Separation of Nitrito- and Nitropentamminecobalt (III) Chloride by High Performance Liquid Chromatography

Tisha Hutchinson
tisha.hutchinson@student.shu.edu

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Separation of Nitrito- and Nitropentamminecobalt(III) Chloride by High Performance Liquid Chromatography

by

Tisha Hutchinson

Master of Science Thesis

Submitted to the Department of Chemistry and Biochemistry of Seton Hall University in partial fulfillment of the requirements for the degree of Master of Science

December 2016

South Orange, NJ
We certify that we have read this thesis and that in our opinion it is sufficient in scientific scope and quality as a dissertation for the degree of Master of Science

APPROVED

Wyatt R. Murphy, Jr., Ph.D.
Mentor, Seton Hall University

Cecilia Marzabadi, Ph.D.
Member of Thesis Committee, Seton Hall University

Nicholas H. Snow, Ph.D.
Member of Thesis Committee, Seton Hall University

Cecilia Marzabadi, Ph.D.
Chairperson, Department of Chemistry and Biochemistry, Seton Hall University
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Abstract

Reversed phase high performance liquid chromatography, chosen because of its ability to collect many data points over a long period of time with minimal involvement, was used to separate pentaamminenitritocobalt(III) chloride and pentaamminenitrocobalt(III) chloride. Pentaamminenitritocobalt(III) chloride was prepared, made into a concentrated solution, divided into aliquots and allowed to isomerize to pentaamminenitrocobalt(III) chloride over a period of twelve to eighteen hours. An injection was made approximately every forty-five minutes. The samples were analyzed at three wavelengths, 254 nm, 460 nm and 490 nm. The absorbance was collected and was used to determine the kinetics of the reaction.

The isomerization from the nitrito to the nitro complex is a first-order reaction. The concentration of the nitrito isomer plotted against the time yields a line with a negative slope. The average rate constant determined was $6.6 \pm 0.1 \times 10^{-5} \text{ s}^{-1}$ which is in excellent agreement with previously reported data for this reaction.
Acknowledgements

I would like to thank Samantha Gribben and Samona Hall for all their help over the past few years. I would like to thank my mother for all of her support. I would also like to thank Dr. Murphy for all his help with this project.
Introduction

Pentaamminenitritocobalt(III) chloride (\([\text{Co(NH}_3\text{)}_5\text{ONO}]\text{Cl}_2\))
and pentaamminenitrocobalt(III) chloride (\([\text{Co(NH}_3\text{)}_5\text{NO}_2\]Cl\)_2) were two of the earliest pairs of inorganic linkage isomers isolated. In the past, these complexes were analyzed using techniques such as infrared spectroscopy (IR), ultraviolet visible spectroscopy (UV vis), x-ray crystallography, nuclear magnetic resonance spectroscopy (NMR), ion-exchange chromatography (IEC) and differential scanning calorimetry (DSC). Reversed phase high performance liquid chromatography (HPLC) has been used to separate the complexes seemingly only for characterization purposes but modern separation techniques make it possible to simultaneously track and identify the components of the reaction throughout the entire process of isomerization.

The physical characteristics of the complexes have been well documented with the most notable characteristic being the color of the complexes. The nitrito complex is a dark reddish orange color while the nitro isomer imparts a yellow color to the complex. Aside from the color change, infrared (IR) or electronic absorption (UV-vis) spectroscopies can be used to confirm what happens within the molecule during the conversion of the nitrito isomer to the nitro isomer. NH\(_3\) vibrations occur at approximately 1600 cm\(^{-1}\), 1320 cm\(^{-1}\) and 850 cm\(^{-1}\). In the case of the NH\(_3\) bond in both the nitrito and nitro isomers, degenerate deformation is responsible for the peak seen at 1595 cm\(^{-1}\), symmetric deformation (bending) of the nitro and nitrito isomers respectively create peaks seen at 1315 cm\(^{-1}\) and 1320 cm\(^{-1}\) and finally NH\(_3\) rocking causes peaks in both complexes at 850 cm\(^{-1}\). In addition to its
three peaks at 1595 cm\(^{-1}\), 1315 cm\(^{-1}\) and 850 cm\(^{-1}\), the O-bound vibrations in the nitrito isomer are responsible for three exclusive peaks at 1460 cm\(^{-1}\), 1065 cm\(^{-1}\) and 825 cm\(^{-1}\),\(^1\),\(^4\) which decrease in intensity over time simultaneously while three new peaks caused by N-bound vibrations appear at 1430 cm\(^{-1}\), 1310 cm\(^{-1}\) and 825 cm\(^{-1}\),\(^1\),\(^4\) Absorption of the nitro complex occurs in the visible spectrum around 460 nanometers while absorption of the nitrito complex occurs around 490 nanometers.\(^4\)

