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Lab notebooks

Orderly record keeping is essential in a laboratory. A well kept notebook makes writing lab reports much easier. It also helps when trying to troubleshoot problems that may arise during an experiment. In an industrial setting a laboratory notebook is a legal record of research activities. Inventions and ideas that are not properly documented may not hold up in patent litigation. A portion of your grade will be based on your lab notebook. Some points to note are given below.

- Write legibly
- Your notebook should have printed numbered pages
- Use tables for data when appropriate
- Each page should be dated and signed
- Mistakes should be crossed out with a single line, initialed and dated
- Observations should be made as you go
- Changes to procedures should be noted
- Write legibly

The lab notebook is not meant to be a picture perfect record of what you did. Many students take 'notes' on scrap paper with the intent of transferring the data to their notebooks later. This practice should be avoided at all times. Copies of the relevant notebook pages will be turned in along with your report. A duplicating page notebook is preferable to photocopies. The notebooks used for chem. 1A are sufficient.

Safety/Hazardous Waste

In any laboratory safety is paramount. Take note of the location of safety showers, eye wash stations and fire extinguishers when entering the lab. Safety glasses are required and must be worn at all times in the laboratory, no exceptions, ever. Lab coats are also recommended. Lab coats protect your clothes and offer an extra layer of protection between you and the reagents you are working with. The bookstore sells goggles and lab coats at a reasonable cost.

All chemical waste should be disposed of in a properly labeled waste
container. If you fill up a waste container it is your responsibility to go to the stockroom and obtain a replacement. Be sure the label is correct for your experiment. Accidents can happen if wastes are improperly mixed. For example, in chemistry 116AL many samples are digested in nitric acid. Nitric acid is an oxidizer and should never be mixed with organic materials. Violent and explosive reactions may occur if wastes are mixed improperly.

Additional waste containers and labels are available in the undergraduate stockroom. Do not leave solutions out in the lab. If a solution is left out between lab periods it will be disposed.

Lab reports

Lab reports are the written synthesis of the work that you performed in the laboratory. An outsider skilled in chemistry should be able to read your report and understand what you did, why you did it and what you discovered. You will find that good written and verbal communications skills are keys to a successful career in the chemical sciences. There are many ways to format and present data from laboratory work. The format depends upon the intended audience. A report may be in the form of a publication in a journal or your Ph.D. thesis in a research laboratory. Industrial reports may consist of progress reports, patent applications or analytical results for a client.

When writing your lab reports, envision yourself as graduate student writing up your research for publication in a journal. The lab report should be divided into logical sections according the outline discussed below. Details on what should be included in each section are listed. As with all scientific reports, it should be written in past tense (since you presumably are writing the report after you did the work) and with passive voice. Grammatically, passive voice is a bit awkward, however it lends objectivity to the report. For example, one may choose to write “I found the weight percent to be 0.59%.” It is not important that you as an individual made the measurement. Properly phrased one would simply write “The weight percent was found to be 0.59%.” Avoid the use of words such as “I” or “we”, the experimental result should not depend on the experimentalist. If only one individual is capable of getting a certain result, then the result has no meaning. The ACS Style Guide is a great resource for scientific writing in the chemical literature.1

Your reports should be prepared on a word processor. Spelling and grammar count. Additional figures and calibration curves not included in the main lab report along with copies of your lab notebook should be added in an appendix. Label your figures and tables. Keep in mind that the reader may not be familiar with your experiment. The following sections should appear in your lab reports.

Experiment Title:

A short description of the experiment is sufficient

Name:

Your name and the name of your group members. Identify yourself as the primary author.

Sample ID:

For example, KHP 01-03 (This is only needed in Chemistry 116AL)

Experimental Methods:

This section describes the physical work that you performed in the lab. Since the procedures are already written for you it is not necessary to re-write them in detail. The exception is when you deviate from the published method. Often times seemingly trivial points such as adding B to A instead of adding A to B are critical in explaining anomalous results. You should write the procedure in brief form without any experimental details. For example one may write “Carbonate free sodium hydroxide was prepared and standardized by titration with dried KHP using a phenolphthalain endpoint”.

Results:

This section contains, like the title suggests, your results and that’s it. What it does not contain is an in depth discussion of what the results mean. You should include data tables of raw data where appropriate (see table 1 below), calibration plots and figures (spectra, diagrams etc…). If you pooled data with group members, indicate which data belongs to you and which is from your lab partners. The results section is where you will report analytical results with confidence intervals and values you determined such as rate constants.

If the experiment analyzes a value by multiple methods, break the results into subsections according to analyte then method. A sentence such as “The weight percent of KHP in sample # 01-03 was found to be 25.28 ± 0.13 at 95% confidence by titration with standard NaOH.” should be at the end of each subsection when appropriate. It is sometimes useful to put your results into a table. This makes it easy for the reader (or grader) to find important values. Tables always contain a caption above the table itself. Grid lines should be limited to separations between headers (and footers) and the data. The table below illustrates some of these ideas. If you are unsure of how to format a data table consult a style guide or refer to journals for examples. An example table is shown on the following page.
Table 1. Sodium hydroxide standardization results.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Mass KHP (g)</th>
<th>Vol NaOH (mL)</th>
<th>[NaOH] (mol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>0.5426</td>
<td>28.04</td>
<td>0.09476</td>
</tr>
<tr>
<td>Trial 2</td>
<td>0.6137</td>
<td>31.58</td>
<td>0.09514</td>
</tr>
<tr>
<td>Trial 3</td>
<td>0.5973</td>
<td>30.63</td>
<td>0.09547</td>
</tr>
<tr>
<td>Trial 4*</td>
<td>0.5011</td>
<td>25.83</td>
<td>0.09497</td>
</tr>
<tr>
<td>Trial 5*</td>
<td>0.4973</td>
<td>25.16</td>
<td>0.09679</td>
</tr>
</tbody>
</table>

Final Result (± 95% C.I.) [NaOH] = 0.0954 ± 0.0001

* data from lab partner

Figures are an efficient way to present numerical data in an easy to visualize format. It is important to format your plots so that the data is clearly and easily identified. The choice of fonts, symbols and sizes of these features should be well proportioned. Use your best judgement here. As with tables, reference to a style guide or journals for examples is a good way to learn how to do this. All figures should be captioned below the figure.

Figure 1. Calibration curve for zinc determined by AAS.

**Discussion:**

This section is a chance to comment on the experiment as a whole. In other words, what do the results signify? What conclusions or predictions can be made based on your results. If you obtained inconsistent or anomalous results this is your chance to attempt to explain why. Do your results agree with literature values?
The square of the standard deviation is called the variance.

The RSD is also known as the coefficient of variation.
Often data are simply reported as the mean plus or minus the standard deviation, $\bar{x} \pm s$. Reporting the proper number of significant figures is sometimes ambiguous. For example a data set may return a mean of 264 and a standard deviation of 18. One may report $2.64 \times 10^2 \pm 0.18 \times 10^2$. However, since the standard deviation is in the second decimal of our mean the third significant figure in the mean looses significance. In practice the number of significant figures varies depending upon the intended use of the data. If $2.64 \times 10^2 \pm 0.18 \times 10^2$ is the final result for a series of measurements one may reasonably report $2.6 \times 10^2 \pm 0.2 \times 10^2$ as a result. If however, your value is intended to be used in further calculations it is desirable to retain more significant figures than are “technically correct” in order to avoid round-off errors. Generally in a series of calculations all of the decimal points (i.e. as many as your calculator holds) are used until the final result is reached, at which time the proper number of significant figures is determined and reported.

### Probabilities and Reporting Results

When we report a result as $\bar{x} \pm s$ we gain some insight into the noise level for a given set of measurements. What we seek in a measurement is in fact the value of $\mu$ and $\sigma$. Since it is unreasonable to make an infinite set of measurements, we would like to express our mean ($\bar{x}$) in terms of probabilities that the value lies within a certain interval of the population mean ($\mu$). The confidence interval is a value that states that the population mean lies within a certain interval relative to our sample mean with a given probability (certainty). For example, we may report that the population mean ($\mu$) lies within ±0.2 of 2.6 with 95% certainty. In other words, we are 95% sure that the true value is somewhere within the range of 2.4 to 2.8. The confidence interval is calculated according to Eq. 1.4

$$\mu = \bar{x} \pm \frac{ts}{\sqrt{n}}$$  \hspace{1cm} (1.4)

where $\bar{x}$ and $s$ are the sample mean and standard deviation, $n$ is the number of data points and $t$ is the appropriate value of the student’s $t$ at the desired confidence at $n - 1$ degrees of freedom. An abbreviated table of student’s $t$ values is given in table 2.

**Table 2. Selected values of Student’s $t$.**

<table>
<thead>
<tr>
<th>DOF</th>
<th>95%</th>
<th>99%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.706</td>
<td>63.657</td>
</tr>
<tr>
<td>2</td>
<td>4.303</td>
<td>9.925</td>
</tr>
<tr>
<td>3</td>
<td>3.182</td>
<td>5.841</td>
</tr>
<tr>
<td>4</td>
<td>2.776</td>
<td>4.604</td>
</tr>
<tr>
<td>5</td>
<td>2.571</td>
<td>4.032</td>
</tr>
<tr>
<td>6</td>
<td>2.447</td>
<td>3.707</td>
</tr>
<tr>
<td>7</td>
<td>2.365</td>
<td>3.500</td>
</tr>
<tr>
<td>8</td>
<td>2.306</td>
<td>3.355</td>
</tr>
<tr>
<td>9</td>
<td>2.262</td>
<td>3.250</td>
</tr>
</tbody>
</table>
Calibration Curves

Instrumental analysis methods involve a signal being measured in response to an analyte of interest. For quantitative analysis measurements the value of the signal must be proportional, preferable linearly, to the amount of analyte present. One must measure the response of an instrument relative to known concentrations of analyte over an appropriate concentration range. It is not logical to calibrate a response on the molar scale when the analyte in an unknown sample is in the milli or micromolar region. Solutions with known concentrations of analytes are called standard solutions or simply standards. Most instrumental methods rely upon measurements of some electrical signal, e.g. current from a photomultiplier tube or potential of an electrode. Modern electronics allow us to make accurate and precise measurements of current, voltage or resistance easily.

The limiting factor for many analytical measurements is the accuracy of the standards. Careful preparation of standards is paramount in quantitative analysis. Poorly prepared standards cannot be expected to give accurate results. A typical procedure for preparing standards is to accurately weigh a quantity of a substance with known composition. Note here that moisture can greatly alter the composition of a substance. The known mass is then dissolved in a volumetric flask, diluted to the mark and thoroughly mixed resulting in a stock solution that can be used to prepare additional standards. Subsequent standard solutions are prepared by taking known volumes (aliquots) of this stock solution and diluting them in volumetric flasks. Many different concentrations can be prepared in this manner. Volumetric pipettes are typically used to deliver known volumes of liquids. Beakers and graduated cylinders are not accurate or precise and should never be used in quantitative analysis.

Measurements of instrument response are made for each standard solution. Each standard should be measured several times if time permits. The instrument response is then plotted as a function of analyte concentration resulting in a calibration curve. An example calibration curve is shown below in figure 2. The error bars on each data point are the standard deviation of several measurements at each known concentration. The linear least squares fit to the data is given along with the 99% confidence intervals for the data. Note that the most accurate region is in the central portion of the calibration range. Extrapolations beyond the calibration range can result in unacceptable confidence intervals.
Figure 2. Example calibration curve. The linear least squares fit (solid), and the 99% confidence intervals (dashed) for the data are shown.

Concentrations of analytes in unknown solutions can be determined using the slope and intercept values calculated from the linear least squares analysis of the calibration data according to Eq. 1.5

$$x = \frac{y - b}{m}$$  \hspace{1cm} (1.5)

where \(x\) is the concentration of the analyte in an unknown solution, \(y\) is the instrument response for an unknown solution, and \(m\) and \(b\) are the slope and intercept of the calibration curve respectively.

Confidence intervals should be reported for results determined using a calibration curve. The procedure can be quite lengthy to do by hand but can easily be incorporated into a spreadsheet. Students are strongly encouraged to take advantage of the calculating power of spreadsheets when applicable. The uncertainty in the \(x\) value from a calibration curve (\(s_x\)) is calculated according to Eq. 1.6.

$$s_x = \frac{s_y}{|m| \sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y - \bar{y})^2}{m^2 \sum (x_i - \bar{x})^2}}}$$ \hspace{1cm} (1.6)

Here \(s_y\) is the standard deviation of the \(y\) values, \(|m|\) is the absolute value of the slope, \(k\) is the number of replicate measurements of the unknown, \(n\) is the number of calibration points, \(\bar{x}\) is the mean value of \(x\) for the calibration points, \(x_i\) are the individual calibration data \(x\) values and \(\bar{y}\) is mean value of \(y\) for the calibration points and \(y\) is the mean \(y\) value of the unknown.

The standard deviation of the \(y\) values (\(s_y\)) is calculated according to Eq. 1.7

$$s_y = \sqrt{\frac{\sum d_i^2}{n-2}}$$ \hspace{1cm} (1.7)

where \(d_i\) is the \(y\) deviation of a data point from the best fit line and \(n\) is number of calibration points. Note two degrees of freedom are lost in determining the slope and intercept and therefor the denominator
is \( n - 2 \). The \( y \) deviations are calculated according to Eq. 1.8

\[
d_i = y_i - (mx_i + b)
\]  
(1.8)

where \( x_i \) and \( y_i \) are the \( x \) and \( y \) values for a calibration data point.

The calculated standard deviation in the \( x \) value is shown graphically in figure 3.

**Figure 3.** An unknown sample (open circle) is plotted on the calibration curve. The resulting uncertainty is shown graphically (vertical dashed lines).

