. Synthetic Methodology

This was fundamentally an "analytical chemistry" project because we developed a method to measure the amount of NO in solvents. However, the project also involved the synthesis, purification, and characterization of the CoTPP sensor molecule. First, I prepared the tetraphenylprophyrin (TPP) from commercially available pyrrole and benzaldehyde following reaction scheme 1 which followed the method of Adler (Adler, **1970** and Adler, **1967**). Pyrrole was freshly distilled, by running it through a rotary evaporator (rotovap) with a water bath with a temperature of ~ 60ºC, on the day of the synthesis of TPP. Reaction scheme 2 shows the initial preparation of the un-metallated TPP by reflux in propionic acid. The synthesis was accomplished by bringing 180 mL of propionic acid solvent to almost boiling (~140°C), adding 4.8 mL of benzaldehyde followed by 3.4 mL of the freshly distilled pyrrole, and allowing the acid solution to boil for thirty minutes. A water-cooled condensing column prevented evaporation of propionic acid solvent and the benzaldehyde and pyrrole were added through the top of the condenser. In a second reaction I inserted the cobalt(II)



Reaction Schemes 2&3: These figures show the chemical reactions done to create CoTPP.

atom by refluxing the TPP with cobalt(II) acetate (Baker Analyzed Reagents) by adding ~0.2 g of the TPP to 80mL of freshly distilled chloroform and this was heated. Then 1.0 g of the cobalt(II) acetate was dissolved in 50 mL of methanol, and the methanol solution was heated to almost boiling (~64.7^{o}C). Finally, the methanol and cobalt(II) solution was added to the chloroform and the mixture was allowed to boil for an hour. This reaction is outlined in reaction scheme 3.

Crude CoTPP was purified with a form of column chromatography where a combination of a solvent (the mobile phase) and a solid (the stationary phase) were used to separate molecules in the crude product based on differences in polarity. A glass Buchner funnel (Z175811), a funnel that uses vacuum to pull the solvent through, was used as a miniature column to separate the CoTPP from the other compounds mixed with it. The stationary phase, in this case alumina (Al_2O_3) filled the top of the funnel. A thin layer of sand covered the top of the alumina. At that point, the mobile phase, DCM (CH_2Cl_2) in this case, was poured into the funnel until it saturated the stationary phase.

The next step for the chromatography was to place a portion of the crude CoTPP (in mobile phase) on the top of the sand in the funnel. After the sample soaked into the sand more DCM added into the funnel moved pure CoTPP through the "column" as a distinct colored band while the other compounds mixed with the crude CoTPP stayed at the top of the "column". This separation of compounds derived from the differences in the polarity of the compounds and the differences in polarity between the mobile and stationary phases. The CoTPP that eluted down the "column" emerged cleaned of impurities and dissolved in DCM (Harris, **2010** ch. 22**)**.

Measurement of Molar Absorptivity (ε)

At this point I used ultraviolet – visible spectrometry (UV-Vis) to test whether CoTPP was successfully prepared. Specifically, figure 3 overlays the UV-Vis spectra of the newly created compound with a known CoTPP sample provided by Dr. D. Randall (obtained from the N. Lehnert group at University of Michigan). Since the wavelengths and relative intensities for the peaks match, the newly created compound was confirmed to be CoTPP. I recorded the spectrum by placing a small amount (1-2 crystals) of the CoTPP in a cuvette and dissolved it in DCM. I placed the cuvette, (quartz with an open top), in the UV-Vis instrument, the Cary 5000, created by Agilent Technologies. To facilitate comparison of relative intensities, the spectra were scaled to have the same intensity.

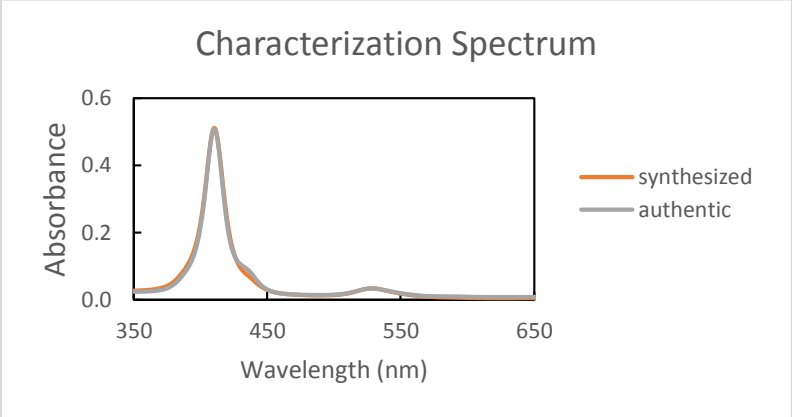


Figure 3: The successful characterization of CoTPP is shown here. The shape of both of the curves matches almost perfectly and thus confirms the creation of CoTPP.

To further confirm the characterization, an epsilon (ε) value, or the molar absorptivity, was calculated at 528 nm for DCM and at 543 for THF. I precisely weighed out over 10 mg of my CoTPP on an 0.1 mg analytical balance to give three significant digits in the mass. Dissolving this in a 10 mL volumetric flask with DCM created a stock solution with a concentration of 2.07 mM. Three dilutions of this stock solution were used to collect UV-Vis spectra. The dilution history used is outlined in table 1. A similar set of three dilutions was done using THF as the solvent but the same stock solution with DCM was used

Dilution 1	50 µL stock with 1450 µL DCM
Dilution 2	100 µL stock with 1400 µL DCM
Dilution 3	200 µL dilution 2 with 1300 µL DCM

Table 1: The dilution history for the dilutions used for the DCM E calculation. The history for the THF dilutions can be found in the appendix.

initially. The THF dilution history is can be found in table 5 in the appendix for reference.

The Beer-Lambert law predicts a linear dependence between the absorbance from the spectrum and the concentration of the solution (eq. 1). With precisely known CoTPP concentrations, the intensity of the spectrum for each dilution can be used to find the epsilon of CoTPP in DCM (Harris 2010 ch. 17). The precision of the concentration allowed the ε values to be recorded with three significant figures.

$$A = \varepsilon l C \qquad \text{[eq1]}$$

A is the absorbance (unitless), l is the cell length (1.00 cm), C is the concentration in Molarity, and ε is the molar absorptivity (M^{-1} cm^{-1}). Taking the slope of the graph of the absorbance intensity vs. the concentration for each solvent gave epsilon value **(**Figure 4). The ε values for the two solvents used in this study are in table 2.

Solvent	ε (M^{-1} cm^{-1}) Value
DCM	13300 ± 250
THF	12000 ± 160

Table 2: These are the values calculated for epsilon for each of the solvents. Uncertainties are the standard error in the slope from the linear regression.

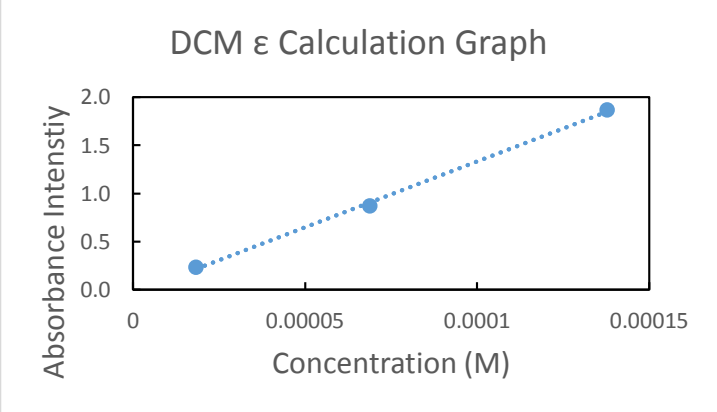


Figure 4: The ε value for DCM was calculated at 528 nm using the slope of the line in the graph above. The cell length, is 1.00 cm so the slope is equal to the ε value in units of M^{-1} cm^{-1}.