Differential Scanning Calorimetry (DSC) has been used to establish the equilibrium between the isomers by analyzing the solid-state isomerization as well as the reversibility of the reaction between the isomers.\(^5\) DSC has also been used to determine the thermodynamic and kinetic properties of the reaction in the solid state.\(^5\),\(^10\) Results from experiments using X-ray crystallography to assess the thermal interconversion between the nitro and nitrito isomers indicate that in the solid state, interconversion occurs without a distinct change in the phase of the compound.\(^5\) The amount of heat generated or absorbed during isomerization is taken into consideration when observing the chemical transformation through progress of thermal isomerization.\(^3\) Isomerization of nitrito to nitro is enthalpy driven while the reverse reaction is entropy-driven. Past experiments have proved that the isomers undergo an intramolecular reaction that can be accelerated at higher temperatures.\(^3\) Thermodynamic results from the analysis of the isomers by DSC have shown that upon heating pure pentaaminecobalt(III) complexes, there is a small endothermic DSC peak for the nitro isomer and a large exothermic DSC peak for the nitrito isomer, an indication that there is an unequal amount of either isomer
during equilibrium. The stable state of both compounds is closer to the semi-stable state of pure nitro than of pure nitrito which is an indication that there may be more of the nitro isomer present in a sample when the reaction reaches equilibrium. The reason for this may be because the Co-N bond in the nitro isomer is stronger than the Co-O bound of the nitrito isomer.

Single-crystal X-ray diffraction has been used to study the structural distortion of and calculate the strain lattice within cobalt complexes. Lattice strain upon cooling was found to be anisotropic. The cobalt complex with chloride was compared with the cobalt complex with bromide and while both complexes had the same values for mean volume coefficients of thermal expansion, directions of expansion in \([\text{Co(NH}_3\text{)}_5\text{NO}_2]\text{Cl}_2\) were observed during cooling, increasing pressure and isomerization while directions of expansion were observed for \([\text{Co(NH}_3\text{)}_5\text{NO}_2]\text{Br}_2\) upon increasing pressure and isomerization, but not during cooling. Distortion of the same structure is different depending on the type of strain. In the structure of \([\text{Co(NH}_3\text{)}_5\text{NO}_2]\text{Cl}_2\) the halide anions are located below and above the nitrogen atoms of the nitrite ion. The oxygen atoms of the nitrite ions for weak hydrogen bonds with \textit{cis-NH}_3 ligands of nearby cations and \textit{trans-NH}_3 ligands form hydrogen bonds with the anions. Anisotropy of the structural strain of the chloride upon cooling is directly related to the directions of hydrogen bonds and hydrogen-bonded chains in the structures.

Either complex will isomerize under various conditions. The nitro isomer can be photochemically converted to the nitrito form upon UV irradiation or exposure to sunlight. Able to occur either by chemical or energy transfer mechanisms,
sensitization has been used to investigate the photochemical behavior of Co(III) complexes. Sensitization processes can occur either by way of chemical mechanisms (involving a bimolecular reaction between the excited sensitizer and the substrate) or by energy transfer mechanisms (involving electronic excitation of the substrate). The study of the direct photochemistry of Co(NH$_3$)$_5$NO$_2^{2+}$ and more pointedly, the direct irradiation of this complex in aqueous solution indicated that redox decomposition and linkage isomerization occurred simultaneously and that the linkage isomerization occurs via cage recombination of the primary radical pair Co(NH$_3$)$_5^{2+}$•NO$_2$.

Organic molecules that were known triplet energy donors were used as sensitizers of the photochemistry of Co(NH$_3$)$_5$NO$_2^{2+}$.

Quantum yield values decrease as the energy of the irradiated band decreases.