The confidence interval for the unknown \( x \) value can be calculated according to 1.9 where the student’s \( t \) value is taken from \( n - 2 \) degrees of freedom.

\[
\mu = x \pm ts_x
\]  
(1.9)

Unknown results obtained using a calibration curve should be reported according to Eq. 1.9.

**Standard Additions**

The method of standard additions is used when matrix effects from an unknown may interfere with the determination of an analyte. In this method standards are prepared in which a constant amount of unknown has been added to each solution, see figure 4 below. The response of each solution is measured and plotted in the usual way. The signal at zero added analyte is due entirely to the unknown analyte and the sample matrix. Extrapolation to the \( x \)-intercept gives the concentration of analyte in the unknown as shown in figure 4. The \( x \)-intercept is calculated according to 1.10.

\[
I_x = \frac{-b}{m}
\]  
(1.10)
Figure 4. Steps to prepare solutions for standard additions. (A) Add an aliquot of unknown to several volumetric flasks e.g. 10 mL (B) Add various amounts of standard solution to all the flasks but one (C) dilute to the mark and mix the solutions well.

Figure 5. Calibration curve for the method of standard additions. The concentration of added analyte is plotted vs. instrument response.

Results obtained by standard additions should be reported with 95% confidence intervals. The confidence interval is calculated according to Eq. 1.9 above, however the standard deviation of the x intercept ($s_x$) is calculated using Eq. 1.11 rather than equation 1.6.

$$s_x = \frac{s_y}{m} \sqrt{\frac{1}{n} + \frac{\bar{y}^2}{m^2 \sum (x_i - \bar{x})^2}} \quad (1.11)$$
**Unknown KHP Determination**

**Introduction**

Volumetric analysis is a quantitative method in which an unknown amount or concentration is calculated by reaction with a known amount of reagent. This type of analysis requires accurate measurements of delivered volume. Commonly available 50 mL burets are graduated in 0.1 mL increments. With practice, you should be able to interpolate between the marks and read to the nearest 0.01 mL. The volume is read at the bottom of the meniscus of the liquid in the buret as shown in the figure below. An index card with a small piece of black electrical tape held behind the buret with the tape just below the meniscus aids in reading the volume. Your eye should be at the same level as the meniscus to avoid parallax errors. Do not stand on chairs or stools to read burets. This a dangerous practice and should be avoided. If you are not tall enough to read the buret at eye level, simply lower the buret over the edge of the lab bench while you read the volume.

![Buret Reading](image)

**Figure 1.** The reading of the buret above is 32.64 mL.

Clean your buret thoroughly before use and rinse with your titrant. When water is drained from a clean buret no drops should adhere to the sides. A few mL of solution should be drained from the buret after filling to remove any air pockets in the tip. Rinse the tip with distilled water from a wash bottle and record the volume before beginning your titration. The tip should be rinsed again into the Erlenmeyer flask containing the titration solution before recording the final volume.

A drop of aqueous solution is approximately 0.05 mL. Sometimes less than a full drop of titrant is required as the endpoint is approached. Partial drops can be delivered by forming a small droplet on the tip of the buret and rinsing the droplet into your Erlenmeyer flask with a wash bottle. It is also good practice to rinse down the sides of the flask periodically during a titration with a small amount of water from a wash bottle.
Preparation of NaOH solution

Preparing solutions of NaOH with known concentration is difficult because solid NaOH is very hygroscopic and absorbs CO$_2$ from the atmosphere. This makes it impossible to obtain an accurate mass reading under normal laboratory conditions. Therefore, it is necessary to standardize NaOH against a primary standard acid such as potassium hydrogen phthalate (KHP).

Carbon dioxide reacts with NaOH in solution to form carbonate ion (CO$_3^{2-}$), a weak base. Carbonates are insoluble in concentrated NaOH so stock solutions of 50% NaOH can be used to prepare carbonate free NaOH. Distilled water contains dissolved CO$_2$ that must be removed. CO$_2$ is removed by boiling distilled water for 5-10 minutes and cooling. While you are preparing the carbonate free NaOH solution, add ~3 grams of potassium hydrogen phthalate (KHP) standard to a weighing bottle and your unknown KHP sample to another weighing bottle and place them in a 110°C oven for use later (dry your samples for 2 hours). Work in groups of two for the next step. Each student should boil at least 1 liter of distilled water. You may choose to boil an extra liter of water at this time so that you don’t run out during the titrations that follow. One member of the group will transfer 3-4 mL of 50% NaOH to one liter of cooled water being careful not to disturb any carbonate precipitates at the bottom of the concentrated base. The resulting solution will be approximately 0.1 M NaOH. Transfer the NaOH solution to a labeled polyethylene bottle and mix thoroughly. The other group member will transfer their cooled water to a labeled glass bottle with a tight seal.

Standardization of NaOH solution

KHP is dried at 110°C and stored in a desiccator to prevent the solid from absorbing moisture. When performing titrations using KHP, phenolphthalein is suitable as an endpoint indicator. The phenolphthalein endpoint is the first pale pink color that persists for 30 seconds. Rinse and then fill a clean 50 mL buret with the NaOH solution prepared above (~0.1 M). Prepare a titration blank by adding a two drops of phenolphthalein indicator to 100 mL of water and titrating to the endpoint as demonstrated by your TA. Repeat the blank titration two additional times. Subtract the average volume required to titrate the blanks from the subsequent titrations of NaOH solution.

Weigh at least three 0.7 – 0.8 gram samples of dry KHP and place them in three labeled Erlenmeyer flasks. Record the mass to the nearest 0.1 mg. Add 50 mL of freshly boiled and cooled water to one of the KHP samples, dissolve the solid completely and add two drops of phenolphthalein indicator solution. Titrate to the phenolphthalein endpoint with your NaOH solution. Repeat this procedure with the remaining two KHP samples. Calculate the molarity of the NaOH solution. Report the concentration with a 95% confidence interval.

Caution! 50% NaOH is highly caustic, avoid contact with your skin.

Place the boiled solutions in an ice bath to speed up the cooling process.

NaOH reacts with glass and solutions of NaOH should not be stored in glass containers for extended periods of time.
Unknown KHP Determination

Your unknown KHP sample contains KHP and a neutral diluent. Using volumetric analysis you will determine the mass percent of KHP in your unknown solid sample. Weigh three appropriate size samples of your unknown KHP solid and add to three labeled Erlenmeyer flasks. Record the mass to the nearest 0.1 mg. Add 50 mL of freshly boiled and cooled distilled water to one of your unknown samples, dissolve the solid and add two drops of phenolphthalein indicator solution. Dissolve only one sample at a time to reduce the influence of carbon dioxide. Titrate to the phenolphthalein endpoint. Calculate the mass percent of KHP in your unknown sample.

*Report your values to 0.01% with 95% confidence intervals.*

*The titration volume should be ~40 mL. Unknowns labeled “LO” should use 2.9-3.1 gram samples. Unknowns labeled “HI” should use 1.6-1.8 gram samples.*
Determination of Mn in Steel

Standard Additions

Introduction

Steel is an alloy of iron with small amounts of transition metals such as Mn, Cr, Cu etc.. Steel is digested in hot concentrated (4-5 M) nitric acid and analyzed for transition metals by a variety of techniques. Upon digestion manganese is converted to the colorless Mn$^{2+}$ ion. An oxidant such as the periodate anion is added to oxidize Mn$^{2+}$ to the familiar deep purple MnO$_4$\(^{-}\) ion (Eq. 1 below). The concentration of the MnO$_4$\(^{-}\) ion is then quantitatively detected by visible spectroscopy. Other metals may interfere with the analysis of Mn by this method and must be removed or masked. The primary constituent of steel is iron which can be masked by the addition of phosphoric acid (H$_3$PO$_4$) forming a colorless phosphate complex in aqueous solution. Other metals such as chromium and cerium can be oxidized with iodate forming potentially interfering colored complexes. To account for these potential interfering ions, the method of standard addition is used.

\[
2\text{Mn}^{2+} + 5 \text{IO}_4^- + 3\text{H}_2\text{O} \longrightarrow 5 \text{IO}_3^- + 6 \text{H}^+ + 2\text{MnO}_4^- \tag{1}
\]

Steel Digestion

Weigh a ~1 g sample of steel and transfer it to a 250 mL beaker. Record the mass to the nearest 0.1 mg. Add 50 mL of 4 M HNO$_3$ to the beaker and boil gently for a few minutes or until the sample is dissolved. Keep your sample covered with a watch glass during the digestion process to prevent loss of material through splattering. Slowly add 1.0 gram of ammonium peroxydisulfate [(NH$_4$)$_2$S$_2$O$_8$] and boil for 10 to 15 minutes to oxidize any carbon in the sample. If your sample is pink or contains a brown precipitate at this point, add ~0.1 grams of sodium bisulfite (NaHSO$_3$) and heat for an additional 5 minutes. Allow the solution to cool to room temperature and quantitatively transfer the solution to a 250 mL volumetric flask. Dilute with distilled water to the mark.

Preparation of Standard Mn Solution

Dissolve ~ 100 mg of Mn metal in 10 mL of 4 M HNO$_3$ and boil for a several minutes to remove the nitrogen oxides that are generated. Record the mass of Mn to the nearest 0.1 mg. Quantitatively transfer the solution to a 1 L volumetric flask and dilute to the mark with distilled water. Each group in the class should prepare one standard Mn$^{2+}$ solution.
Standard Additions

Transfer a 20 mL aliquot of steel solution to a 250 beaker using a volumetric pipette. Add 5 mL of 85% phosphoric acid. Add aliquots of standard Mn$^{2+}$ and solid KIO$_4$ to the beaker according to table 1. Boil each solution for 5 minutes and allow to cool to room temperature. Quantitatively transfer the solutions to 50 mL volumetric flasks and dilute to the mark. Measure the absorbance of each of the purple solutions using the colorless blank as a reference. Record the absorbance at $\lambda_{\text{max}}$ for the permanganate ion. Your TA will demonstrate the proper use of the UV-Visible spectrometer. A plot of absorbance vs. the concentration of added standard Mn$^{2+}$ yields a straight line with an x intercept equal to the concentration of Mn from the steel unknown. The maximum absorbance for any solution should not exceed a value of 1.0.

The extinction coefficient of permanganate$^1$ at 525 nm is 2455 M$^{-1}$.cm$^{-1}$. A few simple calculations ahead of time can help prevent mistakes in solution concentrations.

Table 1. Estimated sample volumes to use when preparing calibration standards.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Steel</th>
<th>H$_3$PO$_4$</th>
<th>Std. Mn</th>
<th>KIO$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-blank</td>
<td>20 mL</td>
<td>5 mL</td>
<td>0 mL</td>
<td>0 g</td>
</tr>
<tr>
<td>2</td>
<td>20 mL</td>
<td>5 mL</td>
<td>0 mL</td>
<td>0.4 g</td>
</tr>
<tr>
<td>3</td>
<td>20 mL</td>
<td>5 mL</td>
<td>1 mL</td>
<td>0.4 g</td>
</tr>
<tr>
<td>4</td>
<td>20 mL</td>
<td>5 mL</td>
<td>2 mL</td>
<td>0.4 g</td>
</tr>
<tr>
<td>5</td>
<td>20 mL</td>
<td>5 mL</td>
<td>3 mL</td>
<td>0.4 g</td>
</tr>
<tr>
<td>6</td>
<td>20 mL</td>
<td>5 mL</td>
<td>4 mL</td>
<td>0.4 g</td>
</tr>
<tr>
<td>7</td>
<td>20 mL</td>
<td>5 mL</td>
<td>5 mL</td>
<td>0.4 g</td>
</tr>
</tbody>
</table>

Refer to the discussion of the method of standard additions in the lab manual introduction or in your analytical textbook. You should report the 95% confidence interval using equation 1.11 in the introduction.

Report the percent by mass of Mn in your steel sample to 0.01% along with the 95% confidence interval.

---

Determination of Mn in Steel

Volumetric Analysis

Introduction

This method involves digestion of steel samples in hot nitric acid followed by the selective oxidation of Mn ions to MnO$_4^-$ with sodium bismuthate (NaBiO$_3$) at reduced temperature. An excess of ferrous ions are added to the sample which react with MnO$_4^-$ according to the redox reaction below.

\[
\text{MnO}_4^- + 5 \text{Fe}^{2+} + 8 \text{H}^+ \rightarrow \text{Mn}^{2+} + 5 \text{Fe}^{3+} + 4 \text{H}_2\text{O}
\]  

(1)

The excess ferrous ions are then back titrated with a standard solution of potassium permanganate (KMnO$_4$). Permanganate solutions are highly oxidizing and react with any carbonaceous matter in solution causing uncertainties in the concentration. Concentrated stock solutions of permanganate will be provided for you. It is necessary to filter these solutions to remove any precipitate of MnO$_2$ that may have formed prior to dilution. The dilute solution of permanganate is then standardized against the primary standard sodium oxalate. Permanganate reacts slowly with oxalate at room temperature so the titration must be carried out above 70°C. The endpoint is signaled by the persistence of a pale pink color due to the presence of unreacted permanganate.

Required Solutions

- 1:30 (v:v) HNO$_3$ – free from nitrous acid
- 1:3 (v:v) HNO$_3$
- 1:20 (v:v) H$_2$SO$_4$
- 0.9 F H$_2$SO$_4$ (150 mL of 6 F H$_2$SO$_4$ in 1.0 L)
- 0.01 M KMnO$_4$ (see below)
- 0.03 M Fe$^{2+}$ (see below)

Day 1

- Prepare all required solutions
- Standardized the KMnO$_4$ solution

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To prepare nitrous acid free HNO$_3$ carefully boil concentrated nitric acid for one to two minutes in the fume hood. Allow the acid to cool before dilution to the desired concentration.