Spectrophotometric titration

The original plan involved setting up a Schlenk line at Andrews University. Issues occurred with obtaining the NO and with purification of the gas. Our adapted plan required a trip to University of Michigan, Ann Arbor. There Dr. N. Lehnert's lab had the needed experimental apparatus available and we were allowed to use it.

The general setup and use of a Schlenk line is described in the literature. (Linn, **2012**) A

Schlenk line started as piece of glass tubing that has a hole on each end, as well as several holes along the side. One end of the glass manifold connected to nitrogen gas and the other end of the glass manifold connected to a vacuum system. This allowed the manipulation of gases when used correctly. Each connection on the side of the tube had a stopcock. When the stopcock opened gases moved from inside the glass tube and into the atmosphere and from the atmosphere inside the tube, when all the stopcocks are closed the system became airtight to the atmosphere. A Schlenk flask was attached to one of the side connections on the Schlenk line. (Wayda, **1985**) A Schlenk flask was a special flask that had three openings. One of the openings had a stopcock so that it could be connected to the vacuum line. One of the holes was plugged with a rubber stopper to allow the solvent inside to be withdrawn using a gas-tight micro syringe. The final hole was sealed with a glass stopper and grease.

Running the experiment itself involved several steps. The first step required the preparation and purification of the solvent by distillation. Boiling the solvent and allowing the vapors to condense in a maze of glass tubing with the condensed solvent dripping out the end of the tubing opposite where it entered as vapor accomplished this. Distillation is based on the concept of impurities in a solvent boiling at different temperatures than the solvent itself.

Preparing NO-saturated solvents

The distilled solvent contained dissolved atmospheric gases, including oxygen that can react with NO, which needed to be removed upon arrival at University of Michigan. Even solvents stored in an O_2 free glove box are saturated with N_2 and the N_2 needed to be removed as well. Some (approx. 10 mL) of each solvent to be saturated with NO was placed in a Schlenk flask and attached to the Schlenk line. A freeze-pump-thaw cycle was used to remove dissolved gas (N_2 and O_2) from the solvent. The vacuum pump first evacuated all the gasses out of the manifold. Then liquid nitrogen (which is at –196 °C = –321 °F) was used to fill a canister under

the flask and the temperature froze the solvent until it became completely solid. Then the stopcock for the Schlenk flask was opened and the vacuum pump ran to remove the gases that were once dissolved in the solvent. Then the stopcock for the Schlenk flask was closed and the flask was moved from the liquid nitrogen to water to allow the solvent to thaw. Three cycles of this freeze – pump – thaw process was standard practice to remove dissolved gases in the solvent. (Lamarque, **2005**) On the day of the experiment toluene, DCM, THF, and acetonitrile were put through this cycle and were ready to be "saturated" with NO. The atmosphere inside each Schlenk flask contained solvent at its vapor pressure, and no other gas.

Finally, at the time to perform the experiment, NO at slightly over atmospheric pressure (bubbles in a silicone oil bubbler at a depth of 1-2 mm) was introduced into each Schlenk flask while the solvent was stirred for approximately 10 minutes with a magnetic stir bar. All solvents were saturated at the same time.

At this point the toluene flask turned blue which indicated that oxygen entered the flask at some point and that it, unfortunately, could not be used. The acetonitrile, DCM, and THF continued to the next step. The entire process of saturating the solvents occurred in a fume hood for safety reasons.

Spectrophotometric titration

The preparation of the samples for the spectrophotometric titration occurred in a glove box to prevent contamination with atmospheric gases. These "samples" were a known number of moles of CoTPP in a cuvette. Each sample is referred to as a run throughout the course of this paper. To prepare a stock solution, a known mass of CoTPP was placed in an Eppendorf tube and dissolved in 1.00 mL of DCM (measured with solvent-inert tuberculin syringe). This stock solution was then diluted with two one-to-ten dilutions (0.100 mL solution and 0.900 mL gas-free solvent), to create the solution used for the titration. The solution after the second dilution

(1:100 diluted from stock) was placed in the gas-tight, septum-capped cuvette (Starna 9B-Q-10-GL14-S) and removed from the glove box. The exact dilution history for DCM (table 6) and THF (table 7) can be found in the appendix.

The titration required accurately knowing the mass of CoTPP in the cuvette and the volume of the sample. The number of moles of CoTPP present in the cuvette could be determined from the dilution process outlined above. Diluting the solvent was necessary so that sufficient CoTPP could be weighted out to give 2 sig figs while keeping the concentration of CoTPP low enough so that the UV-Vis absorbance was between 0.4 and 2.0 consistent with best analytical use (Harris, **2010** Ch. 17). While making the dilutions, an issue arose with acetonitrile. Judging from the precipitate that formed, it appeared that the CoTPP is insoluble in acetonitrile. Due to this issue, acetonitrile was discontinued.

After taking a UV-Vis spectrum of the sample containing only CoTPP, the spectrophotometric titration was initiated by adding a microliter (1.000 µL) of the NO-saturated solvent with a 10 µL gastight syringe (Hamilton). Following this addition of NO-saturated

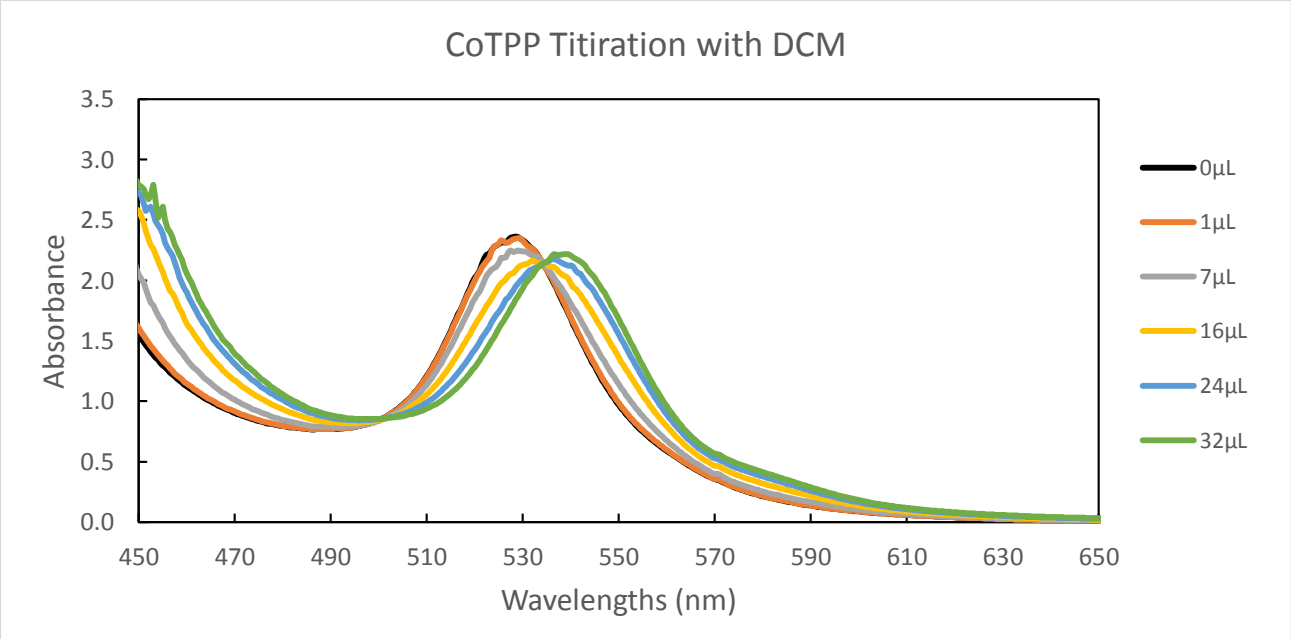


Figure 5: This figure shows the data from a trial with DCM

solvent, a second UV-Vis spectrum was recorded. This spectrum showed some shift in the shape of the spectrum compared to the first. Specifically, the peak at 523 nm representing the CoTPP shrunk in height, and the peak at 536.5 nm representing the CoTPP·NO grew in height. Then another µL of the NO in solvent was added and another spectrum was taken. This process continued until the UV-Vis spectrum no longer changed (Figure 5).