Acid- or base-catalyzed isomerization and hydrolysis has frequently been of interest. In one experiment, base-catalyzed linkage isomerization of the cobalt complex (as well as complexes for rhodium and iridium) was studied at different base strengths between 0.01 and 0.1 M over a temperature range of about 20°C. In addition to analyzing the reaction under base catalysis, one experiment focused on the reduction of the cobalt complex by titanium (III) in aqueous acidic solutions. Ti$^{III}$ was in a 15-fold excess over Co$^{III}$ and so this reaction was studied under pseudo-first order conditions. The reaction was found to proceed by an outer-sphere mechanism: proof of the outer-sphere mechanism was determined upon analysis of the kinetics of the aquation rate. The rate of aquation, determined to be in the order of 10$^{-5}$ s$^{-1}$, was less than the rate of electron transfer. The second-order rate constants were consistent and proved that the reductions of the Co$^{III}$ complexes by
$\text{Ti}^{\text{III}}$ were not affected by the aquation process.\textsuperscript{18} Values of the hydrolytic equilibrium deduced from this study were in the range of previously reported values that lie in the range ascribed to outer-sphere reaction mechanisms.\textsuperscript{18}

The reaction is faster in an aqueous solution\textsuperscript{4} and is an intramolecular reaction: it remains unaffected by any outside factors during isomerization and has been confirmed to be an intramolecular reaction using $^{18}$O tracer techniques.\textsuperscript{8,17} It has been proven in experiments that the oxygen in the cobalt/oxygen bond and nitrogen/oxygen bond do not exchange with the solvent during isomerization.\textsuperscript{8} Also, prior studies have shown that the kinetics of the reaction remained the same in an aqueous solution regardless of the amount of the NO\textsubscript{2}\textsuperscript{$-$} added.\textsuperscript{8}

In the past, RP-HPLC as well as other chromatographic techniques have been used to identify and characterize different cobalt isomers. Although ion exchange chromatography had been used to successfully separate cobalt (III) coordination complexes, long columns and subsequently longer run times were needed.\textsuperscript{9} Also, on earlier HPLC systems, each sample had to be injected into the HPLC manually. Over time, this process has been made simpler with the introduction of an autosampler that can be programmed to inject each sample at a specific time.

The inspiration for this project came from an experiment done by Nicholas H. Snow ("Kinetics of Transition Metal Complex Linkage Isomerization by HPLC") at the University of Virginia. The isomerization of the pentaammminecobalt(III) complex allows for great versatility in analysis techniques and subsequently in the determination of the kinetics of the reaction.
Experimental

Materials: The following solvents and reagents were used as received: sodium acetate trihydrate (Sigma Ultra Sigma Aldrich), ammonium chloride (Sigma Aldrich), concentrated aqueous ammonia (Mallinckrodt Chemicals), 30% hydrogen peroxide (BDH), glacial acetic acid (Pharmco Aaper) and hydrochloric acid (Pharmco Aaper and Commercial Alcohols) and cobalt(II) chloride 6-hydrate (Alfa Aesar).

Syntheses: All complexes were prepared as described in literature. The starting material [Co(NH$_3$)$_5$Cl]Cl$_2$ was prepared by dissolving 10.0 g of ammonium chloride in 60.0 mL of concentrated aqueous ammonia in an Erlenmeyer flask. Finely powdered cobalt(II) chloride 6-hydrate (20.0 g) was added in small portions. Hydrogen peroxide (16.0 mL) was slowly added using a dropping funnel followed by 60.0 mL of concentrated hydrochloric acid. The solution was stirred on a hot plate and the temperature was held at approximately 85 °C for ca. 20 min. The mixture was cooled to room temperature. The product [Co(NH$_3$)$_5$Cl]Cl$_2$ was precipitated and isolated by filtration.