Day 2

- Digest and workup steel unknown
- Standardize Fe²⁺ solution
- Titrate unknown

Work in groups of two for this experiment. Each student will perform all of the titrations indicated. You may pool your standardization results if you desire.

**Preparation of standard KMnO₄ solution**

Dry ~0.5 grams of sodium oxalate at 105°C for one hour. Filter 100 mL of ~0.1 M KMnO₄ solution through a clean sintered glass filter. Do not use paper to filter the solution. Organic material is oxidized by the permanganate ion. Transfer the solution to a 1000 mL volumetric flask and dilute to the mark with distilled water. Mix the solution well.

Weigh out several 100 mg samples of dried sodium oxalate and transfer to 250 mL beakers. Add 100 mL of 0.9 F H₂SO₄ and heat to 80-90°C while stirring. It is not necessary to boil the solutions. Fill a buret with the permanganate solution. The meniscus is difficult to read due to the dark color of permanganate solutions. A light held behind the buret will aid in volume readings. Record the initial volume of solution in your buret then add one drop of permanganate to the solution oxalate while you are heating. When the color disappears and all of the solid is dissolved you may begin the titration. Titrate the oxalate solution until a pale pink color persists. The reaction of permanganate with oxalate proceeds according to the following reaction

\[
2 \text{MnO}_4^- + 5 \text{C}_2\text{O}_4^{2-} + 16 \text{H}^+ \rightarrow 2 \text{Mn}^{2+} + 10 \text{CO}_2 + 8 \text{H}_2\text{O} \quad (2)
\]

Calculate the concentration of MnO₄⁻ in this solution. Tightly stopper the solution and store in your drawer until the next lab period. The endpoint is very sharp in this titration so take care not to overtitrate!

**Preparation of Standard Ferrous Ammonium Sulfate Solution**

Add ~12 grams of ferrous ammonium sulfate hexahydrate (Fe(NH₄)₂(SO₄)₂ · 6 H₂O) to a 1000 mL flask and dissolve in 1:20 sulfuric acid. Dilute to the mark with 1:20 sulfuric acid. Mix well, tightly stopper the solution and store in your drawer until the next session. Take care to use the correct reagent for this step. Read the label! Transfer 25 mL of 1:30 nitric acid to a 250 mL Erlenmeyer flask with a volumetric pipette. Add 25 mL of the ferrous ammonium sulfate solution to the flask with another volumetric pipette. Fill a
buret with the previously standardized KMnO₄ solution and titrate to a pale pink endpoint. Repeat this procedure several times. Calculate the concentration of Fe²⁺ in the ferrous ammonium sulfate solution using the stoichiometric relationships in equation 1.

### Preparation of Steel Unknown Sample

Weigh a 1.0 gram of your steel unknown and transfer to a 250 mL beaker (record the mass to 0.1 mg). Do not dry the steel unknown. Add 50 mL of nitrous acid free 1:3 HNO₃ to the beaker in the fume hood (do not use 1:30 HNO₃ for this step!) Cover the beaker with a watch glass and gently boil for 5-10 minutes. All of the solid should be dissolved. Remove the sample from the heat and carefully add ~0.5 grams of NaBiO₃ solid to the solution. This preoxidation step removes any carbonaceous reductants from the solution. Return the solution to the heat and boil for an additional 5 minutes. The solution should turn purplish and/or a brown precipitate should appear. If not, add an additional 0.2 grams of NaBiO₃. Note, The color change is subtle so watch the solution carefully. Do not add more than one additional aliquot of NaBiO₃. Remove the solution from the heat and add dropwise a solution of saturated sodium sulfite (NaSO₃) until the purple tint and/or precipitate disappears. Return to the heat and boil an additional 5 minutes. The solution should be rust orange in color and contain no precipitates at this point. Allow the solution to cool for several minutes. Carefully place the solution in an ice bath and cool until the temperature is 15°C. Add ~0.7 grams of NaBiO₃ and stir for 5 minutes. A purple color should develop and solid NaBiO₃ should remain in the beaker.

\[
2 \text{Mn}^{2+} + 5 \text{BiO}_3^- + 14 \text{H}^+ \rightarrow 2 \text{MnO}_4^- + 5 \text{Bi}^{3+} + 7 \text{H}_2\text{O}
\]

Wash a sintered glass filter with 1:30 nitric acid. Discard the filtrate in the proper waste container. Filter your unknown solution into a clean filter flask. A folded paper towel placed so that the flask is slightly tilted away from the sidearm will help keep the solution from being pulled into the suction hose. Rinse the NaBiO₃ solid with several 1–2 mL aliquots of cold 1:30 nitric acid. Add 3–4 mL of 85% phosphoric acid (H₃PO₄) to the filtrate and mix well. Quantitatively transfer the solution to a 100 mL volumetric flask. Rinse the filter flask with several small portions of 1:30 nitric acid and add to the volumetric flask. Dilute to the mark with additional 1:30 nitric acid.

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*Nitric acid generates NO(g) when oxidizing metals. NO is a reactive and toxic gas and should only be generated in a fume hood.*

*85% phosphoric acid is very viscous. Use a 10 mL graduated cylinder to measure the volume of acid.*
Titration of Steel Unknown

Transfer 25 mL of the ferrous ammonium sulfate solution to a clean Erlenmeyer flask using a volumetric pipette. Transfer 25 mL of the steel unknown solution to the flask with a volumetric pipette. The purple color will disappear as it enters the solution and reacts with the ferrous ions. Back titrate this solution with the standard permanganate solution. The endpoint is reached when a pale pink color persists in solution.

Calculations

There are several steps in this procedure and good notebook practices will facilitate the many calculations required. The Mn content of the unknowns varies from approximately 0.10% to 1.00% All of the required stoichiometric relationships are given in the above instructions. Refer to your analytical text for discussions of back titrations.

Report the percent by mass of Mn in your steel sample to 0.01% along with the 95% confidence interval.
**Introduction**

Chelation ion chromatography can be used to determine trace amounts of heavy metals in µg/L concentrations. The instrument used for this type of chromatography is an HPLC system. The column in this setup consists of an ion exchange resin from which transition metals are separated using complexing eluents. An acidic solution of oxalic acid is used as the eluent for the determination of Pb²⁺, Cu²⁺, Cd²⁺, Mn²⁺, Co²⁺, Zn²⁺ and Ni²⁺. The eluted metals are quantified by indirect spectrophotometric detection. After elution the stream is mixed with a solution containing (PAR). The ligand changes color from orange to red when bound to transition metals metal. This technique is called post-column derivatization and allows for sensitive detection of trace amount of a variety of transition metals.

**Preparation of Pb²⁺ Stock Solution**

Add a weighed amount of Pb shot to a 150 mL beaker (do not dry the lead in an oven). In a fume hood add 20 mL of distilled water and 20 mL of concentrated nitric acid. Cover with a watch glass and boil gently until the metal has dissolved and the solution is colorless. If a white precipitate forms, cool the solution and add 20 mL of water. Remove from the heat and allow to cool slightly. Rinse the watch glass and the sides of the beaker with a small amount of distilled water and boil for an additional 10 minutes. Allow the solution to cool and quantitatively transfer it to a 1000 mL volumetric flask. The amount of lead added should yield a final concentration of lead of ~500 ppm (mg Pb²⁺ / L).

**Preparation of Cu²⁺ Stock Solution**

Weigh a ~0.5 gram sample of copper foil and digest with 50 mL of dilute HNO₃ (3 M) in a fume hood. Record the mass of copper to the nearest 0.1 mg. Handle the copper sheet with gloves to avoid fingerprints and do not dry in an oven before weighing. Gently boil the solution for 10 minutes after the last of the copper has been dissolved and the solution is blue and transparent. Keep the beaker covered with a watch glass and do not let the solution evaporate to dryness. Cool the sample to room temperature and quantitatively transfer the solution to a 1000 mL volumetric flask. Dilute to the mark with distilled water.
Preparation of Unknown Brass Stock Solution

Do not dry the brass before weighing. Weigh a 1.0 gram sample of brass and add it to a 150 mL beaker. Record the mass to the nearest 0.1 mg. Add 10 mL of water followed by 15 mL of concentrated nitric acid in a fume hood. After the evolution of gas slows, add an additional 10 mL of water. Heat the solution and continue to carefully boil the covered solution for an additional 20 minutes after all of brass has dissolved. The solution will be reduced in volume but do not allow it to evaporate to dryness. Allow the solution to cool to room temperature. A fluffy white precipitate may be visible in the blue solution. The precipitate is hydrated stannic oxide (H$_2$SnO$_3$). This precipitate may be collected with ashless filter paper and ignited to constant composition for gravimetric determination of tin. Carefully filter the solution through a cone of filter paper into a clean beaker. The filtration step may take a long time so plan accordingly. Quantitatively transfer the solution to a 500 mL volumetric flask.

Calibration Method

Using the stock solutions of lead and copper create calibration solutions containing both lead and copper. The calibration solutions should contain both lead and copper over the range of 0 to 50 ppm. You will use these solutions to calibrate the instrument response. The detector for the HPLC does not obey Beer’s law when the absorbance maximum is much above 1.0. If your higher calibration standards exceed this level you will need to prepare additional solutions at lower concentration. Mix all of your diluted solutions well.

Transfer a small amount of one of the standard solutions to a clean and dry beaker. Fill the injection syringe several times with this solution to thoroughly rinse the syringe and needle. Fill the syringe with 1 mL of solution and slowly load the sample loop on the injection valve as instructed by your T.A (when changing solutions you may want to repeat this several times to thoroughly rinse the sample loop). Take care not to inject air bubbles into the HPLC. Initiate the chromatographic analysis with the instrument software. A typical chromatogram of a standard sample is shown below in Figure 1. Your T.A. will demonstrate how to operate the HPLC software. You may wish to include a blank of distilled water to check for background metal contamination if time permits. You have one four hour lab period to complete the calibration and analysis of your unknowns. **Plan your HPLC injections accordingly.**
**Figure 1.** Chromatogram showing the separation of lead and copper using chelation ion chromatography.

Most of the brass samples contain zinc ions which elute at a much longer retention time than lead or copper. For runs with brass use the longer method. Your TA will demonstrate how to load new methods on the instrument. Completed chromatogram will automatically be printed as a PDF file with the peak areas and heights listed.

**Analysis**

Construct a calibration curve by plotting the response vs. the concentration of metal. Using linear regression methods determine the slope and intercept for this data. Determine the concentration of copper and lead in your injected unknown sample. Calculate the mass percent of lead and copper in your unknown brass sample. Use the equations given in the lab manual introduction to determine the appropriate confidence intervals. The standard deviation of the result for your unknown solution is found by using equation 1.6 in the introduction. Be sure to convert this to a relative deviation to determine the deviation of the weight percent.

*Report your results for the weight percent of copper and lead to 0.01% along with 95% confidence limits.*
Introduction

Flame ionization atomic absorbance spectroscopy (AAS) consists of probing electronic transitions of atoms in the gas phase. Samples are atomized, introduced into a gas stream of acetylene and air and passed to a burner (Figure 1). The resulting flame (2500K) decomposes the sample leaving atoms in the gas phase. Because the electronic transitions of atoms are very sharp (bandwidth ~0.001 nm), conventional lamps and monochromators cannot be used to probe absorbance. Hollow cathode lamps containing the analyte of interest are used as light sources with narrow bandwidth and correct energy. The light is passed through the flame and changes in intensity are monitored as the sample is introduced. Absorbance is defined as \( \log \frac{I_o}{I} \). We define \( I_o \) as the intensity of light reaching the detector when a blank solution is being introduced to the flame. The signal \( I \) is measured when a sample containing the analyte is present and the absorbance is calculated.

![Figure 1. Schematic of a flame ionization atomic absorbance spectrometer. (A) hollow cathode lamp (B) acetylene flame (C) sample introduction / atomizer (D) monochromator/detector assembly.](image)

Beer’s law holds for AAS just as it does in other spectroscopic methods. A plot of absorbance vs. concentration should result in a straight line that can be used to calibrate the instrument response. The calibration curve is then used in the determination of unknown analyte concentrations.

Solution Preparation

Prepare stock solutions of copper, lead and brass as described in the procedure for HPLC. The HPLC method and AAS method required similar concentration ranges for calibration so you may use the same standards and dilutions for both experiments. Adjust the dilution of the brass unknown so that the concentration of Cu and Pb fall within the instrument calibration limits.
AAS Procedure

The AAS has separate hollow cathode lamps for copper and lead. Monitor the absorbance of lead at 283.3 nm. Copper should be monitored at 324.8 nm. Fill labeled test tubes with your calibration solutions and unknowns and place them in the autosampler. Your TA will demonstrate how to use the instrument and software. If your calibration curve is not linear, rotate the flame so that the effective light path is smaller. Repeat the analysis. The software will automatically create a calibration curve and report the concentration of analytes in your unknown solutions.

Analysis

Construct a calibration curve by plotting the average absorbance vs. the concentration of metal. Include error bars for each point (standard deviations). Using linear regression determine the slope and intercept for this data. Determine the concentration of Pb and Cu in your unknown sample using the calibration curves.

Report your results for the weight percent of copper and lead to 0.01% along with 95% confidence limits.
Introduction

Gravimetric analysis is one of the oldest analytical techniques. It remains a very accurate method of analysis when a large sample is available. Modern techniques require much less sample but may not be as accurate. Under the proper conditions, lead may be separated quantitatively from other metals as an insoluble sulfate. Precipitation of lead sulfate is accomplished by the addition of concentrated sulfuric acid. Copper and zinc sulfates are soluble and do not coprecipitate with lead. The method is fairly straightforward with two caveats to the experimentalist. The solubility of lead sulfate increases with temperature and is dependant upon the weight percent of sulfuric acid in solution (figure 1). In addition, PbSO₄ is somewhat soluble in nitric acid. Evaporation of the solution to fumes of SO₃ effectively removes nitric acid from solution reducing the solubility of lead sulfate.