The UV-Vis spectrum was collected from 300 nm to 900 nm with absorption readings every 0.5 nm. There are two peaks that appear in the spectrum, but the one used for analysis was the peak at 523 nm. The total spectral line showed one isosbestic point, where the spectrum is constant as the NO-saturated solvent is added. The existence of the isosbestic point implied that there is a simple conversion of one chemical species into another as in scheme 1.

Analysis Methodology

The raw data from the UV-Vis software, when opened in Excel, contained the wavelengths and the absorption intensities for each of the sets of data collected. By converting the wavelengths (nm) into wavenumbers (cm^{-1}) using equation 2 the absorption curves displayed broad absorption shapes with a width of approximately 1000 cm^{-1}.

$$\frac{10{,}000{,}000}{nm} = cm^{-1} \qquad \text{[eq2]}$$

The first absorption spectrum of the spectrophotometric titration of pure CoTPP, was saved as a CSV file. Similarly, the final absorption curve of pure CoTPP·NO was saved as a CSV file. This file type, which stores only the cm^{-1} and absorbance pairs in order, could be opened in a program called Fityik (Wojdyr, **2010**) so that the experimental spectral line shapes could be simulated.

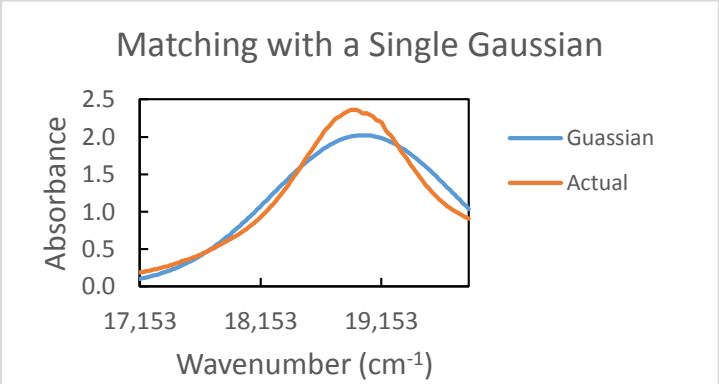
To prepare a "synthetic spectrum" for pure CoTPP in Fityk computer software (Wojdyr, **2010**), Gaussian curves were fit to the actual UV-Vis spectrum for the sample that contains pure

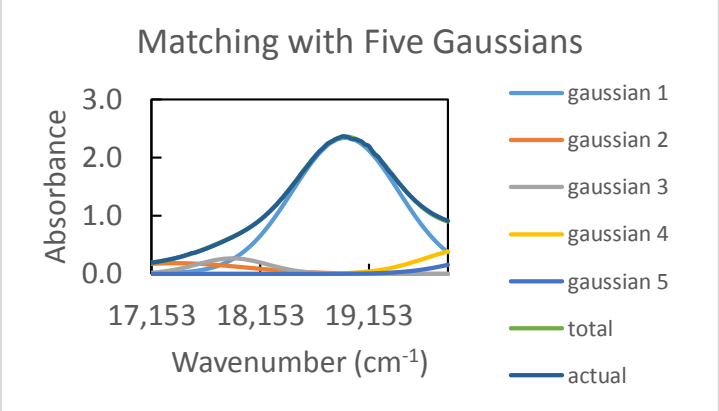
CoTPP. As eq. 3 shows, the three parameters of the Gaussian curve employed by Fityk were: an amplitude named a_0, a center named a_1 and a half-width-half-max value named a_2.

$$a_0 \exp\left[-\ln(2)\left(\frac{x - a_1}{a_2}\right)^2\right] \qquad \text{[eq3]}$$

An initial guess Gaussian is added by hand and is set to have a peak height equal to that of the experimental data. The Fityk program then has an algorithm to adjust $a_0, a_1,$ and a_2 so that the curve best matches the experimental spectrum, see figure 6.

Figure 6 shows the experimental spectrum fit in Fityk with one Gaussian, with three Gaussians, and with five Gaussians. The single Gaussian does not match the data well (79% match). The five Gaussian curve matches the data well (99.95% match), but the half-widths of the curves are quite different (from 383 cm^{-1} to 751 cm^{-1}). In this case the extra Gaussian





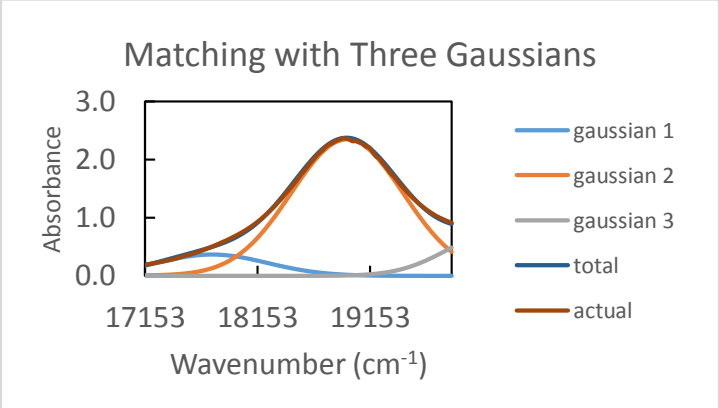


Figure 6: The different Gaussian variations are shown here to explain why three Gaussians were chosen for the analysis.

curves do not physically improve the fit. The fit with three Gaussians (99% match) matched the experiment much better than with one Gaussian. The use of three Gaussian curves was estimated to be the minimal number of parameters that it provided as well as a similarity in the half-width-half-max values. Using the half-width-half-max, amplitude, and center for each of the three Gaussians to create a synthetic spectrum in Excel, I summed the three Gaussians for each wavenumber. Following the same process, a synthetic spectrum for CoTPP·NO was created (figure 15 in the appendix). The complete sets of parameters for all of the DCM Gaussians can be seen in Table 3.

	a_0	a_1	a_3
CoTPP Gaussians			
Gaussian 1	2.344	18950	585.6
Gaussian 2	0.7222	20270	512.9
Gaussian 3	0.3678	17750	580.1
CoTPP·NO Gaussians			
Gaussian 1	2.180	18560	600.3
Gaussian 2	0.7636	19850	601.3
Gaussian 3	0.3392	17270	465.9

Table 3: The parameters for the Gaussians fit to the CoTPP and the CoTPP·NO spectra.

After fitting the first spectrum (pure CoTPP) and the last spectrum (pure CoTPP·NO) with synthetic spectra, a process to analyze the intermediate spectra was developed. The intermediate spectra were assumed to arise from a fraction of unreacted CoTPP starting material and CoTPP·NO product. Equation 4 shows how the synthetic spectra for pure CoTPP and pure CoTPP·NO were combined to create a synthetic spectrum for each intermediate.

$$modeled\ spectrum = a \times spectrum_{CoTPP} + (1 - a) \times spectrum_{CoTPP \cdot NO} \quad \text{[eq4]}$$

In order to find exact values for (a) using the "solver function", I used a "Least Sum of Squares (LSS)" (Miller, **2010**) methodology where the sum of the squares of differences in the actual value and the synthetic value for each point (wavenumber), equation 5.

$$LSS = \sum (actual - calculated)^2 \quad \text{[eq5]}$$

Then I wanted to minimize the LSS value so that the actual spectrum and the synthetic spectrum would be as close as possible. To do this, the percentage of the CoTPP spectrum (a), and thus by default the percentage of the CoTPP·NO ($1 - a$), were allowed to float.