[Co(NH$_3$)$_5$Cl]Cl$_2$ was used to synthesize [Co(NH$_3$)$_5$ONO]Cl$_2$, [Co(NH$_3$)$_5$Cl]Cl$_2$(5.0 g) was dissolved in a solution of 15 mL of concentrated aqueous ammonia in 80 mL of water while stirring and heating. The solution was cooled to ca. 10 °C and titrated with 2 M HCl until the solution had a neutral pH by pH paper. Sodium nitrite (5.0 g) was dissolved in the solution and 5.0 mL of 6 M HCl added. The solution was placed in an ice bath for one hour. Crystals of the nitrito complex formed and were isolated by filtration. The crystals were washed with 25.0 mL of ice water and 25.0 mL of ethyl alcohol.
**Liquid chromatography mobile phase** - The mobile phase buffer (pH 3.00) was prepared from 17.7 mL of 0.1 M sodium acetate in 982.3 mL of 0.1 M glacial acetic acid. The mobile phase consisted of 10% MeOH and 90% 0.05 M pentane sulfonate in pH 3 acetate buffer. The sample concentration was 0.4 mg/mL and the injection volume was 20 µL.

**Instrumentation** - A Hewlett Packard 8452A diode array spectrophotometer rebuilt by OLIS was used to obtain spectra of the initial and final products. Samples were filtered using 10 mL Norm-Ject syringes fitted with a 0.45 µm Pall Acrodisc filter. The separations for this project were carried out on a C\textsubscript{18} 5 µ, 100 x 4.60 mm Columbus reversed phase column. A Waters 2695 separations module was used in conjunction with a Waters 2998 photodiode array detector, set for wavelengths at 254, 460, and 490 nm. Chromatograms for this project were taken approximately every 45 min.
Results and Discussion

Synthesis, yields and analyses - Identification of the sample involved assessing the initial and final color prior to running the sample as well as measuring the absorption spectrum using ultraviolet spectroscopy. The color of the nitrito isomer can be described as a darker reddish orange color while the nitro isomer is a much lighter orange and can almost be described as yellow. The samples were dissolved in the mobile phase for analysis in the HPLC and the color change was the same in solution as it was in the solid state.

Nitritopentaamminecobalt (III) chloride begins to isomerize after synthesis is complete; therefore the samples had to be prepared immediately after drying and analysis begun soon after. Four chromatographic runs were attempted, where ten to fifteen sample vials were prepared and analyzed. In all cases, no absorbance was observed in the chromatograms during the first or second samples, taken at 0 min and approximately 45 min. By 90 min, a clear peak could be seen for the nitrito isomer with a smaller peak or bump attached signifying the nitro isomer – this was consistent with Snow’s results. It was not possible to program the software to run a sample at a specific time (completely stopping the flow rate and starting it back up again), so a very slow flow rate was introduced between sampling times at 0.01 mL/min. The sample run time was ten minutes and initially, chromatograms were taken every twenty to thirty minutes. However, this was too fast. It appeared that the instrument could not handle jumping between such a slow flow rate and a faster flow rate so quickly. Taking samples approximately every forty-five minutes seemed to leave enough time for the flow rate to change without having an effect on the
injection - the instrument had enough time to flow steadily before being required to switch methods.

Prior to the start of each run, the isomers were analyzed using absorption spectroscopy. Figures 1 and 2 on page 13 are graphs compiled from the data points of such runs. The low energy $\lambda_{\text{max}}$ of the nitrito isomer is at 490 nm while the $\lambda_{\text{max}}$ of the nitro isomer is 460 nm. As is typical of Co(III) octahedral complexes, the extinction coefficient for these ligand field bands are small (ca. 10 M$^{-1}$cm$^{-1}$). This complicates the use of these characteristics but poorly absorbing transitions to follow the course of the reaction. The ligand to metal charge transfer bands observed in the ultraviolet are much more intense (ca. 20,000 M$^{-1}$cm$^{-1}$) and provide a much better measure of the reaction progress (vide infra). The absorption spectrum of the sample eluting at a particular retention time can be obtained. Examples of the spectra and chromatograms can be found in Figures 3 through 10.
Figure 1. Visible spectrum of \([\text{Co(NH}_3\text{)}_5(\text{ONO})]\)^{2+} in the mobile phase (MeOH:0.05 M pentane sulfonate (10:90) pH 3.00).