Figure 1. Solubility of PbSO₄ in sulfuric acid as a function of wt% and temperature.²

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2. Data from H.D. Crockford and D.J. Brawley J. Am. Chem. Soc., 56, (1934), 2600
Brass Digestion

Do not dry the brass before weighing. Weigh three ~1.0 gram samples of brass and add them to three 250 mL beakers. Record the mass to the nearest 0.1 mg. Add 10 mL of water followed by 15 mL of concentrated nitric acid in a fume hood. After the evolution of gas slows, add an additional 10 mL of water. Heat the solution and continue to carefully boil the covered solution for an additional 20 minutes after all of brass has dissolved. The solution should be reduced in volume but do not allow to evaporate to dryness. Allow the solution to cool to room temperature. A fluffy white precipitate may be visible in the blue solution. Carefully filter the solution through a cone of filter paper into a clean beaker. Add 4 mL of concentrated sulfuric acid to the filtrate and heat the solution to fumes of SO\textsubscript{3}. Cool the solution and rinse down the sides of the beaker with distilled water. Return to the heat and bring to fumes of SO\textsubscript{3} again. Cool the solution to room temperature and dilute with 25 mL of distilled water. Heat the solution to nearly boiling then add 50 mL of distilled water. Allow the solution to stand at room temperature for one hour.

PbSO\textsubscript{4} Collection

While you are digesting your brass samples prepare three filter crucibles for later use. Add enough Celite to form a layer on the bottom of the crucible about 5 mm deep. Rinse the Celite with dilute nitric acid followed by distilled water. Place the crucibles in a 200°C oven and allow to dry to constant mass. Store and cool the crucibles in a desiccator. When the crucibles have reached a constant mass (be sure to record the mass in your notebook), filter the brass solutions being sure to transfer all of the lead sulfate solids. Wash the filtrate thoroughly with cold 1:20 (v:v) sulfuric acid. Place the crucible in a 200°C oven and dry to constant mass. Calculate the weight percent of lead from the mass of lead sulfate and the mass of the unknown sample.

*Report your results for the weight percent of lead to 0.01% along with the 95% confidence interval.*
Introduction

Anodic stripping voltammetry (ASV) is a useful technique for determination of very low levels of metals in solution. The analytes of interest are concentrated at the working electrode by reduction in stirring solution that can last up to 30 minutes. The voltage is subsequently swept towards more positive potential and the amount of current that flows during the oxidation waves is proportional to the amount of analyte in solution.

![Graph of Anodic Stripping Voltammetry](image)

Figure 1. Anodic stripping voltammograms of copper at various concentrations using a mercury film electrode.

A hanging mercury drop electrode (HMDE) is often used to facilitate a reproducible amount of analyte that is reduced. Mercury forms amalgams with many other metals and is a very good electrode material for ASV. We will be using a modified mercury film electrode (MFE) for our experiments. A MFE is constructed by reducing mercury ions to mercury metal at the surface of a working electrode to form a thin film of mercury. The modified electrode is then transferred to another solution to be used for the analysis. Care must be taken with an electrode of this type so as not to dislodge the mercury film. An alternative method is to co-deposit mercury and the analytes during the reduction step. This generates a reproducible film in situ and avoids the problem of having to transfer the MFE to a separate solution.
Solutions

Prepare stock solutions of copper and brass as described in the method. Use a solution of 4% (v:v) acetic acid to prepare all dilute samples for this procedure. Prepare several standards of copper in the range of 1 to 50 ppm in 100 mL volumetric flasks. As in all other calibration techniques you want your analyte concentration to fall within the calibration range. If your brass dilution does not fall within this range, adjust the dilution accordingly.

ASV Method

Pipette 25 mL of a copper or brass solution into a 50 mL beaker. Add a one inch stir bar and spike the solution with one drop of a 0.1 M Hg$^{2+}$ solution. Dispose these solutions in a properly labeled container only. Assemble the electrochemistry apparatus as directed by your TA. The green wire is the working electrode (glassy carbon electrode), the white wire is for the reference electrode (Ag/AgCl) and the red wire is for the counter electrode (platinum wire). Reproducible stirring is required for this technique. Stir the solution at a setting of 125 rpm on the electronic stir plate provided. Your TA will demonstrate the use of the electrochemistry software.

The linear sweep voltammetry method is used with the following parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conditioning time</td>
<td>90 sec</td>
</tr>
<tr>
<td>Conditioning voltage</td>
<td>-0.800 V</td>
</tr>
<tr>
<td>Equilibration time</td>
<td>30 sec</td>
</tr>
<tr>
<td>Initial potential</td>
<td>-0.800 V</td>
</tr>
<tr>
<td>Final potential</td>
<td>+0.500 V</td>
</tr>
<tr>
<td>Scan rate</td>
<td>10 mV/sec</td>
</tr>
</tbody>
</table>

Start the method with the solution stirring. A timer will count down for 90 seconds. At the end of this time turn the stirring off. Try to stop the stirring as close to zero as possible. The working electrode is held at –800 mV for 30 seconds to allow the solution to settle. The voltage will then be scanned to +500 mV at a rate of 10 mV/sec. Voltammograms like those shown in figure 1 will be recorded. The software will allow you to measure the peak areas which will be used to construct a calibration curve. A plot of the peak area (mC) vs. copper concentration (ppm) should yield a straight line. Record the voltammogram for your brass unknown and use the calibration curve to determine the concentration of copper in the unknown solution. Calculate the mass percent of copper in your brass unknown.

Hints for Better Results

The reproducibility of this experiment can be improved with the following precautions. The state of the electrode surface should be as identical as possible for each experiment. The working electrode should be polished every few runs to remove residual materials from the previous experiment. Bubbles on the surface of the electrode
will cause significant problems in reproducibility. You may need to decrease the stirring rate to prevent bubbles from forming on the electrode surface. Bubbles prevent the solution from reaching the working electrode and effectively reduce the surface area for reduction to occur and lead to inconsistent results. Be sure to check all of the electrical connections. If the electrodes are not connected properly you will see the current/potential overload indicator light up on the potentiostat.

Report your results for the weight percent of copper to 0.01% along with the 95% confidence interval.
**Introduction**

Photo-induced electron transfer reactions in metal complexes are an important class of reactions in inorganic and biochemistry. These electron transfer reactions are typically initiated by light absorption into charge transfer bands of metal complex. A metal to ligand charge transfer (MLCT) is one type of reactive state that has been extensively studied. \([Ru(bpy)_3]^{2+}\), \((bpy = 2,2´-bipyridine)\), is an inorganic metal complex with an MLCT absorption in the visible region of the spectrum. These excited state complexes are of interest for their insight into the electron transfer process.

The synthesis of \([Ru(bpy)_3](PF_6)_2\) utilizes \(Ru(DMSO)_4Cl_2\) as a precursor, since it binds bidentate ligands more readily than other available starting materials, such as \(RuCl_3\cdot xH_2O\). Once purified, the \(Ru(DMSO)_4Cl_2\) is refluxed in ethanol with 2,2´-bipyridine in the proper molar ratio to generate \([Ru(bpy)_3]Cl_2\). For this experiment several polypyridyl ligands will be available for you to choose from. Following its synthesis, \([Ru(bpy)_3](PF_6)_2\) will be characterized by UV-vis and fluorescence spectroscopy, as well as cyclic voltammetry.

**Synthesis of \(Ru(DMSO)_4Cl_2\)**

Place 0.50 g \(RuCl_3\cdot 3H_2O\) in a 10 mL round-bottomed flask equipped with a magnetic stirring bar. A reflux condenser is attached to the flask and the apparatus is placed on a heating mantle set on a magnetic stirring hot plate. Bubble nitrogen or argon gas through 1.8 mL of DMSO (DMSO = dimethyl sulfoxide, \((CH_3)2SO\) placed in a graduated cylinder. Add the degassed DMSO to the round-bottomed flask, and heat to reflux with continuous stirring. Allow the mixture to boil for five minutes only. Do not overheat. The mixture will turn a brown-orange color when the reaction is complete.

The solution is cooled and transferred, using a Pasteur filter pipet, to a 25 mL Erlenmeyer flask. Yellow crystals may separate out at this point; if this is the case, the crystals can be collected as stated below. Otherwise, the volume of the solution is reduced to 0.5-1.0 mL by passing a gentle stream of nitrogen gas over the warmed liquid. 20.0 mL of dry, reagent grade acetone is carefully added to the DMSO solution to form two phases. Cool the mixture in an ice bath. Yellow crystals should form upon standing for 10-15 minutes. Alternatively the solution may be stored covered until the next lab period. Collect the crystals by suction filtration using a Hirsch funnel. Wash the crystals with 1 mL of acetone followed by 1 mL diethyl ether. Allow

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the crystals to dry then weigh to calculate a percent yield. Determine the melting point and obtain an infrared spectrum using a KBr pellet. Prepare a KBr pellet by grinding 100-200 mg of dry IR grade KBr in a mortar along with 1-3% by weight of your sample until a fine powder is produced. Load the press with enough of the powder to cover the bottom with a layer 1-2 mm deep. Record the spectrum using air as the background signal. Locate and identify the absorption frequency of the S-O band.

**Synthesis of [Ru(L)3](PF6)_2**

[Ru(L)3](PF6)_2 is synthesized from Ru(DMSO)4Cl2 by refluxing with a polypyridyl ligand (L), in a stoichiometric ratio slightly less than 1:3, i.e. a slight excess of ligand. Representative ligands are shown in figure 1. Check with your TA for the ligands available. Each group in a section should choose a different ligand. Please note that there is a limited amount of certain ligands. **Check your calculations with your TA before proceeding.**

![A B C D](image)

**Figure 1.** Representative polypyridyl ligands. A) 4,4’-dimethyl-2,2’-bipyridine (dmbpy) B) 4,7-diphenyl-1,10-phenanthroline (dpphen) C) 2,2’-bipyridine, (bpy) D) 1,10-phenanthroline (phen)

A 100 mL round-bottom flask is equipped with a magnetic stirring bar, into which 0.5 g of Ru(DMSO)4Cl2 and an appropriate amount of ligand are placed. 25 mL of reagent grade ethanol are then added to the flask. The mixture is heated at reflux, with continuous stirring overnight. The solution changes in color from yellow to dark red. The solution is first dried by rotary evaporation to isolate the product. The solid is collected by vacuum filtration and washed with toluene to remove excess ligand. The crystals are then dried under vacuum. Further purification may be achieved by column chromatography, with ethanol as the solvent and alumina as the stationary phase.

The final step in the synthesis is the conversion of [Ru(L)3]Cl2 to its hexafluorophosphate (PF6-) salt by metathesis. Heat 0.5 grams of [Ru(L)3]Cl2 in a beaker with 10 mL of distilled water. In a separate beaker, a five-fold molar excess of ammonium hexafluorophosphate

A ratio of 3.6 moles of ligand per 1 mole of Ru(DMSO)4Cl2 should be used.
(NH₄PF₆) is heated in 10 mL of distilled water. Check your calculations with your TA before weighing the NH₄PF₆, this salt is expensive. When both solutions are warm (not boiling), the NH₄PF₆ solution is slowly added to the [Ru(L)₃]Cl₂ solution. The color of the solution changes from red to orange and a precipitate should form. Continue to gently heat the resulting solution for 5 minutes (be careful not to bring the solution to a boil). Cool in an ice bath and collect the crystals by suction filtration. Dry the crystals under vacuum and calculate a percent yield starting from the Ru(DMSO)₄Cl₂ starting product.

**Spectroscopic Characterization**

Prepare 50 mL of an acetonitrile solution of your product that is approximately 0.03 mM. Obtain the UV-Visible spectrum of Ru(L)₃²⁺ and calculate the extinction coefficient of the MLCT band at λ_{max} (400-500 nm). Literature values for complexes of this type are generally in the range of 14,000 – 32,000 M⁻¹·cm⁻¹. Knowledge of the extinction coefficient you will also be able to determine the appropriate concentration for an acetonitrile stock solution of Ru(L)₃²⁺ for use in a following experiment. The stock solution will be diluted to make several solutions (25 mL each) that have an absorbance of 0.1 – 0.3 in the MLCT region.

The measured λ_{max} in the MLCT region will be used as the excitation wavelength in the luminescence measurement. The emission spectrum should be recorded over the 500-900 nm range. The absorption and emission spectrum of Ru(bpy)₃²⁺ is shown above in figure 2. The charge transfer bands have been assigned as ligand centered (LC, π → π*), metal centered (MC, d → d) or metal to ligand charge transfer (MLCT).²

In a later experiment we will need to obtain the E₀₀ energy for the triplet state in the ruthenium complex. The E₀₀ energy is the energy difference between an electron in the ground electronic state in the lowest vibronic level and an electron in the excited triplet state in the lowest vibronic level (see figure 3). The energy for this transition can be estimated by the “10% rule”.³ That is, the point at which the emission intensity on the high energy side is 10% of that at λ_{max} (figure 4). Convert this wavelength into eV for use later (Remember that $E = \frac{hc}{\lambda}$ and $1 \text{eV} = 1.6022 \times 10^{-19} \text{J}$).

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² Kalyanasundaram, K. *Coord. Chem. Rev.*, 46, 1982, 159-244
Figure 2. Absorption (blue line) and emission (red line) spectrum of Ru(bpy)$_3^{2+}$ in acetonitrile solution with assignments of the electronic absorption bands. The absorbance in the MLCT region has been multiplied by a factor for four for clarity.