The solver function in Excel was configured to minimize the LSS value in equation 5 by changing the value for the single variable a (fraction of CoTPP). This of course also changes the value for $1 - a$ (fraction of CoTPP·NO), implicit in this analysis approach is the assumption that CoTPP and CoTPP·NO are the only two forms in which CoTPP is present. The presence of an isosbestic point in figure 3 suggests this is the case. With the value of LSS minimized, the values for the percent of CoTPP and the percent of CoTPP·NO in the intermediate spectra were determined after for a particular volume of NO-saturated solvent had been added to the cuvette. This process was repeated for each intermediate spectrum. An example of an intermediate can be seen in figure 7.

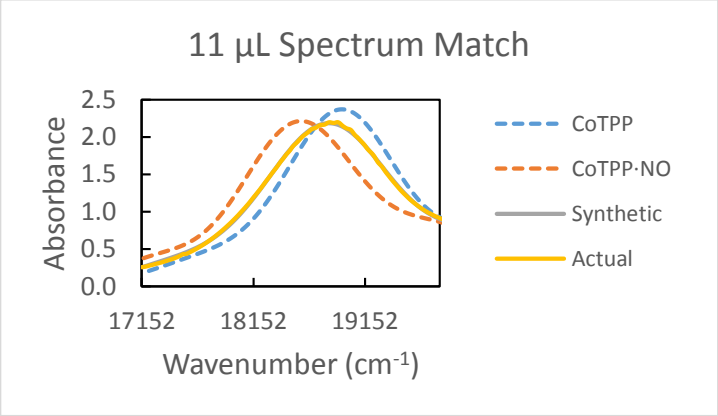


Figure 7: An example of matching an intermediate with the CoTPP and the CoTPP·NO synthetic spectra.

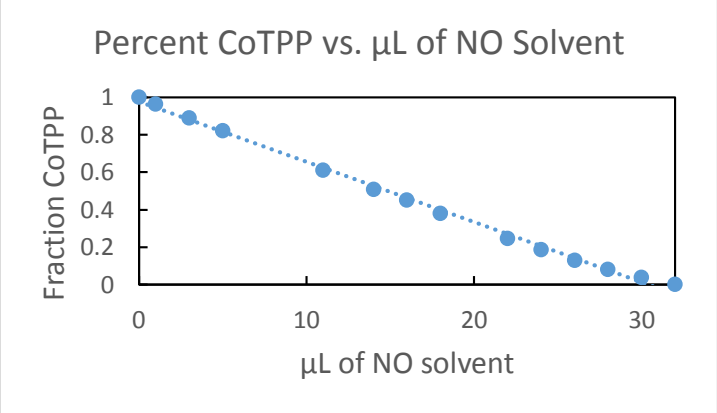


Figure 8: The trend line whose slope could be converted into the concentration of NO in the solvent.

Graphing the fraction of CoTPP (a) vs. the µL of NO solvent (figure 8) gave a straight line. The slope of that line, through unit conversion, gave the concentration of NO in solvent for that data run. The unit conversion is outlined in equation 6.

$$slope \times \frac{moles\ CoTPP}{100\%\ CoTPP} \times \frac{1\ \mu L}{10^{-6}\ L} \times -1 = \frac{moles}{L} = M \qquad \text{[eq6]}$$

The first term is the slope, in this case, –0.0321 / µL NO-saturated solvent. The second term is the number of moles of CoTPP in the cuvette. This method found the concentration of NO in DCM to be 2.29 mM ± 0.04 mM. The uncertainty is from the standard deviation of the slope and is converted to mM using the same conversion outlined in equation 6. The method I developed and described above was also used to analyze data for THF.

III. Results

The analysis method was developed on data previously collected by Dr. Randall at University of Michigan. The data for the results below was collected on one day during the process of this project. Results are reported for DCM and for THF.

Data analysis: DCM (Feb 2016)

The collection method above was followed for three samples of DCM with the goal of assessing the reproducibility with the previously collected data. The spectrophotometric titration of each sample is described as a "run". Each run used a new cuvette filled with a fresh and precisely known number of moles of CoTPP. For the DCM data, each run came from a separate dilution 2 vial. (For a detailed dilution history see table 6 in the appendix.) The Schlenk flask filled with NO-saturated solvent remained constant for all three runs of the DCM collection. After

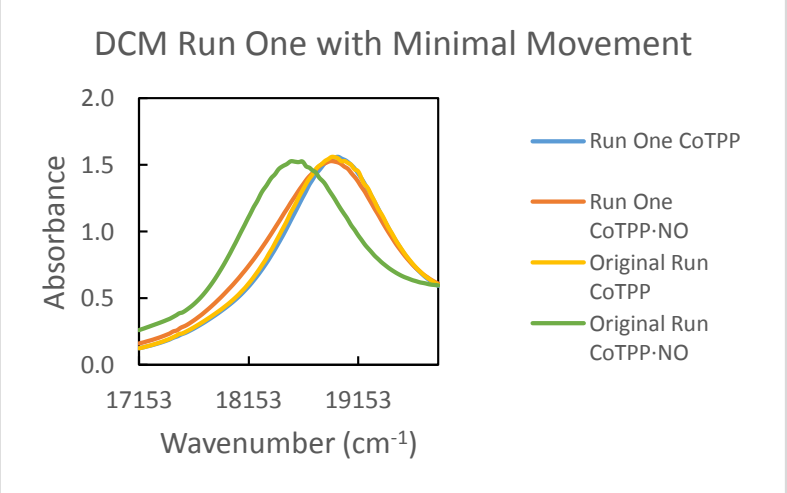


Figure 9: The comparison between the shift experienced in the original data collected by Dr. Randall and the much small shift that the data from Feb 2016 showed a significant size difference. This difference is the reason this data was disqualified.

finishing the titrations on the solvent DCM, I noted that the NO-saturated solvent had turned yellow, indicating that the sample may have become contaminated with atmospheric oxygen, or some other form of contamination occurred. Further, the ~530nm peak in spectra of the three DCM titrations showed little to no shift compared to the previously collected data (figures 9, 10). This implies that the solvent did not get saturated with NO or that the NO came out of solvent.

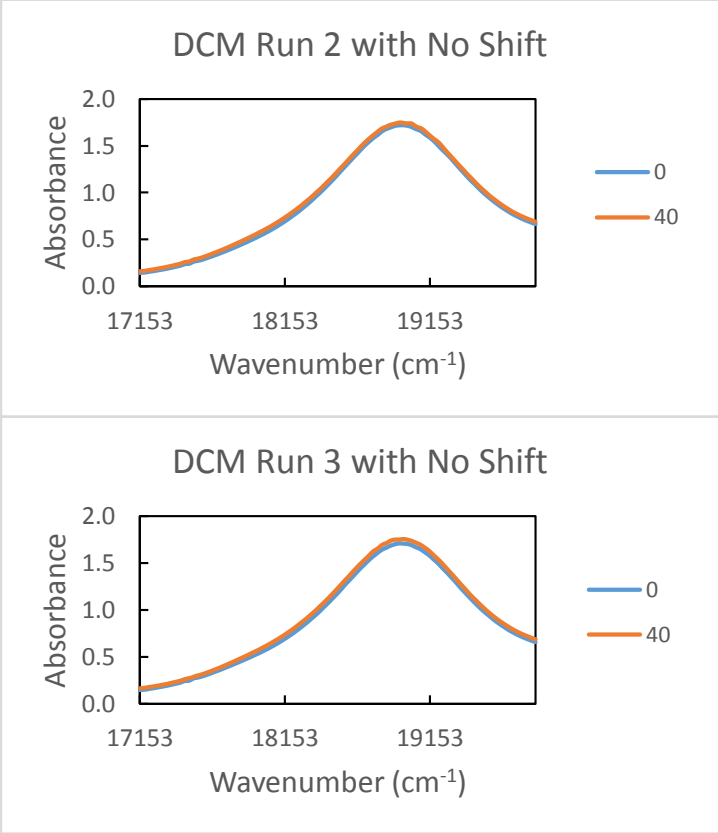


Figure 10: These two graphs show that in the second and third attempts at collecting data for DCM there was no shift at all.