Figure 2. Visible spectrum of \([\text{Co(NH}_3\text{)}_5(\text{NO}_2)]\)^{2+} in the mobile phase (MeOH:0.05 M pentane sulfonate (10:90) pH 3.00).
Figure 3: Chromatogram at 45 minutes. The retention time of the nitrito complex is 8.410 minutes. Note the shoulder on the left side of the peak – this will ultimately deepen, split and grow into a separate peak for the nitro complex. The earlier bumps in the chromatograms with retention times could be impurities in the solution or from the column.
Figure 4: Extraction of peak at 45 minutes, 8.410 minutes into the sample run time. This is created by selecting and extracting the peak of interest in Empower. The HPLC uses a diode array to measure results; this is simply a spectrum indicating the wavelength of the analyte at this particular retention time. Note the wavelengths of interest: 462.2 nm and 484.1 nm. These are the wavelengths of the analytes being studied and can be seen as a full peak and shoulder in the previous chromatogram.
Figure 5: Chromatogram at 90 minutes, nitrito peak appears at 7.274 minutes and nitro peak at 8.547 minutes. The peak for the nitro complex is beginning to separate from the nitrito complex peak. Note the decreasing height of the peak at 8.547 minutes – this is the solution beginning to isomerize from nitrito to nitro. This chromatogram shows that at this particular time, there is about an equal amount of complexes in solution.
Figure 6: Extraction of peak at 90 minutes, 7.274 and 8.547 minutes into the sample run time. The nitrito complex peak has the higher wavelength at 484.1 while the nitro complex peak has a strong wavelength at 457.4 nm.
Figure 7: Chromatogram at 135 minutes, nitrito peak appears at 8.472 minutes and nitro peak at 7.136 minutes. The nitrito complex peak is decreasing while the nitro complex peak is increasing. Both peaks are still connected as the retention times are very close.
Figure 8: Extraction of peak at 135 minutes, 7.136 and 8.472 minutes into the sample run time. The nitrito complex peak has the higher wavelength at 485.3 while the nitro complex peak has a stronger wavelength at 457.4 nm. The nitrito complex peak is decreasing and makes up a smaller percentage of the solution. The wavelength is also decreasing.
Figure 9: Chromatogram at 765 minutes, nitrito peak is gone (while a slight bump is still present) and nitro peak at 6.859 minutes.
Figure 10: Extraction of peak at 765 minutes, 6.859 minutes into the sample run time. Nitro complex peak is at its strongest with a wavelength of 457.4 nm. The signal at 484.1 nm is still there – this may be evidence of the presence of the nitrito complex still in solution and is a reflection of the small bump seen to the right of the peak in the previous chromatogram (Figure 9).
In an experiment done at UVa studying the same reaction, Snow concluded that UV detection at 254 nanometers is preferred and, indeed, these chromatograms have the sharpest peaks and highest absorbance values. The differences in absorbance and quality can easily be seen when comparing the three chromatograms. Analysis of the data and any subsequent conclusions were derived from the best three runs carried out for this experiment.

Interpretation of the data depends on analyzing the chromatograms at all three wavelengths. The chromatograms taken at 254 nanometers are the easiest to interpret, with a high absorbance and little to no noise - however, the chromatograms taken at 490 and 460 nanometers hone in on the true characteristics of the sample at that particular moment in time. Towards the end of each run, the shapes of the peaks in the chromatograms were nearly identical, despite the smoothness of the baseline and the peaks. The chromatograms taken at 460 nanometers are similar to those taken at 254 nanometers but the chromatograms taken at 490 nanometers indicate that the presence of the nitrito isomer, while not nearly as strong as that of the nitro isomer, as well as the state of equilibrium between the two may last longer than perhaps previously believed.

The chromatograms taken at 254 nanometers have the least amount of noise and have a higher absorbance, however the HPLC has the ability to capture the samples at different wavelengths and gives a complete picture of the identity of the sample at different time points. In Figure 3, the chromatogram taken at 490 nanometers shows a single peak with a slight bump. Based on this chromatogram alone one could assume that the sample only consisted of the nitrito isomer but was
already beginning to isomerize shortly after the solutions were made and analysis begun.

The nitro isomer has a stronger solitary peak at every wavelength with a higher absorbance at the end of every run compared to the solitary nitrito peak at the beginning of the run. For example: absorbance values of the nitrito isomer at the beginning of Run 2 are 0.00065, 0.00050 and 0.060 at 490 nm, 460 nm and 254 nm, respectively. The absorbance values of the nitro isomer at the end of the run are 0.00085, 0.0022 and 0.22 at 490 nm, 460 nm and 254 nm, respectively. Note that even taken at 490 nm, the nitro isomer has a higher absorbance than the nitrito isomer taken at this same wavelength. This indicates the preference of the isomer to take the nitro form.