Figure 3. Energy level diagram depicting the triplet to singlet phosphorescence transition ($E^{00}$ energy).
Figure 4. Luminescence spectrum of Ru(bpy)$_3^{2+}$ in acetonitrile solution. The $E^{00}$ energy is estimated to be where the emission intensity is 10% that of the emission at $\lambda_{\text{max}}$.

Electrochemical Characterization

You will be performing several electrochemical measurements in acetonitrile (CH$_3$CN) solution. A supporting electrolyte must be added to the otherwise non-conducting solvent. Prepare 50 mL of an acetonitrile solution that is 0.1 M in n-tetrabutyl ammonium hexafluorophosphate (TBAH) in a volumetric flask. Note: TBAH is moisture sensitive and exposure to the room air should be minimized. The TBAH salt is also expensive. Do not waste your solutions. Add 5-10 mL of this solution to a clean and dry electrochemical cell containing a magnetic stir bar. Set up the electrodes on the potentiostat as instructed by your TA. Bubble the stirring solution with nitrogen or argon for 5-15 minutes. Stop the stirring and remove the gas line from the liquid but keep it suspended above the solution. The gas from the line should not disturb the surface of the solution. A slow flow should gently blanket the cell with inert gas.

Record a blank cyclic voltammogram of the electrolyte solution over the range of +1.75 to –2.25 V at a scan rate of 200 mV/sec. A reduction peak near –0.8 V indicates the presence of oxygen and further sparging is required. Add a small amount of your ruthenium complex to the acetonitrile solution (~50-100 mg), sparge for several minutes and record a cyclic voltammogram of this solution. Export your data as an ASCII file for inclusion in your lab report. The voltammogram should look similar to the one below in figure 5. Record the differential pulse polarogram as instructed below before discarding this solution. Repeat the above procedure to record the cyclic voltammogram of phenothiazine (PTZ), N,N,N’,N’-Tetramethyl-p-phenylenediamine (TMPD) and p-chloro nitrobenze (PCNB). Record the signal for these compounds over the range of -0.250 to +1.0 volts at a scan rate of 100 mV/sec. The trace should resemble that shown in figure 6. Record the $E_{1/2}$ value in your notebook for use in a later experiment.
Figure 5. Cyclic voltammogram of Ru(L)$_3$ in acetonitrile solution with 0.1 M TBAH supporting electrolyte. The $E_{1/2}$ values for each wave are calculated as the potential halfway between the cathodic ($E_{pc}$) and anodic ($E_{pa}$) waves as shown in figure 7.

Figure 6. Cyclic voltammogram of phenothiazine in acetonitrile solution.
Figure 7. Cyclic voltammogram for a quasi-reversible process. The 
$E_{1/2}$ value is the mean of the cathodic peak ($E_{pc}$) and the anodic peak 
($E_{pa}$).

**Differential Pulse Voltammetry**

Reduction and oxidation potentials can also be measured using 
differential pulse voltammetry. In this method a saw-tooth potential 
waveform is scanned giving rise to greater sensitivity. Refer to 
your analytical text for more information regarding various types 
of electrochemical measurements. A representative differential 
pulse polarogram is shown in figure 8. The first reduction in each 
voltammetry method corresponds to the reduction of Ru(L)$_3^{2+}$ to 
Ru(L)$_3^+$. The ground state reduction potentials and the $E^{00}$ energy 
estimated above will be used to estimate the excited state reduction 
potentials in a following experiment.

Figure 8. Differential pulse polarogram of Ru(L)$_3^{2+}$ in acetonitrile 
solution with 0.1 M TBAH supporting electrolyte. The arrow indicates 
the direction of the scan.
**Luminescence Quenching**

**Stern-Volmer Kinetics and Marcus Electron Transfer Theory**

**Introduction**

Luminescence quenching of a molecule can be used to directly measure bi-molecular processes. In the following experiment, we will investigate an electron transfer induced quenching event from an organic electron donor to [Ru^{II}(L)^3]^{2+}. In many cases this process is diffusion controlled ($k_{\text{diff}} \sim 10^{10} \text{ s}^{-1}$) and a bimolecular quenching rate ($k_q$) will be determined by calibration of the quenching mechanism using a series of prepared standards.

The emission intensity of the fluorophore can be quenched by an excited-state fluorophore quencher reaction (dynamic). The following general scheme illustrates the nature of processes that deactivate an electronically excited state of a molecule.

$$
A + h\nu \rightarrow A^* \quad \text{(excitation process)}
$$

$$
A^* \rightarrow A + h\nu \quad \text{(radiative)}
$$

$$
A^* \rightarrow A + \text{heat} \quad \text{(non-radiative)}
$$

$$
A^* + Q \rightarrow A + Q^* \quad \text{(energy transfer)}
$$

$$
A^* + Q \rightarrow A^- + Q^+ \quad \text{(electron transfer)}
$$

The absorption of a photon by $A$ produces an excited state species $A^*$. The excited species has several ways to get rid of the excess energy – for example, emission of light (luminescence), conversion of energy to heat (radiationless deactivation), or interaction with another species present in solution (bimolecular quenching). If one takes the reciprocal of the radiative decay rate constant $k_r$, the result is the radiative lifetime ($\tau$) of the lumophore. A relatively long lifetime of the excited state is important to facilitate an efficient quenching process. In other words $k_q$ must be much larger than $k_r$. When this is the case, the quencher molecule (Q) can interact with the excited state species and undergo energy or electron transfer reactions.

A Stern-Volmer kinetics analysis measures the competition between the bi-molecular quenching of a molecule in its excited state with the unimolecular decay of the molecule from its excited state. The steady-state approximation assumes that the rate of formation of $A^*$ is equal to the rate of its disappearance. This is a valid assumption for systems in which an intermediate species reacts or returns to starting material rapidly. For the system described here the following expression is obeyed:

$$
\frac{I_0}{I} = 1 + k_{SV}[Q] \quad , \quad k_{SV} = k_q \tau_0 \quad (1)
$$

where $I_0$ is the integrated intensity of emission without a quencher present, $I$ is the integrated intensity in the presence of quencher, $[Q]$
is the concentration of quencher, and $k_{SV}$ is the Stern-Volmer rate constant defined as $k_q \tau_0$. Here $k_q$ is the bimolecular quenching rate constant and $\tau_0$ is the natural radiative lifetime in the absence of quencher. Reactions that are thermodynamically favorable (i.e. large $-\Delta G$) have $k_q$ values that approach the diffusion controlled limit, $k_D \sim 10^{10}$ s$^{-1}$.

The observed emission intensity is directly proportional to the lifetime of the emitting species so that expression [1] above may be redefined in terms of lifetimes as follows.

$$\frac{\tau_0}{\tau} = 1 + k_{SV}[Q] \quad (2)$$

Notice that in each case (1) and (2) the equation is in the form of a straight line. If the ratio $I_0/I$ or $\tau_0/\tau$ is plotted vs. [Q] a straight line should result with the slope equal to the Stern-Volmer quenching constant, $k_{SV}$, and an intercept of 1. Very fast bimolecular rate constants may be determined by analysis of this type.

**Electron Transfer Theory**

Electron transfer reactions are a class of reactions in which an electron donor transfers an electron to an electron acceptor. These simple redox reactions are common in inorganic and biochemical systems. One may view electron transfer processes along a reaction coordinate in which two overlapping potential energy surfaces cross as shown in figure 1. The electron transfer reaction can occur through an inner sphere or outer sphere mechanism as in Eq. 3 and 4 respectively. In both cases there is no net change in the reactants or products so the free energy of the system remains unchanged, i.e. $\Delta G = 0$.

![Reaction coordinate diagrams for electron transfer when A) $\Delta G = 0$ and B) $\Delta G < 0$.](image)

$$\begin{align*}
[H_2N]_3Ru^{II} \rightarrow \rightarrow \rightarrow N \rightarrow Ru^{III}(NH_3)_5 \rightleftharpoons [H_2N]_3Ru^{III} \rightarrow \rightarrow \rightarrow N \rightarrow Ru^{II}(NH_3)_5 \\
\text{Ru}^{II}(NH_3)_6^{2+} + \text{Ru}^{III}(NH_3)_6^{3+} \rightarrow \text{Ru}^{III}(NH_3)_6^{3+} + \text{Ru}^{II}(NH_3)_6^{2+}
\end{align*} \quad (3) \quad (4)$$

The thermally activated electron transfer pathway in figure 1A proceeds through points ABCD with an activation barrier ($E_a$) equal to the difference in energy between points A and the crossing point B.
The electron transfer rate is given by

$$k_{ET} = \nu_{ET} \exp \left( \frac{-E_b}{RT} \right)$$

(5)

where $\nu_{ET}$ is the frequency factor that depends on the overlap of the two states, electron tunneling, or resonance between states. The reaction coordinate diagram for an unsymmetrical electron transfer is shown in figure 1B. In such a case the electron transfer rate is related to the free energy for the overall process and a reorganization term, $\lambda$, which takes into account changes in bond length and solvent polarization induced by changes in charge distributions, equation 6.

$$k_{ET} = A \exp \left[ \frac{(-\Delta G^\circ + \lambda)^2}{4\lambda k_BT} \right]$$

(6)

As with a symmetrical electron transfer, the frequency factor, $A$, is related to frequency of the system reaching the crossing point. The value for $\lambda$ (figure 2.) can be estimated theoretically but is beyond the scope of this experiment. For a given related series of electron transfer reactions in the same solvent, the value of $\lambda$ will be fit to experimental data.

![Reaction coordinate diagram for an unsymmetrical diabatic electron transfer with labeled free energy terms.](image)

**Figure 2.** Reaction coordinate diagram for an unsymmetrical diabatic electron transfer with labeled free energy terms.

Since many electron transfer reactions are quite rapid in solution, we must take into account the theoretical limit for bimolecular reactions. For two species to react, they must be close enough to interact with one another. In solution this is limited by the diffusion process. Imagine a large container full of balls. A few of the balls are colored red and a few are colored blue. Now shake the container. Reactions between red and blue balls can only occur if they collide with one another. The frequency at which they collide is governed by the viscosity of the solution and the average kinetic energy of the particles in solution (proportional to temperature). The Debye equation can be used to estimate the diffusion rate in solutions when one or more of the reacting species is uncharged.

$$k_D \approx \frac{8RT}{3000\eta}$$

(7)

Where $\eta$ is the viscosity of the solvent in poise and $R$ is $8.31 \times 10^7$ erg mol$^{-1}$ K$^{-1}$. Solvent viscosities can be found in the reference books such as the CRC Handbook of Chemistry and Physics.
Taking diffusion into account, the overall quenching rate constant is given in Eq. 8

\[ k_q = \frac{1}{k_D} + \frac{1}{K_a k_{ET}} \quad K_a \approx 1 \tag{8} \]

where \( K_a \) is a pre-association constant, \( k_D \) is the diffusion rate constant and \( k_{ET} \) is the electron transfer rate constant given by Eq. 6. Because our quencher molecules are not charged we don’t expect any sort of pre-association in our system and is set equal to 1.

**Experimental**

All measurements will be conducted in acetonitrile solutions under oxygen free conditions (oxygen acts as a quencher). The samples are sparged by inserting a needle into the septa of the cell and slowly bubbling N\(_2\) through the samples. Be sure to provide a second needle as a vent or the cuvette will build up pressure and crack. Sparging times of ~5 min are sufficient for 2-3 mL of solution.

Prepare 100 mL stock solutions of ~1 mM of two of the following quenchers: phenothiazine (PTZ), N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) or parachloronitrobenzene (PCNB). Be sure to record the exact concentrations in your lab notebook. Add enough quencher solution to four 50 mL volumetric flasks so that the quencher concentration will range from 0.00 to ~0.1 mM after dilution. Partially dilute the samples with 15-20 mL of acetonitrile before proceeding. Add an aliquot of \([\text{Ru(L)}_3]^{2+}\) to one flask using a volumetric pipette so that the \(\lambda_{max}\) is between 0.1 and 0.3 in the MLCT region of the visible absorbance spectrum. Check a single solution before you make them all in case your calculated amount is incorrect. If the absorbance value is acceptable, proceed to add the \([\text{Ru(bpy)}_3]^{2+}\) to the other flasks diluting to the mark as required. It is important that the exact same volume of ruthenium stock solution is added to each flask. Note: the TMPD solutions are photosensitive. Prepare them in dimmed room light. You should also prepare these solutions one at a time to minimize thermal reactions. There are a limited number of volumetric flasks. Please, clean the flasks and replace them when you have finished your experiment so others may use them.

Fill a cuvette with ~2.5 mL of one of your solutions, cap the cuvette with a septum and sparge with N\(_2\) for 5 minutes to remove oxygen. Be sure there is a second needle to vent the gas. Record the fluorescence spectrum of your sample over the 500 to 900 nm region. Using the available software record the integrated peak area for your sample. Make a note of the emission \(\lambda_{max}\) for use in the time resolved emission experiment. The next step is to record the time resolved luminescence of your sample. Excitation will occur using a pulsed N\(_2\) laser. The laser emits short (< 4 ns) pulses of 337 nm light. Caution should be exercised around any laser, you only have one set of eyes. Laser safety goggles are required as all times in the laser room – **NO EXCEPTIONS**. Failure to follow safety rules will result in a failing grade. Your TA will instruct you how to operate the laser and data collection system. Set the monochromator on the instrument to the wavelength of the emission maximum that you recorded on the fluorimeter. Transfer
the data to the computer when the data on the digital oscilloscope displays acceptable signal to noise. You should use the curve fitting routine in the software to obtain the observed decay rate constant and the lifetime. You are required to enter a “guess” value for the fitting algorithm. A guess value of 500 ns is a good place to start. Record the lifetime of your sample in your lab notebook.