Unfortunately, no data on DCM in Feb 2016 data set was judged viable to pursue further data analysis. Because of these issues, it was not possible to check for reproducible values between runs for DCM.

Data analysis: THF (Feb 2016)

For THF, four "runs" were performed. The spectra for the first two THF solutions showed inconsistencies in absorbance intensity though they were prepared similarly. This means that they came from the same stock solution, the same first dilution, and the same second dilution and an equal amount of the second dilution created a third dilution for THF. (A table explaining the dilution and run history for THF can be found in table 7 in the appendix.) A third dilution, a 3 to 10 dilution from dilution 2, was needed because the absorbance was much higher than the dilutions done in DCM. For the THF data used, the total dilution factor from the stock was

3:1000 (1:10 followed by 1:10 followed by 3:10). The first two runs of the THF data were discarded due to unknown differences in preparation that resulted in inconsistent initial absorption spectra of CoTPP in THF. Only the third and fourth trials of THF were analyzed. Figure 11 shows the selected spectra for run 3. The shift of the 536 nm peak is more difficult to see than in the preliminary DCM data because the peak moved about 16 cm^{-1} in THF: from 18651 cm^{-1} (536.16 nm) for pure CoTPP spectrum to 18635 cm-1 (536.62 nm) for pure CoTPP·NO. Figure 11 shows that the line shape is changing somewhat, so more does appear to be occurring than an 0.5 nm peak shift. (The similar graph for run 4 can be found in the appendix as figure 17).

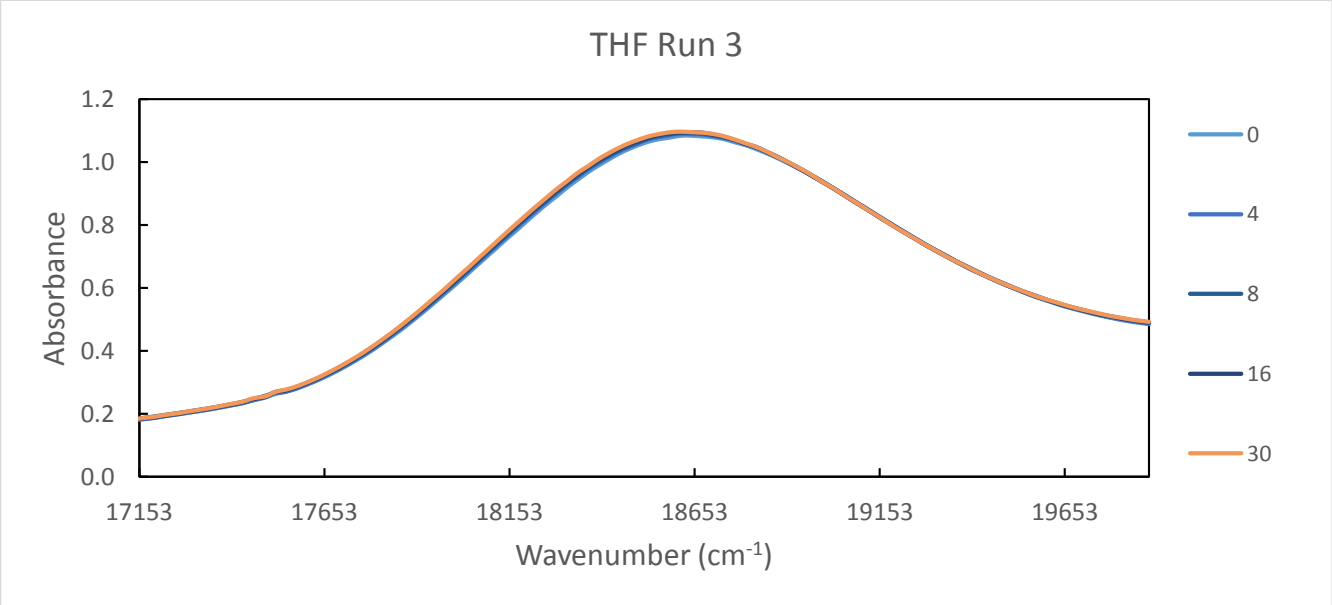


Figure 11: This graph shows the data collected for run 3 of THF. The graph for run 4 (figure 17) is viewable in the appendix.

Following the analysis method explained above, I analyzed each THF run individually. For each run the pure CoTPP and pure CoTPP·NO were each fit with their own set of three Gaussian curves (see figure 12). The figure for CoTPP·NO for run 3 as well as CoTPP and CoTPP·NO can be found in the appendix as figures 16, 18, and 19 respectively. The values for the centers of the Gaussians between the runs were kept consistent, while the half-width and

intensity were allowed to float. The latter allows for the possibility that one of the samples might be slightly more concentrated. Table 4 summarizes the parameters. (The parameters for THF Run 4 can be found in table 8 in the appendix.) As above, each intermediate sample (those after adding some µL of NO-saturated solvent) was analyzed and the fraction of CoTPP and the complementary fraction of CoTPP·NO were calculated by minimizing the LSS function (eq. 5) using Excel's solver function. Then the fractions of CoTPP in the run 3 and run 4 were graphed and the slope of the line for the points was determined as seen in figure 13 (figure 20 in the appendix shows the graph for THF run 4). Using equation 6 the slope was then converted to find the concentration of NO in THF. The slope for run 3 was found to be -0.04841. The slope for run 4 was found to be -0.05197. Using unit conversion and taking the average of the two values, the concentration of NO in THF was calculated to be 0.9 ± 0.1 mM. The uncertainty is based on the standard

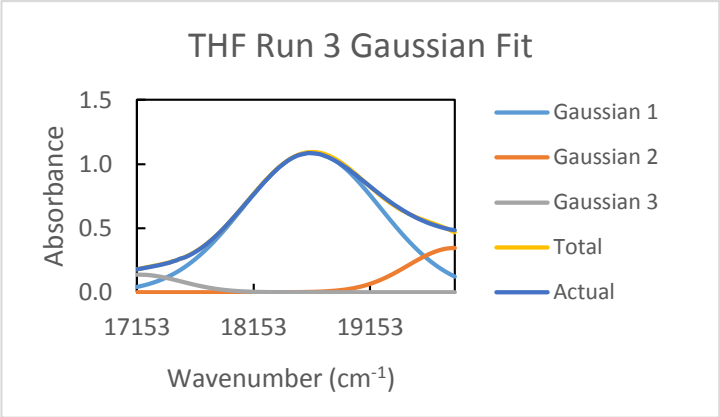
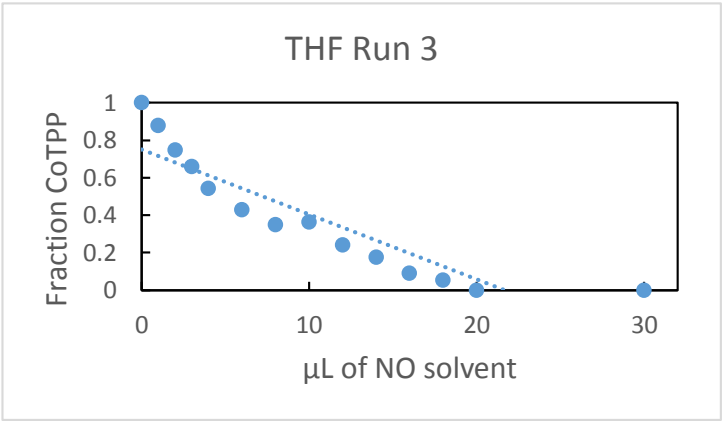


Figure 12: The fit of the Gaussians for the third data run for THF. The graph for the fourth run looks similar and can be seen in figure 17 in the appendix.