In addition to low absorbance values, preference for the nitro isomer can also be proven by quality of the chromatograms taken at 490 nanometers. Each of these peaks in these chromatograms has a lower absorbance and, as a result, noise is present in each chromatogram. Approximately ten to twelve hr later, near the end of each run, the most prominent peak is that of the nitro isomer. This is the case regardless of the wavelength being analyzed. It is interesting to note that even in the chromatograms taken at 254 and 460 nanometers, there is a small and separate band indicating the presence of the nitrito isomer. In each run, a similar band could be seen. This raises the question of whether the compound ever completely isomerizes or if there is always a small amount of the nitrito isomer present - while it may possess most of the physical characteristics of the nitro isomer (namely the
color) as well as the quantitative characteristics, it does not become the pure nitro isomer. This is consistent with observations made in the solid state.4

The data in Table 1 were compiled from the three best runs and will be referred to from this point on as Runs 1, 2 and 3. The chromatograms selected and featured in Figures 3-10 are from Run 3. Data points for Run 3 chromatograms collected at 490 nm were also analyzed and included in the calculations, graphs and charts that follow. The data for Run 3 at 490 nm uses a manual measurement of the chromatograms for analysis. Measurements for this particular set of data points were collected using printouts of the chromatograms and measuring the height of the nitrito peak with a 6-inch steel ruler with 1/64th inch graduations. Note that some data points were omitted because the resulting chromatogram did not produce a coherent result.
<table>
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Table 1: Peak area (µA×s) of the nitrito starting material vs time (min) for Runs 1, 2 and 3 measured at 254 nm. Run 3 at 490 is the peak height of the chromatograms measured at 490 nm.

A first order reaction follows the rate law in the first line of the Analysis on the next page. Integrating the rate law yields the linear equation \( \ln[A] = \ln[A]_0 - kt \).

**Analysis**

\[
Rate = k[A]
\]

Rate is proportional to the rate constant and concentration of A.

\[
-\frac{d[A]}{dt} = k[A]
\]

Rate is rewritten as the rate of disappearance of the concentration over time.

\[
\frac{d[A]}{[A]} = -k \, dt
\]
Concentration is divided on both sides, time is multiplied.

\[ \int \frac{d[A]}{[A]} = -k \int dt \]

Both sides are integrated.

\[ \int \frac{1}{[A]} d[A] = -k \int dt \]

Rule of integrals: constants are allowed to be moved.

\[ \ln[A] = -kt \]

Integral of the first part of the equation is equal to ln of the concentration. integral of second part of equation is simply time.

\[ \ln[A]_t - \ln[A]_0 = -k(t - 0) \]

Equation is written out according to the notation.

\[ \ln[A]_t = -kt + \ln[A]_0 \]

Graph ln[A] on y axis and and t on x asix, line will be straight with a negative slope. Y intercept will be at ln[A]_0.

If ln[[Co(NH₃)₅(ONO)]²⁺] is graphed on the y-axis and t on the x-axis then the graph will have a straight line with a negative slope (= -k) and a y-intercept of ln[A]_0 if a first order reaction mechanism is followed. In Runs 1-3, the area under the peak measured at 254 nm was taken as directly proportional to the concentration of [Co(NH₃)₅(ONO)]²⁺, while in Run 4, the peak height measured at 490 nm was used
for the concentration [Co(NH$_3$)$_5$(ONO)$_2$]$^{2+}$. Figures 11 and 12 from Run 1 present the first order analysis of the peak area of the 254 nm absorbance (diamonds) and the predicted y-value (solid line) plotted against time. Figures for Runs 2 through 4 can be found in the addendum.
Figure 11: First order regression analysis of the disappearance of the nitrito isomer of Run 1. Detector wavelength is 254 nm. The slope of the line is 
-3.99±0.05 × 10⁻³ and the rate constant is 6.65 × 10⁻⁵ s⁻¹.
Figure 12: Corresponding first order residual plot of the disappearance of the nitrito isomer of Run 2. Detector wavelength is 254 nm. Points are randomly dispersed around the x-axis which indicates a first order reaction.
Table 2. Statistical analysis for Graphs 1-4.