Data Analysis

Using EXCEL or another data analysis program (IGOR, SIGMA PLOT, KALEIDAGRAPH etc…) plot $I_0/I$ and $\tau_0/\tau$ vs. the concentration of quencher (inset figure 3). For each experiment fit the data to a straight line using linear least squares regression and record the slope of each line. From these results determine the second order bimolecular quenching constant $k_q$. Report these results with along with their standard deviations. Include in your LAB report the plots generated above, the values for the $k_q$ (with the standard deviation) from each experiment. Compare your results to literature values if available. Many quenching rate constants can be found in a review article by Hoffman et. al. Not all complexes will have data in this source. Search the primary literature for your specific compound and quencher combinations.

Class data will be compiled and posted on the course website for analysis. Send your group results to your instructor using the report form available on the course website. Be sure to include all requested information.

Figure 3. Emission intensity as a function of quencher concentration. Inset: Stern-Volmer plot for the emission quenching by PTZ.

Calculating $\Delta G_{el}$

The following table contains example values for calculation of $\Delta G_{el}$. Use the values you measured in the laboratory for your compound. The boldface type indicates the calculation required to obtain the

---

correct value. Note the free energy calculation gives you units of J mol\(^{-1}\), please convert the energy units to kJ. The “\(n\)” in the free energy calculation is the number of electrons transferred and is equal to 1 for the reactions we are studying here.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triplet Energy</td>
<td>(E^{00}) 2.16 eV</td>
</tr>
<tr>
<td>Ground State reduction potential ((\text{Ru}^{2+/+} \text{ from CV data}))</td>
<td>(GS -1.38 \text{ V})</td>
</tr>
<tr>
<td>Excited State reduction potential ((\text{*Ru}^{2+/+})) ((\text{ES} = \text{GS} + E^{00}))</td>
<td>(ES +0.78 \text{ V})</td>
</tr>
<tr>
<td>Quencher reduction potential ((E_{1/2} \text{ of Q}^{+/0} \text{ from CV data}))</td>
<td>(Q +0.53 \text{ V})</td>
</tr>
<tr>
<td>Potential for electron transfer</td>
<td>(E_{el} +0.25 \text{ V})</td>
</tr>
<tr>
<td>Free energy change for electron transfer ((\Delta G_{el} = -n \text{ F } E_{el}))</td>
<td>(-24.1 \text{ kJ mol}^{-1})</td>
</tr>
</tbody>
</table>

**One step beyond**

There is some work that takes place during the electron transfer process.\(^2\) There is a work term that comes from bringing two molecules together to a radius of \(r\), \(w_R(r)\), and another for pulling the two molecules apart after the reaction, \(w_P(r)\). The work corrected potential for electron transfer is more properly given by the following

\[
E_{el} = ES - Q + w_P(r) - w_R(r)
\]

Each of the work terms can be calculated by the following equation

\[
w_{P,R}(r) = z_A z_B e^2 N / [\epsilon r (1 + A r \mu^\frac{1}{2})]
\]

where \(z_A\) and \(z_B\) are the charges on molecules A and B, \(\epsilon\) is the charge on an electron, \(\epsilon\) is the dielectric constant, \(\mu\) is the ionic strength and \(A = (8\pi Ne^2/1000kT)^\frac{1}{2}\). Since one of the reactants (the organic quencher) is uncharged \(w_R(r) = 0\). The remaining work term, \(w_P(r) \approx 0.03 \text{ eV}\). Since the correction is fairly small we will neglect it in our studies, however you should be aware that these corrections do exist.

---

Curve Fitting

Set up a spreadsheet in EXCEL as follows:

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ΔG (J/mol)</td>
<td>log ( k_q )</td>
<td>log ( k_q ) fit</td>
<td>( A )</td>
<td>5.00E+10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-22730</td>
<td>9.98</td>
<td>10.03</td>
<td>( \lambda )</td>
<td>40000</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-5510</td>
<td>9.71</td>
<td>9.33</td>
<td>( k_D )</td>
<td>2.00E+10</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-22580</td>
<td>10.03</td>
<td>10.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-13140</td>
<td>9.68</td>
<td>9.75</td>
<td>( \chi^2 )</td>
<td>2.778965</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-2400</td>
<td>9.78</td>
<td>9.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-67540</td>
<td>10.17</td>
<td>9.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>-54060</td>
<td>10.32</td>
<td>10.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>-20340</td>
<td>9.92</td>
<td>9.98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-73080</td>
<td>10.02</td>
<td>9.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>-52300</td>
<td>10.26</td>
<td>10.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2000</td>
<td>8.00</td>
<td>8.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>9000</td>
<td>7.50</td>
<td>8.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>11000</td>
<td>7.00</td>
<td>7.82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>18000</td>
<td>6.80</td>
<td>6.98</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data in columns A and B are the class experimental values for \( \Delta G_{EL} \) and log \( k_q \) provided on the course website. The values in column C are the calculated log \( k_q \) values from Eq. 8. The expression for cell C2 is:

\[
=\text{LOG}(1/(1/\$F$3+1/(\$F$1*EXP((-1*((\$F$2+A2)^2)/4*F$2*8.314*295)))))
\]

Copy and paste this expression in cells C3... for all data points. The values in cells F1, F2 and F3 are the guess values for \( A \), \( \lambda \) and \( k_D \). Use the values in the table above as starting points. Cell F6 is our measure of the goodness of fit given by \( \chi^2 \). EXCEL has a built in function that calculates \( \chi^2 \) values (SUMXMY2). The expression for cell F6 is:

\[
=\text{SUMXMY2(B2:B100,C2:C100)}
\]

Change B100 and C100 to match the number of data points you have on your spreadsheet. The goal now is to vary the parameters \( A \), \( \lambda \) and \( k_D \) in order to minimize the \( \chi^2 \) value. Doing this by hand would be time consuming and we would never be sure we reached a minimum. We will use the SOLVER add-in for EXCEL to find the best values of \( A \), \( \lambda \) and \( k_D \) automatically for us. The procedure for using the SOLVER is outlined on the following page.
1. Under the Tools menu (or Data tab in Office v. 2007 or later) select Solver. A pop-up window will appear.

2. In the box labeled “Set Target Cell” type in $F5$

3. Below this select “Equal To” the min function since we are trying to minimize the value in cell F5.

4. In the box labeled By “Changing Cells” type $F1:F3$. This allows the solver to vary the values for $A$, $\lambda$ and $k_D$ to minimize the sum of $\chi^2$.

5. Click the Options button and check the box labeled “Use automatic scaling” then click OK.

6. Now click on “Solve”. The program will alter your guess values to fit the data to minimize $\chi^2$.

7. A new pop-up will appear asking if you want to keep the new values or revert to your original values. Select keep solver solution and click the OK button.

8. The best fit values for $A$, $\lambda$ and $k_D$ will now be in cells F1, F2 and F3. The fitted values should match very closely to the experimental data (see below). If they do not, then you need to make better guesses for $A$, $\lambda$ and $k_D$ to start with. Enter new guess values and repeat the procedure.

**Figure 4.** Plot of $\Delta G_{EL}$ vs. log $k_q$. Data is compiled class data from 2005-2010 and literature data. The line is a fit of the data to Eq. 8.

It is nice to show the fitted line through the data points as a smooth function. To accomplish this we will generate data using the fitted values for $A$, $\lambda$ and $k_D$. In column G on your spreadsheet enter values

---

3. If you do not see Solver as an option, the add-in needs to be installed. To do this select “Add-Ins” under the “Tools” menu and check the solver add-in. Note: You may need the Excel installation disk to add this feature.
for DG from 20 000 to -100 000 in increments of 2000. Column H will contain the new data for log kq. Enter the following expression into cell H1

\[ \text{LOG} \left( \frac{1}{\left\{ \frac{1}{F3} + \frac{1}{F1 \times \exp(-1 \times (F2 + G1)^2 / (4 \times F2 \times 8.314 \times 295))} \right\}} \right) \]

Then copy and paste this expression from H2 to the end of the data in column G. The resulting values in columns H and G are added to the plot and shown as a smooth line (red line in figure 4). Compare the fitted value for \( k_D \) in cell F3 to the theoretical value calculated with Eq. 7. Do these values agree with each other? Why or why not?
Introduction

Electronic transitions induced by the absorption of UV or visible light result in an excited state molecule. The energy absorbed must be released to return the excited species back to the original ground state. There are numerous unimolecular pathways for the excess energy to be dissipated. A molecule may loose energy through nonradiative processes such as vibrational cooling. Energy may also be lost through radiative process such as fluorescence or phosphorescence. Both of these processes involve the emission of a photon of light. The various pathways for relaxation of the excited state to the ground state are shown in figure 1.

Figure 1. Relaxation pathways for a typical molecule. Fluorescence (k_r), phosphorescence (k_{r'}), non-radiative decay (k_{nr}, k_{nr'}) and intersystem crossing (k_{isc}) are all unimolecular deactivation processes. Bimolecular quenching (k_{q}[Q]) is a deactivation process available in the presence of quenching molecules.

In a typical molecule the ground electronic state is a singlet (all electrons are paired, 2S+1). Electronic excitation results in an excited...
state singlet that may undergo internal conversion to a triplet state. Because the triplet to singlet transition is a forbidden process, the triplet state may exist for a substantial length of time (some molecules have lifetimes over 10 seconds). Emission from the triplet-singlet state is termed phosphorescence. In contrast, fluorescence is the emission resulting from a singlet to singlet transition. This allowed process is marked by short lifetimes often less that one nanosecond ($10^{-9}$ sec). If an excited state species exists for a long enough time, bimolecular deactivation pathways must also be considered. The triplet state can be deactivated by electron or energy transfer to another molecule in solution according to the following expression

$$A^* + Q \rightarrow A + Q^*$$

where $A^*$ is the excited molecule and $Q$ is a quencher molecule. The rate constant $k_q$ is a measure of the efficiency of the above process. This process is called quenching because it is a nonradiative pathway for deactivation. An emitting compound will emit fewer photons in the presence of a quenching compound and the emission intensity will be subsequently "quenched".

Molecular oxygen in the ground state is a triplet species. Energy transfer is allowed when the energy donor and the energy acceptor have the same spin multiplicity. For this reason, O$_2$ is a good energy acceptor for compounds which form excited state triplets efficiently. One class of compounds that has been extensively studied is the inorganic dye molecule ruthenium (II) tris-bypyridine [Ru(bpy)$_3$]$^{2+}$ and various analogs. In the presence of oxygen the Ru(bpy)$_3$]$^{2+}$ emission is efficiently quenched. We can exploit this observation to build an oxygen sensor using the emission intensity of Ru(bpy)$_3$]$^{2+}$ as a measure of O$_2$ concentration. According to the relationships developed by Stern and Volmer the following expression relates the concentration of oxygen to the emission intensity

$$\frac{I_0}{I} = 1 + k_{SV} [Q], \quad k_{SV} = k_q \tau_0$$

where $I_0$ is the integrated intensity of emission without a quencher present, $I$ is the integrated intensity in the presence of quencher, [Q] is the concentration of quencher, and $k_{SV}$ is the Stern-Volmer rate constant defined as $k_q \tau_0$. Here $k_q$ is the bimolecular quenching rate constant and $\tau_0$ is the natural radiative lifetime in the absence of quencher. Reactions that are thermodynamically favorable (large -$\Delta G$) have $k_q$ values that approach the diffusion controlled limit, $k_d$$ \sim 10^{10}$ s$^{-1}$. This relationship can be used to quantitatively determine the concentration of a quencher molecule if $k_{SV}$ has been determined previously.

**Experimental**

You will prepare thin oxygen sensing films and calibrate the response using known mixtures of oxygen and nitrogen. Polymers, glasses and crystals have all been used in sensing applications. For this experiment we will use silicone rubber as the matrix to support the inorganic dye. Silicone rubber (aquarium sealant) is a clear, pliable and inexpensive
material to work with. The first step is prepare a thin film of silicone to act as a support. This step should be completed the week before to allow the silicone time to cure. Press a drop (about 1 mL) of silicone rubber between two pieces of Parafilm. You may make several films of various thickness.

Remove the Parafilm from the silicone rubber and cut out a 0.75 cm × 1.5 cm rectangle of material. Soak this film in a beaker of methylene chloride that has been saturated your ruthenium material for several minutes. The film will swell to several times its original size. Remove the film with forceps and allow the solvent to evaporate. The film will shrink to its original size and leave the dye embedded within the silicone matrix. The sensor element is to be constructed from a cuvette as shown in figure 2.

Assemble the apparatus shown in figure 2 as instructed by your TA. The emission intensity is monitored by a photodiode connected to a lock-in amplifier. Details on how this instrument works are provided below. Pass a stream of N₂ over the film and observe the changes in emission intensity. Pass an air stream over the sample after the signal stabilizes. You may also pass O₂ over the film and observe the changes. The response should resemble that shown in figure 3 below. The time constant and sensitivity of the lock-in amplifier can be controlled using a LabView interface. The program will also allow you to collect data as a function of time and export this data as an ASCII text file for plotting and analysis in other programs. Your TA will demonstrate the use of the lock-in amplifier and the LabView program. To generate signals we will be passing different gasses over our sensing film, Air, N₂ (or Ar) and O₂.
Sensor films may be calibrated by passing known mixtures of N$_2$ (or Ar) and O$_2$ over the film. Use the mass flow meters to measure the volume flow of nitrogen and oxygen. Purge the cuvette with gas mixtures ranging from 0 to 35% O$_2$. Plot the ratio of the signal under pure nitrogen to that under N$_2$/O$_2$ mixtures versus the volume percent of oxygen (Stern-Volmer Plot). Fill the cuvette with room air by pulling air through the cell using the house vacuum. Determine the volume percent of oxygen in air using your sensor. Note: Stop the vacuum before measuring the signal. The sensors respond to the partial pressure of O$_2$ and will be lower than expected if the overall pressure in the cell is low. An example calibration curve is shown in Figure 4.
While experimenting with your thin films you may want to keep the following questions in mind.