THF Run 3	a_0	a_1	a_2
CoTPP Gaussians			
Gaussian 1	1.09	18650	693.4
Gaussian 2	0.3439	19880	466.7
Gaussian 3	0.1358	17170	428.9
CoTPP·NO Gaussians			
Gaussian 1	1.099	18635	691.3
Gaussian 2	0.3599	19880	485.9
Gaussian 3	0.1373	17172	416.6

Table 4: This table shows the Gaussian fit parameters for the third run of THF. The values for run 4 can be found in the appendix.



deviations of the slopes. The standard deviations were converted to mM using equation 6 and then the error was propagated. Inspecting the graphs showed that the first five points for both graphs showed steeper, linear form. These points were graphed and slopes of -0.11342 and -0.12962 for runs three and four respectively. Conversion and averaging for these slopes gave a NO concentration of 2.2 mM ±0.3 mM in THF (uncertainty from standard deviations, conversion, and propagation). Based on our analytical method, we consider the value from all the data points to better represent how NO-saturated THF might be used. However, the 2.2 mM value represents an upper limit to the NO concentration in THF. This highlights the need to do more careful measurements.

Analysis justification

Readers may wonder why the decision was made to create a unique synthetic spectrum (set of Gaussian curves) for each of the THF data runs rather than creating a set of parameters for one set and then analyzing both runs using the same synthetic spectrum (identical set of Gaussians). After plotting the CoTPP spectrum and the CoTPP·NO spectrum, figure 14 shows that the starting points (pure CoTPP) and ending points (pure CoTPP·NO) were not identical

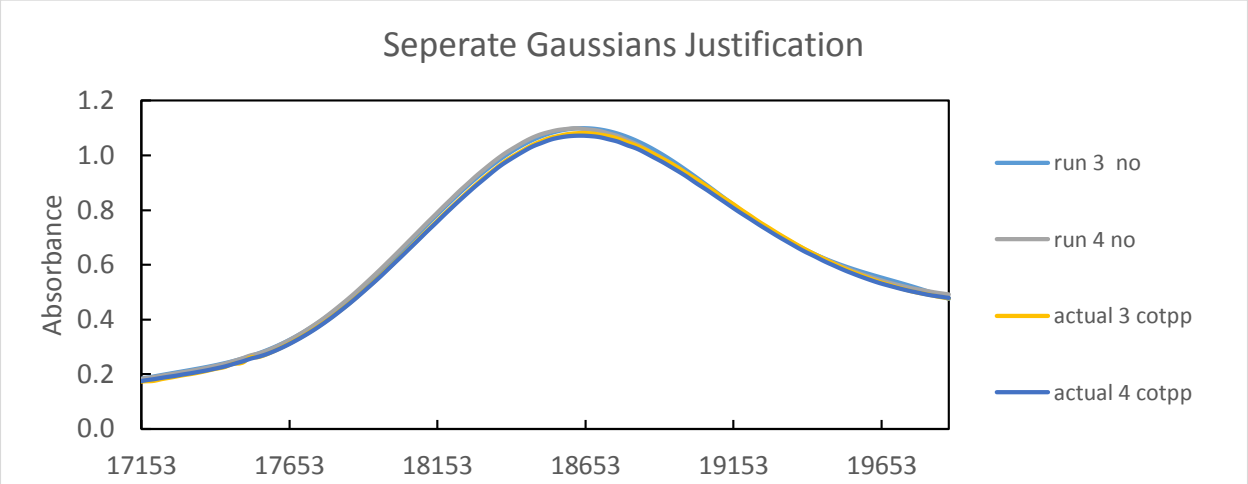


Figure 14: The shifts between the third and fourth runs can be seen here and show and why it was necessary to have separate Gaussians for each run.

it

between runs. This difference justified the preparation of separate synthetic spectra for the two THF runs.

The values for the centers of each Gaussian in the synthetic spectra were kept the same between the runs. Following the analysis method listed above there are three Gaussians for the CoTPP curve and three Gaussians for the CoTPP·NO curve.

IV. Discussion/Next Steps

This project made strides towards finding concentrations of CoTPP in NO in both THF and DCM, but some changes should be made to the experiment to be more successful. The practical difficulty in doing the experiment (18-hour day trip to Ann Arbor, MI), made it impractical to implement these improvements.

Recommendation 1: In the future CoTPP should be tested to see if it is soluble in the solvents to be used. Specifically, acetonitrile should be tested. If CoTPP is soluble in acetonitrile, a stock solution should be made from acetonitrile to avoid immiscibility problems.

Recommendation 2: Additionally, instead of having one flask containing the NO solvent for each type of solvent, it would be better to have one flask of NO-saturated solvent to be used for each titration. This would be beneficial because each time the needle pokes through the rubber septum stopper on the flask a small hole is created that could allow atmospheric gas to slowly seep through. If atmospheric gas seeps in it could slowly change the NO concentration.

Recommendation 3: Another experimental change that could help with reproducibility of the spectrophotometric titrations and the NO concentrations would be to immerse the syringe needle inside the CoTPP solution in the cuvette before injecting the solvent. This would make it more likely that the dissolved nitric oxide reacts with the CoTPP rather than (possibly) remaining in

the atmosphere (headspace) of the cuvette where NO could come out of solvent over the CoTPP solution. This would require the needle to be cleaned between each addition.

Recommendation 4: A successful concentration for nitric oxide in DCM was calculated in this project. There is not, however, any reproducibility on that number. The next step for this should be to redo the titration. Additionally, THF should be analyzed again to ensure reproducibility from separate samples.

Recommendation 5: Summarizing a previous comment, both the titration-to-titration repeatability (as attempted here), as well as the NO-saturation (flask-to-flask) repeatability should be examined. Additionally, different methods used to NO-saturate solvents could be explored: freeze-pump-thaw vs simply over-pressuring with NO pressure while stirring.

V. Conclusions

The synthetic methodology was successfully completed. Throughout this processes a successful process for data analysis was developed and tested. Some problems with the technique for the titration have been discovered and some ideas for correction have been developed for future attempts. This project provided some preliminary results of values for the concentration of nitric oxide in DCM and in THF. A full table of precise values is not yet ready for researchers who work with NO and with model complex research, but with the analysis method developed and tested, steps toward that goal have been successfully taken.

VI. Bibliography

Adler, A.D.; Longo, F.R.; Finarelli, J.D.; Goldmacher, J.; Assour, J.; Korsakoff, L., A Simplified Synthesis for *meso*-Tetraphenylporphrin. *J. Org. Chem.* **1967**, *32(2)*, 476.

This paper describes the method that is still used to synthesize a tetraphenylporphrin ring. Though a very short paper, it includes the details that must be followed to produce the ring including seemingly minor things like distilling the pyrrole before use. The paper also includes ways of purifying the compound including the method of vacuum sublimation and combining the compound with Florex. This source provided helpful synthesis information about the compound that is being used in my project.

Adler, A.D.; Longo, F.R.; Kampas, F.; Kim, J., On the preparation of metalloporphyrins. *J. Inorg. Nucl. Chem*. **1970,** *32*. 2445-2448.