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</tbody>
</table>

The slope of the equation obtained from the linear regression analysis for this project was converted to the rate constant in s⁻¹ by multiplying by -1 min/60 s. The results show an average rate constant of 6.6 ± 0.1 × 10⁻⁵ s⁻¹. For reference, Snow obtained a slope of -0.0048 ± 0.0004, corresponding to a rate constant of 8.0 ± 0.0007 × 10⁻⁵ s⁻¹. This is excellent agreement for a kinetic analysis.

It has been shown in various experiments that the isomerization from nitrito to nitro is a first-order reaction in an aqueous solution (where the reaction goes to completion) as well as in the solid state (where the reaction establishes an equilibrium) and that the isomerization is faster in an aqueous solution. In a study of the effect of base-catalysis on the rate of isomerization, it was found that in a solution with a 1.0M concentration of sodium hydroxide the observed rate of isomerization was 6.35±×10⁻⁵s⁻¹. Increasing the concentration of the base increased the rate of reaction with the averages being approximately 3.85×10⁻⁴s⁻¹ with the concentration increasing from 0.01M to 0.1M and approximately 3.28×10⁻³s⁻¹ with the concentration increasing from 0.1M to 1.0M. In an experiment conducted on both freshly and photochemically prepared [Co(NH₃)₅ONO]Cl₂, the rate constant was found using infrared spectroscopy to focus on the intensity of the N-O
vibrations at 1055 cm\(^{-1}\) at regular time intervals.\(^9\) The rate of isomerization was found equal to \(1.43 \pm 0.02 \times 10^{-6}\) s\(^{-1}\).\(^9\)

As previously stated in this paper, the study of this particular reaction is popular because it is fairly simple to analyze and assess the rate law of the reaction under many different conditions with many different techniques. Currently, there does not seem to be a better way to assess the rate of a reaction than by way of modern HPLC techniques because it has the potential to amass many data points at selected intervals. In this particular experiment, however, it was difficult to get a good chromatogram with just the nitrito peak on the first few runs despite a blank being run before sampling began and also in between the first and second samples. It was only until the third or fourth sample that a clear peak could be distinguished and by this time approximately an hour and a half to two hours had already passed and the nitro peak would be eluting a few minutes before the nitrito peak. At the end of the run, about a day later, the chromatograms from the last samples had nothing but a nitro peak present. It is not clear why an induction period occurs.

Earlier studies commented on the equilibrium between the two samples and indicated that the equilibrium was a mixture of the two, with one species being more prevalent than the other. If a few more runs were to be taken after the final official run from the experiment, one would only see a nitro peak. It may be interesting to track the isomerization under different conditions: i.e. heating the sample to track it from nitro to nitrito. Also, because of the difficulty in arranging shorter times to collect more data points, it may be a useful undergraduate project to attempt to run the sample as fast as and as many times as possible.
HPLC has been frequently used previously to separate and identify the components and as Snow indicated HPLC is very useful in analyzing the kinetics of the reaction. The PDA has the ability to analyze samples at multiple wavelengths at one time, giving the opportunity to see the true absorbance value. Since this isomerization is so versatile, there are not many conflicting opinions or corrections to this area of study. Rather, modern HPLC techniques can be further used to gain an even better picture of the transitions between isomers as well as other reactions.
References

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Addendum

Figure 13: First order regression analysis of the disappearance of the nitrito isomer of Run 2. Detector wavelength is 254 nm.
Figure 14: First order regression analysis of the disappearance of the nitrito isomer of Run 2. Detector wavelength is 254 nm.
Figure 15: First order regression analysis of the disappearance of the nitrito isomer of Run 2. Detector wavelength is 254 nm.
Figure 16: First order regression analysis of the disappearance of the nitrito isomer of Run 2. Detector wavelength is 254 nm.
Figure 17: First order regression analysis of the disappearance of the nitrito isomer of Run 2. Detector wavelength is 254 nm.
Figure 18: First order regression analysis of the disappearance of the nitrito isomer of Run 2. Detector wavelength is 254 nm.