Instrument response time is defined as the time it takes for a signal to reach 90% of the maximum. What is the response time of your film? Suggest ways to decrease the response time.

- Does the response time change with film thickness?
- Do different ruthenium dyes have different response times?
- Are the responses similar for each dye? If there are differences propose an explanation.
- Why is the calibration curve nonlinear (or linear)?

**Supplemental Instrument Background**

Lock-in amplification is a technique that can be used to isolate signals in the presence of high levels of noise. The method involves modulating the signal by some reference frequency ($\omega_{\text{ref}}$) then detecting and amplifying the signal ($\omega_{\text{sig}}$). In principal, a lock-in amplifier simply consists of electronic circuits that first multiply two signals, separate the result into two components then sends the results through a low pass filter. Let’s take as an example two sine waves with frequencies of 20 Hz and 18 Hz respectively. The product of these two signals is shown below in figure 5.

![Figure 5. The product of two sinusoidal waves with 20 Hz and 18 Hz frequencies.](image)

Using the trigonometric identity below the product of any two sinusoidal waves can be expressed as the sum of two waves with frequencies of ($\omega_{\text{ref}} - \omega_{\text{sig}}$) and ($\omega_{\text{ref}} + \omega_{\text{sig}}$) called the difference and sum frequencies respectively.

$$
\cos(\omega_{\text{ref}} t + \phi_{\text{ref}}) \cos(\omega_{\text{sig}} t + \phi_{\text{sig}}) = \frac{1}{2} \cos((\omega_{\text{ref}} - \omega_{\text{sig}}) t + (\phi_{\text{ref}} - \phi_{\text{sig}})) + \frac{1}{2} \cos((\omega_{\text{ref}} + \omega_{\text{sig}}) t + (\phi_{\text{ref}} + \phi_{\text{sig}}))
$$

($\phi$ is the phase of each signal)
A plot of these the sum and difference frequencies is shown in figure 6. Note that one signal is higher in frequency and the other is lower in frequency than either of the two input signals. When $\omega_{\text{sig}} = \omega_{\text{ref}}$, the sum wave is a sine wave at $2\omega$ and the other is a DC signal ($\omega = 0$ Hz). An electronic low pass filter is applied to these two resulting waves. A low pass filter consists of a resistor (R in ohms) and a capacitor (C in farads). The product of these two values gives a value called the RC time constant or time constant for short (units in seconds).

![Figure 6](image)

**Figure 6.** The difference (dashed) and sum (solid) frequencies for 20 Hz and 18 Hz input signals.

The time constant determines the frequencies which are allowed to pass through the filter un-attenuated. Mathematically, a low pass filter can be expressed as the following

$$S_{\text{out}} = \frac{S_{\text{in}}}{\sqrt{1 + (RC\times\omega)^2}}$$

where the magnitude of the signal output ($S_{\text{out}}$) depends upon the input signal ($S_{\text{in}}$), the time constant ($RC$) and the frequency of the signal ($\omega$, the difference and sum frequencies). Applying a low pass filter with a time constant of 1 second to the signals in figure 3 gives the output shown below in figure 7.

![Figure 7](image)

**Figure 7.** Low pass filter (RC = 1 second) applied to the signals shown.
in figure 6. Difference (blue) and sum (red).
Note here that the high frequency signal is greatly attenuated relative
to the low frequency signal. The total output from the lock-in amplifier
is given as the sum of the two filtered signals.

Up to this point we have yet to see the real advantage of a lock-in
amplifier. Let’s take a look at the frequency response of such a system.
Assume that we have a reference frequency of 20 Hz. An analytical
signal may have multiple sources of noise present. For example, 60
Hz noise resulting from the AC signals in standard electrical outlets
(called line noise) may interfere with detection. Computer monitors
operate in the 60-100 Hz range and are a common source of RF noise.
Cables and wires connecting instrument components frequently act
as antennas picking up ambient RF signals. A plot of the output at
time = RC as a function of the signal frequency is shown in figure 8
using a 1 second time constant.

![Figure 8](image)

**Figure 8.** Output of a lock-in amplifier as a function of signal
frequency. The reference frequency was 20 Hz and a one second time
constant was used.

It is clear that frequencies nearest to our reference of 20 Hz are
amplified over those that are further away. However, the amplification
is not all that significant. In order to increase the selectivity of
amplification it is necessary to increase the time constant relative to
our reference frequency. If the time constant is increased to 10 seconds
as shown in figure 9, the value of a lock-in amplifier becomes very
clear. Now only those frequencies that are very close to the reference
frequency are amplified and all other frequencies are suppressed. If
we had problems with 60 Hz line noise before, they all but vanish after
lock-in amplification.
Figure 9. Output of a lock-in amplifier as a function of signal frequency. The reference frequency was 20 Hz and a ten second time constant was used.

Frequently optical signals are modulated using an optical chopper assembly. A chopper is a rapidly rotating plate with slits that act to alternately block and pass light. There is an AC output from the chopper the serves as the reference frequency for the lock-in amplifier. A more convenient (and inexpensive) way to modulate an LED light source is to provide an AC signal to the LED, effectively dimming and brightening the LED with a given frequency. A simple timing circuit provides a square wave voltage profile to the LED and provides a reference signal. The basic layout our the sensing device is shown below in figure 7. Note that there are two filters in the system. One filter serves to remove any low energy light from the LED. While LEDs are fairly monochromatic, the blue LEDs in particular emit a fair amount of light in other regions of the visible spectrum as well. The highpass filter only allows light at <500 nm to pass through. The other filter is a lowpass filter that allows light >590 nm corresponding to the emission maximum of our sensing dye. The combination of the two filters is necessary to eliminate any of the LED excitation light from entering the detector. If we remove the filters, we see a very large signal which is simply the amplification of the LED light source and not the fluorescence signal that we are trying to detect.
**SYNTHESIS AND CHARACTERIZATION OF Mn^{III}(salpn)(AcAc)**

**Introduction**

First row transition elements have d-orbitals that may be partially filled depending on the atomic number and oxidation state of the metal. When surrounded by a spherically symmetric electric field, the five d-orbitals are degenerate and have the same energy. However, when the electric field has octahedral geometry, the orbitals split into two sets. One with higher energy relative to the symmetric field ($e_g$) and one with lower energy ($t_{2g}$). The energy gap between the two states is called $\Delta_o$ (figure 1). In a transition metal complex, we envision the metal cation being approached by negative point charges from the bound ligands. The orientation of these charges depends on the coordination number of the metal. A coordination number of 6 is common for many transition metal complexes and gives rise to octahedral geometry with respect to the ligands surrounding the metal center.

![Figure 1. Splitting of d-orbitals in an octahedral field.](image)

When the magnitude of $\Delta_o$ is small, electron-electron repulsion dominates and the electrons fill the available orbitals one at a time before pairing. If $\Delta_o$ is large then the electrons fill the lower energy $t_{2g}$ set of orbitals fully before populating any of the higher energy $e_g$ orbitals. In first case described, electron spin multiplicity is maximized and such systems are said to be “high-spin”. In the latter case the systems are described as “low-spin” (figure 2). The magnitude of $\Delta_o$ determined by the metal, the oxidation state and the nature of the ligands. Ligands that are strongly $\sigma$ donating (e.g. NH$_3$) or $\pi$ (e.g. CO) accepting are generally strong field ligands the generate large $\Delta_o$ and low spin complexes. The relative ligand field strengths of ligands is listed as the spectrochemical series. A full treatment of this topic can be found in inorganic texbooks in the sections describing ligand field theory. Here, we focus on the experimental methods for determining whether a system is in a high or low spin state.
Pre-Lab Assignment

Look up the Materials Safety Data Sheet (MSDS) information for all of the underlined reagents used in this experiment. MSDS’s can be found from several places online. The environmental health and safety office has links to online MSDS databases. In addition many chemical vendors such as Fisher Scientific and Aldrich Chemicals give MSDS information on the web sites. Bring the MSDS’s with you to class.

Synthesis of H₂salpn ligand

To prepare 40 mmoles of salpn (salpn = 1,3-bis(salicylideneiminato)propane, M.W. = 282.35), dissolve 80 mmoles of salicylaldehyde (M.W. = 122.12, d₂₀ = 1.1674 g·cm⁻³) in 100 ml of methanol in a 500 mL roundbottom flask. While stirring this solution with a magnetic stir bar, add 40 mmoles 1,3-diaminopropane (M.W. = 74.12, d = 0.8880 g·cm⁻³). Stir the solution for 15 minutes. Obtain a UV/visible absorption spectrum from 250 nm to 800 nm in methanol solution. Adjust the concentration to keep the absorbance values below 1.0. Evaporate the solvent by rotary evaporation. Calculate the yield of solid.

Synthesis of Mn³⁺(salpn)(AcAc)

Stir 20 mmol salpn in 50 mL methanol and bring to reflux. Add 20 mmol manganese(III)acetylacetonate [Mn³⁺(AcAc)₃, M.W. = 352.27]. After stirring briefly, let the solution cool to near room temperature. Add ~100 mL of water while stirring. Recover the solid product by suction filtration through filter paper in a Büchner funnel. Rinse the solide with diethyl ether. Determine the yield of the dry solid.

Figure 2. High and low spin electron arrangements for a d⁶ transition metal cation.
Definitions

**Volume Susceptibility**

\[ \chi_v = \frac{I}{H} \]  

where \( I \) is the intensity of induced magnetism and \( H \) is the applied magnetic field.

**Mass Susceptibility**

\[ \chi_g = \frac{\chi_v}{\rho} \]  

where \( \rho \) is the density of the sample.

**Molar Susceptibility**

\[ \chi_m = \chi_g(MW) \]  

where \( MW \) is the molecular weight of the sample.

**Introduction to Magnetic Characterization**

The mass susceptibility (c.g.s units) of a solid sample is calculated using the following expression when using the magnetic susceptibility balance

\[ \chi_g = \frac{lC}{m10^9}(R - R_0) \]  

where \( l \) is the length of the sample (in cm), \( m \) is the mass of the sample (g), \( R_0 \) is the reading for the empty tube and \( R \) is the reading of the tube plus the sample. \( C \) is a calibration constant for the balance and is obtained by comparison to a known standard. Using the standard provided, the calibration constant is determined by the following equation

\[ C = \frac{C_{Tube}}{(R - R_0)} \]  

The \( R_0 \) and \( C_{Tube} \) values are listed on the calibration standard. Your TA will provide you with the calibration constant which should be close to unity.

The effective magnetic moment (\( \mu_{eff} \)) for a substance can be calculated by the following
Here $k$ is Boltzmann’s constant, $T$ is the temperature (in kelvin), $N_0$ is Avagadro’s number, $\beta$ is the Bohr magneton and $\chi_A$ is the magnetic susceptibility corrected for diamagnetism.

Equation 6 reduces to the following when the appropriate values for the constants have been included (Note the value for $\mu_{\text{eff}}$ is given in Bohr magnetons)

$$\mu_{\text{eff}} = 2.828 \sqrt{\chi_A T}$$

(A note concerning $\chi_m$ vs. $\chi_A$)

For transition metal complexes this value can be compared to the spin-only contribution to the magnetic moment, calculated from the following where $n$ is the number of unpaired electrons (also given in Bohr magnetons).

$$\mu_{\text{eff}} = \sqrt{n(n+2)}$$

All substances have a diamagnetic component. This means that the value for $\chi_m$ is lower than the actual paramagnetic susceptibility. Since $\chi_m$ contributions are additive, the diamagnetic influence can be corrected for. First row transition metals have a correction factor of $13 \times 10^{-6}$ and the acac ligand has a correction term of $55 \times 10^{-6}$. Measure the diamagnetic contribution of the salpn ligand using your synthesized product.

**Mn$^{III}$(salpn)(AcAc) magnetic measurement**

In your prelab write-up, calculate the “spin only” magnetic moment for the complex (based on the number of unpaired electrons) according to equation 9 below.

$$\mu_{\text{spin,only}} = g_e \mu_e \sqrt{S(S+1)}$$

Here $g_e$ is the electron $g$ factor ($g_e = 2.00$ for simplicity), $\mu_e$ is the Bohr magneton ($\mu_e = 9.274 \times 10^{-24}$ J T$^{-1}$) and $S = \frac{1}{2}$ for a single unpaired electron. For example, high spin Fe$^{2+}$ in an octahedral field has four unpaired electrons and the spin only magnetic moment is equal to $2.00\mu_e[2(2+1)]^{\frac{1}{2}} = 4.90$ Bohr magneton.

Measure the room temperature magnetic moment by a modified Gouy method for Mn$^{III}$(salpn)(AcAc) and for the salpn ligand. The instructions for using the magnetic susceptibility balance are given on the following page.
Instructions for using the magnetic susceptibility balance

1. Turn the RANGE knob to the ×1 scale and allow a 10 minute (minimum) warm-up period before use.

2. Adjust the ZERO knob until the display reads 000.

3. Place an empty sample tube of known weight into the tube guide and take the reading $R_0$. Be sure to record the mass of the empty tube.

4. Pack the sample into the tube. Be sure that the sample is packed evenly. Add a small amount of sample to the tube and gently tab the bottom of the tube on the lab bench to force the solid to the bottom of the tube. Repeat until the height of the sample is between 2.5 and 3.5 cm. The tubes are very fragile so handle them with care.