This paper starts briefly explains the problems previously encountered in synthesizing metalloprophyrins and then discusses a newly created method for creating these compounds. Several methods previously existed including a popular one which involved using an acid and a large amount of a metallic salt. This method involved several problems so they developed a new method which uses the technique of refluxing and uses a different solvent, DMF. This article was useful for explaining how the CoTPP that was created by a previous student.

Harris, D.C. Quantitative Chemical Analysis. 8th ed. Freeman: New York, **2010**.

Hunt, A.P.; Lehnert, N., Heme-Nitrosyls: Electronic Structure Implications for Function in Biology. *Accounts Chem. Res.* **2015,** *48 (7)*, 2117-2125.

This source discussed some models of NO binding with Heme. Heme is a part of hemoglobin which is what moves oxygen through your body. NO binds with heme and provides signaling and other important functions for the body. The authors of the paper measured energy changes and vibrational densities along with many other characteristics of heme. They used UV-Vis, EPR, and VDOS analysis to make their conclusions. This paper was useful for providing context on why it is necessary to be able to measure the concentration of NO.

Lamarque, G.; Cretenet, M.; Viton, C., Domard, A., New Route of Deacetylation of α – and β – Chitins by Means of Freeze – Pump Out – Thaw Cycles. *Biomacromolecules*. **2005**, *6*. 1380-1388.

This journal article was about the deacetylations of compounds that were extracted from shrimp and squid. The authors were trying a new method because the old method of deacetylation caused hydrolysis. Through these methods followed in this paper, values about kinetics were discovered in addition to some studies about alkaline hydrolysis. This source was helpful because it explained how the Freeze – Pump Out – Thaw cycle works which is a method that is very important to my project.

Lim, M.H.; Lippard, J.S., Metal-Based Turn-On Fluorescent Probes for Sensing Nitric Oxide. *Acc. Chem. Res.* **2007**, *40*. 41-51.

This article begins by explaining why knowing the concentrations of NO can be helpful and what some of the biological implications are. Then the article continues on and describes several different ways in which they are attempting to use metal sensors to sense NO. They have several Co(II) complexes which they explain they were able to use with fluorescence technology to detect NO. They were able to take crystal structures of many of the compounds they used which allows for better understanding of the overall process. This paper provided some context for my paper.

Linn, D.E. Jr., An Accessible Mercury-Free Vacuum Schlenk Line for Air-Free Techniques. *J. Chem. Educ*. **2012**, *89*, 1479-1480.

The paper provided an improvement to the traditional Schlenk Line. Traditionally Mercury bubblers have been used to check for the presence of gasses moving through the system. It is considered advantageous to remove mercury from use whenever possible due to its toxicity. Linn describes a new way to set up a Schlenk line using analog or digital gauges to check for flow instead of the mercury bubbler. He also includes diagrams about how a basic Schlenk line is set up. This paper provided key information about setting up a Schlenk line similar to the one in my project.

Miller, S.J. The Method of Least Squares. *Brown University*. **2010.**

Möller, M.; Botti, H.; Batthyany, C.; Rubbo, H.; Radi, R.; Denicola, A., Direct Measurement of Nitric Oxide and Oxygen Partitioning into Liposomes and Low Density Lipoprotein. *J. Bio. Chem*. **2005**, *280*. 8850-8854.

This paper explains how the NO radical interacts with Liposomes and Lipoprotein. It explains how the Stern-Volmer equation and the Stokes Einstein equation explain the results of their study. They were looking at the concentration of the NO radical overtime in LDL samples and in distilled water. They used fluorescence and quenching to take measurements as they compared the reaction of NO and the reaction of O_2. This journal article provided some context for the project.

Praneeth, C.K.K.; Nather, C.; Peters, G.; Lehnert N., Spectroscopic Properties and Electronic Structure of Five- and Six- Coordinate Iron(II) Porphyrin NO Complexes: Effect of the Axial N-Donor Ligand. *Inorg. Chem.* **2006**, *45(7)*, 2795-2811.

Shaw, A.W.; Vosper, A.J., Solubility of Nitric Oxide in Aqueous and Nonaqueous Solvents. *J. Chem .Soc. Faraday Tans. 1*. **1977**, *73*. 1239-1244.

The fact that NO is soluble in solvents is the key fact behind this paper. It discusses some of the entropy, enthalpy and Gibbs free energy values that enable NO to be soluble in certain solvents. The paper also explains a method which allowed them to calculate the concentration of NO in solvents like water. The scaled particle theory is what supplies these values. This paper was helpful because it supplied proof that the NO will dissolve in the solvents and thus gave some of the background for this project.

Wayda, A.L.; Dye, J.L., A versatile system for vacuum-line manipulations. *J. Chem. Educ*. **1985**, *62 (4)*, 365-359.

This article addresses the process of setting up a vacuum-line system. The authors address many of the set-ups possible and discusses the changes that have to be made depending on the type of materials that the vacuum system will be exposed to. They talk about how certain types of O-rings can be degraded by different types of solvents and because of this it is important to have the correct type of O-ring for you experiment. They also include examples of techniques they were able to complete using the system that they describe. This paper was useful for giving an in-depth explanation of how a gas line can be set up which is very important part of my project.

Wojdyr, M. *J. Appl. Cryst*. **2010,** 43, 1126.

Fityk is a software program created for fitting curves. Data can be loaded into the program and matched with several different kinds of commonly used curves such as a Gaussian or a polynomial. The software will use a fit algorithm to make sure that the curves match the data as closely as possible. This software provided a program capable of analyzing the UV-Vis spectrum absorption curves.

VII. Appendix

This appendix contains extra information that supports or is redundant to data contained in the text of this thesis.

Dilution 1	100 µL stock with 1400 µL THF
Dilution 2	750 µL dilution 1 with 750 µL THF
Dilution 3	750 µL dilution 2 with 750 µL DCM

Table 5: The dilution history for the THF solutions for calculating the epsilon value.

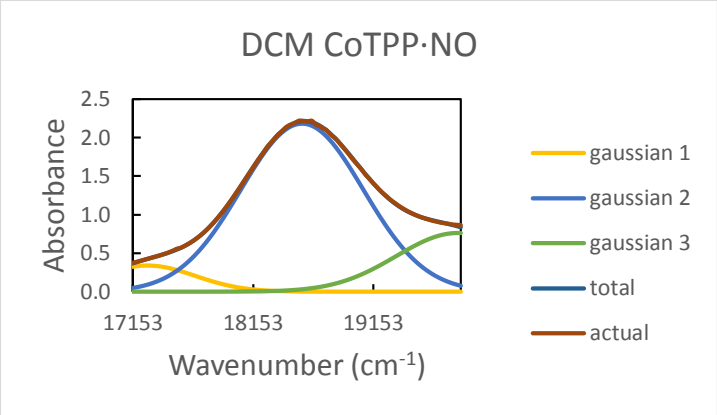


Figure 15: The simulated spectrum and component Gaussians used for CoTPP·NO for the DCM data used in method development.

DCM Stock Solution		
Dilution 1 (1/10 dilution of DCM stock solution in DCM)		
Dilution 2 A [Run 1] (1/10 dilution of dilution 1 in DCM)	Dilution 2 B [Run 2] (1/10 dilution of dilution 1 in DCM)	Dilution 2 C [Run 3] (1/10 dilution of dilution 1 in DCM)

Table 6: The dilution history for Feb 2016 DCM titration.

DCM Stock Solution				
Dilution 1 (1/10 dilution of DCM stock solution in THF)				
Dilution 2 A (1/10 dilution of dilution 1 in THF)	Dilution 2 B (1/10 dilution of dilution 1 in THF)		Dilution 2 C (1/10 dilution of dilution 1 in THF)	
	Dilution 3 A [Run 1] (3/10 dilution of dilution 2B in THF)	Dilution 3 B [Run 2] (3/10 dilution of dilution 2B in THF)	Dilution 3 C [Run 3] (3/10 dilution of dilution 2C in THF)	Dilution 3 D [Run 4] (3/10 dilution of dilution 2C in THF)

Table 7: The dilution history for Feb 2016 THF titration.

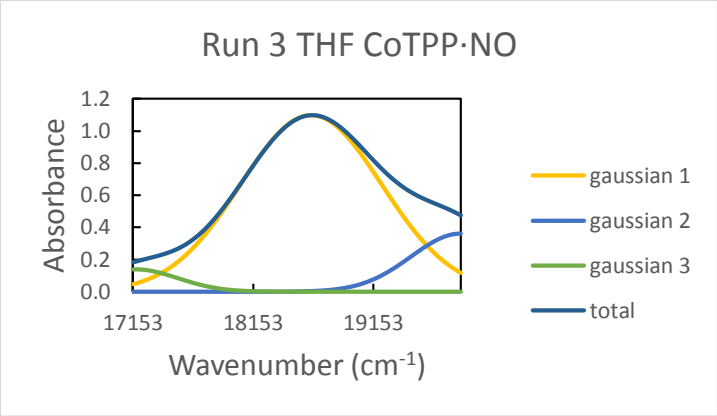


Figure 16: The simulated spectrum and component Gaussians for CoTPP·NO for the third run of the THF data.

THF Run 4	a_0	a_1	a_2
CoTPP Gaussians			
Gaussian 1	1.066	18650	704.0
Gaussian 2	0.3250	19880	451.4
Gaussian 3	0.1217	17170	388.8
CoTPP·NO Gaussians			
Gaussian 1	1.084	18635	702.2
Gaussian 2	0.3452	19880	458.6
Gaussian 3	0.1287	17172	379.5

Table 8: Parameters for the Gaussians for THF Run 4. Parameters for Run 2 are included in the text.

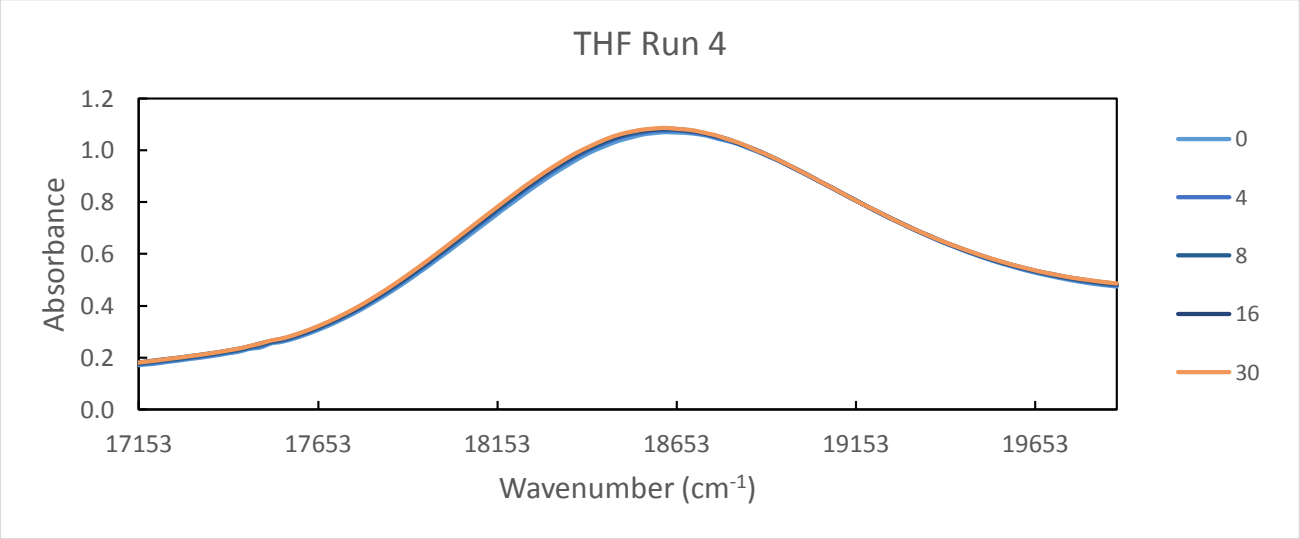


Figure 17: The data from THF Run 4.

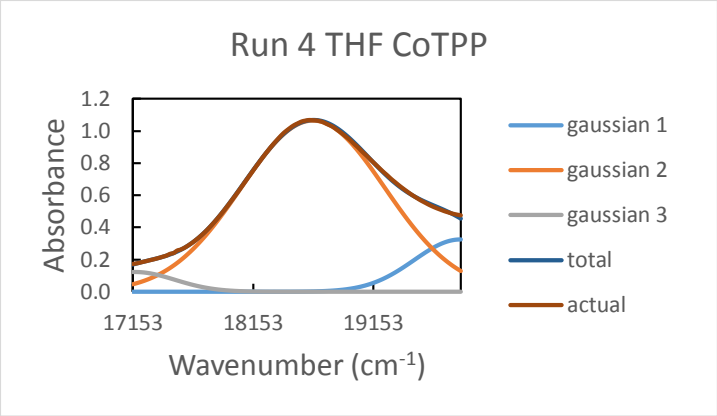


Figure 18: The simulated spectrum and component Gaussians for CoTPP from THF run 4.

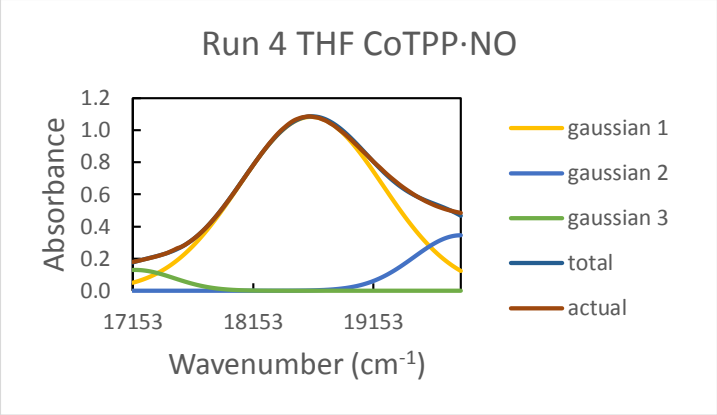


Figure 19: The simulated spectrum and component Gaussians for CoTPP·NO from the THF run 4.

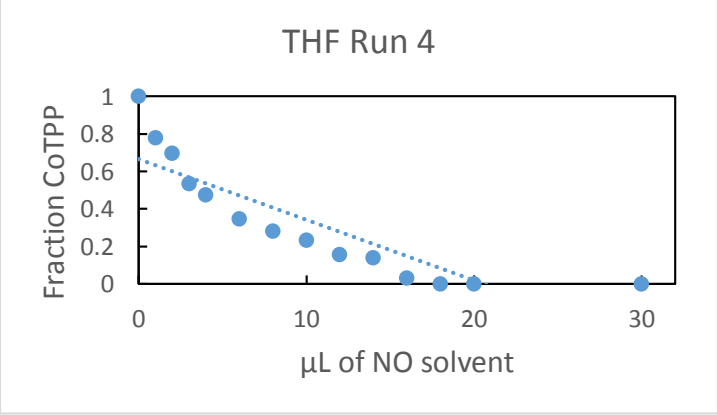


Figure 20: The amount of CoTPP graphed as a function of the volume of NO-saturated THF added for run 4 of THF. The analogous graph for run 3 is in the text.