5. Record the mass of the tube with the sample.

6. Re-zero the balance if needed and record the sample reading $R$.

7. Calculate the gram susceptibility according to equation 4 above.

8. Compare the experimental and theoretical values for the effective magnetic moment. Use Eq. 7 and 8.

Be sure to keep all ferromagnetic materials away from the balance.
**Introduction**

The first homogeneous catalytic hydrogenation of unsaturated organic compounds was achieved using RhCl(P(C₆H₅)₃)₃ (Wilkinson’s catalyst) in 1965.¹ Detailed studies have resulted in the proposed mechanism shown below in figure 1. Molecular hydrogen is activated through oxidative addition to the unsaturated rhodium complex shown in step 2. The resulting hydrido complex allows for addition of an olefin or other unsaturated species with a concomitant loss of phosphine. The rate limiting insertion reaction in step 5 is followed by a reductive elimination step which completes the catalytic cycle.

![Figure 1. Proposed mechanism for hydrogenation of alkenes by Wilkinson’s catalyst.](image)

**Synthesis of RhCl(PPh₃)₃**

\[
\text{RhCl}_3 \cdot 3\text{H}_2\text{O} + \text{P(C}_6\text{H}_{5})_3 \rightarrow \text{RhCl(P(C}_6\text{H}_{5})_3)_3
\]

Place 20 mL of absolute ethanol in a 25-mL round bottom flask equipped with a magnetic stir bar. Attach a reflux condenser and place the apparatus in a heating mantel on a magnetic stir plate. Heat the ethanol to just below the boiling point. Remove the condenser

momentarily, and add ~600 mg (2.29 mmol, a large excess) of triphenylphosphine to the hot ethanol and stir until dissolved. Remove the condenser momentarily once again, and add 100 mg (0.48 mmol) of hydrated rhodium (III) chloride to the solution and continue to stir. Heat the solution to a gentle reflux. Initially, a deep red-brown solution is obtained, which during additional heating under reflux will slowly form yellow crystals. After ~20-30 minutes of reflux, the yellow crystals are converted into shiny burgundy-red crystals.

Collect the product crystals by suction filtration on a Hirsch funnel while the solution is hot. Wash the crystals with three 1-mL portions of hot ethanol followed by three 1 mL portions of diethyl ether. Dry the crystals on the filter by continuous suction. Calculate the percentage yield and determine the melting point of the product.

**Synthesis of RhCl(PPh₃)₃H₂**

\[
\text{RhCl(P(C₆H₅)₃)}_3 + \text{H}_2 \rightarrow \text{RhCl(P(C₆H₅)₃)}_3\text{H}_2
\]

Add ~100 mg of RhCl(P(C₆H₅)₃)₃ to a 3 mL conical vial fitted with a septum cap. Add 1 mL of deoxygenated chloroform using a syringe. Chloroform can be deoxygenated by bubbling with an inert gas such as argon or nitrogen for 2-3 minutes. Bubble H₂ gas through the solution for a 10 minutes, whereupon the red solution will turn yellow (this change may be subtle because the concentration is high). Concentrate the solution under a flow of H₂ gas. When the solution is sufficiently concentrated (~0.2 mL), add deoxygenated diethyl ether drop-wise with a syringe until precipitation occurs, about 2 mL. Cool the flask in an ice-water bath and collect the pale yellow crystals by suction filtration using a Hirsch funnel. Calculate the percent yield. Obtain the IR spectrum of your starting material and the dihydride. Identify the hydride stretching frequencies.

**Catalytic Hydrogenation**

Flush a flask equipped with a magnetic stir bar and a rubber septum with H₂ through a long needle. Add 10 mL of toluene to this flask and saturate it with hydrogen gas by bubbling for 10 minutes. Quickly remove the septum and with stirring dissolve 25 mg of Wilkinson’s catalyst in the solvent. Note: The catalyst is relatively insoluble in toluene, but in the presence of H₂ gas it dissolves fairly rapidly due to the formation of the more soluble dihydrido species. The resulting solution is pale yellow.

Discontinue the stirring, remove the septum, and with a Pasteur pipet, drop-wise, add 1 mL of freshly distilled cyclohexene. As soon as the alkene is added the solution turns deep red-brown in color. Reattach the septum. Flush the flask with hydrogen gas. Upon stirring in an atmosphere of H₂, the color of the solution again lightens to pale yellow. Note: If the H₂ gas flow is stopped and stirring is discontinued even for only 30 seconds, the solution again turns deep red-brown. Remove a small sample of the solution for GC analysis. The presence of cyclohexane formed as a result of the hydrogenation of cyclohexene should be evident in the chromatogram.

You can speed up the evaporation process by placing the vial in a beaker containing hot tap water.
Kinetics of Hydrogenation

In the presence of excess phosphine ligand the loss of hydrogen from species A (refer to figure 2) is suppressed and can be ignored under the conditions of this experiment ($k_{-2} \approx 10^4 \text{ s}^{-1}$). The proposed mechanism begins with A reacting with an alkene (E) to form the intermediate species B with the loss of one phosphine ligand (P). B then reacts in a rate determining step (R.D.S.) to give the hydrogen insertion product C which rapidly eliminates the alkane and in the presence of phosphine and absence of hydrogen reforms the original $(\text{PPh}_3)_3\text{RhCl}$ starting material. Inspection of the above mechanism with regards to species A, B and C results in the following differential equations.

\[
\frac{dA}{dt} = k_4[A][E] - k_{-4}[B][P]
\]
(1)

\[
\frac{dB}{dt} = k_{-4}[B][P] - k_4[A][E] + k_5[B]
\]
(2)

\[
\frac{dC}{dt} = k_5[B]
\]
(3)

The goal is to manipulate these equations so that only one experimentally observable variable remains. In the experimental conditions described below an excess of triphenylphosphine and cyclohexene are added in single turnover conditions. A single turnover in a catalytic cycle means that the conditions are such that the reaction stops after one cycle. Because the reaction is stopped after a single turnover the concentrations of phosphine and cyclohexene do not change during the experiment and can be treated as constants. In equation 1 there are two concentration variables A and B. We will attempt to rewrite this equation so that the B term is eliminated. Any equilibria or mass action expressions can also be used in working with these equations. The equilibrium expression for the first step in the mechanism is shown in Eq 4.

\[
K_4 = \frac{[B][P]}{[A][E]} = \frac{k_4}{k_{-4}}
\]
(4)
At any point during the reaction the total amount of rhodium containing species in solution, \( T \), is the sum of \( A \), \( B \) and \( C \) and the amount of reactive rhodium (\( R \)) is equal to \( A + B \) since once \( C \) is formed it can no longer react to produce \( A \) or \( B \). If this is true then the following relations hold.

\[
[T] = [A] + [B] + [C] \\
[R] = [A] + [B] = [T] - [C]
\]

Manipulation of the equilibrium expression leads to the following:

\[
\frac{1}{K_i} \left[ \frac{[A][E]}{[B][P]} \right] \frac{[P]}{K_i[E]} = \frac{[A]}{[B]} \\
\frac{[P]}{K_i[E]} + 1 = \frac{[A] + [B]}{[B]}
\]

(5)

Note that in the second transformation, \([B]/[B]\) was added to each side of the equation. Substitution of eq. 5 into eq. 6 and rearrangement gives the following expression for \([B]\) in terms of \( R \), \( P \) and \( E \).

\[
[B] = \frac{[P]K_i[E]}{(P) + K_i[E]}
\]

(6)

Thus far no assumptions have been made during these derivations. Let’s take a closer look at how \([B]\) changes during the course of the reaction. Since the equilibrium is rapid, that is \( k_4 \) and \( k_{-4} \) are fast reactions, one may assume that as \( B \) is formed it rapidly reacts to reform \( A \) or goes on to form \( C \). As a result the concentration of \( B \) remains at a small constant value throughout the experiment, until all of \( A \) has been converted to \( C \). In effect we are setting Eq. 2 equal to zero. This assumption is called the steady state approximation and is encountered frequently in chemical kinetics. With this assumption in place one can argue that the rate of disappearance of \( A \) must therefore be equal to the rate of appearance of \( C \). Substitution of Eq. 7 into equation 3 results in the following

\[
\frac{dC}{dt} = K_i k_4[E][R] + K_i k_4[E] (T) - [C]
\]

(7)

Using the relationships in Eq. 5 above and making the assumption that \([B] << [A]\) results in the following equation for the rate of disappearance of:

\[
-\frac{dA}{dt} = \frac{K_i k_4[E]}{[P] + K_i[E]} [A] = k_{obs} [A]
\]

(8)

Since the concentration of alkene and phosphine are constants the resulting expression is in the form of the familiar first order kinetics that we are exposed to in general chemistry. The integrated form of this equation leads one to predict that in the presence of an excess of phosphine and alkene, simple exponential kinetics should be observed. The value for the observed rate constant \( (k_{obs}) \) is predicted to be dependent on the concentrations of alkene and phosphine. One
additional manipulation is required to test this hypothesis.

\[
    k_{obs} = \frac{K_p k_k [E]}{[P] + K_p [E]} \quad \frac{1}{k_{obs}} = \frac{1}{k_i} + \frac{[P]}{K_p k_k [E]}
\]  

(10)

If a series of experiments are performed in which [P] and [E] are varied, a plot of \(1/k_{obs}\) vs. \([P]/[E]\) should result in a straight line with a slope equal to \(1/(K_p k_k)\) and an intercept of \(1/k_i\).

**Kinetics Procedures**

All solutions prepared in this section should be placed in a 25°C water bath in a fume hood as the rates of reaction are highly temperature dependent. Add 25 mL of toluene to a 100 mL roundbottom flask. Cap the flask with a rubber septum and bubble the solvent with argon for 15 minutes. Prepare a similar flask with 20 mL of cyclohexene and bubble with argon for 15 minutes as well. Add 20-30 mg of \(\text{RhCl(P(C_6H_5)3)}_3\) to a 100 mL roundbottom flask equipped with a stir bar. Cap the flask with a rubber septum and purge the flask with hydrogen gas for several minutes. Using a syringe, transfer enough argon saturated toluene to the flask to result in a solution that contains 2 mg of \(\text{RhCl(P(C_6H_5)3)}_3\) per mL of solution. Continue to bubble the solution with \(\text{H}_2\) with stirring until all of the solid has dissolved and a clear yellow solution is formed. This step may take 10-20 minutes. Weigh a sample of triphenylphoshine directly into a septum top cuvette according to table 1 below. The masses of phosphine are approximate in the table so be sure to record the actual mass to the nearest 0.1 mg. Cap the cuvette and purge with argon for several minutes. Transfer 0.5 mL of argon saturated toluene to the cuvette with a syringe. Transfer 0.5 mL of the Wilkinson’s catalyst solution to the cuvette using a syringe and continue to bubble the solution with argon for 2-3 minutes. While purging the cuvette take care that none of the solution is forced out of the vent needle. Add an aliquot of deoxygenated cyclohexene to the cuvette according to table 2 below. Mix the solution well and place the cuvette in the spectrophotometer and monitor the absorbance at 450 nm for 15-50 minutes. The cell holder in the instrument is thermostated and should be set to 25°C. You may stop the data acquisition earlier if the absorbance has stopped changing (figure 3). If the absorbance decreases significantly, this is a sign that the solutions were not adequately degassed and you must repeat the experiment. Be sure to save your data in ASCII format with a “txt” file extension.

**Table 1.** Amounts of cyclohexene and triphenyl phosphine for the kinetics measurement.

<table>
<thead>
<tr>
<th>PPh3 (mg)</th>
<th>cyclohexene (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
</tr>
<tr>
<td>20</td>
<td>1.0</td>
</tr>
<tr>
<td>40</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The density of cyclohexene is 0.81 g cm$^{-3}$.  

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Each group will investigate the effect of triphenylphosphine and cyclohexene concentration on the observed rate of reaction according to table 1. Your TA will assign your group a set of measurements to perform. While your solutions are being prepared determine the observed rate constant according to the instructions below. Report the observed rate constant, volume of cyclohexene, and mass of triphenylphosphine on the board in the lab.

**Data Analysis**

In this experiment we will be monitoring the formation of \( \text{RhCl}(\text{PPh}_3)_3 \) at 450 nm. The experimental data is expected to be in the form of an exponential rise to a maximum value which takes the form as Eq. 11

\[
A_{\text{bs}} = c + A(1 - \exp(-k_{\text{obs}}t))
\]

There are numerous numerical analysis programs that allow for nonlinear regression of experimental data. If you are familiar with nonlinear curve fitting you may use your favorite program to obtain values for \( k_{\text{obs}} \). Described below is a method for nonlinear curve fitting using the Microsoft Excel program. An example dataset is shown below.

![Figure 3. Plot of absorbance vs. time for a single turnover experiment.](image)

**Using “Solver” in Excel for non-linear curve fitting**

Create a spreadsheet similar to the one pictured below. Import your kinetics data into columns A and B. Column A should be the time values (in seconds) and B the absorbance values.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.180</td>
<td>0.185</td>
<td>A</td>
<td>0.185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0.182</td>
<td>0.193</td>
<td>k</td>
<td>6.00E-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.190</td>
<td>0.198</td>
<td>c</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>0.197</td>
<td>0.204</td>
<td>X^2</td>
<td>1.22E-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0.205</td>
<td>0.209</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>0.212</td>
<td>0.214</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The equation for an exponential rise to a maximum value is given in Eq. 11 where A is the amplitude of the curve, c is the offset from zero and \( k_{\text{obs}} \) is the observed rate constant. A and c are easily estimated from inspection of the data. The value for c is estimated as the absorbance at time zero, and the amplitude is estimated as the difference between the absorbance at time zero and the absorbance at infinite time. The rate constant can be estimated as \( 1/t_{1/2} \) (\( t_{1/2} \) = half-life).

Column C is the predicted curve based upon the guess values of A, c and k.

The following formula should be entered into cell C1 and copied into all of column C:

\[
=G3+G1*(1-\text{EXP}(-G2*A1))
\]

Cell G4 is the sum of the chi squares values, \( \chi^2 \), enter the following in cell G4:

\[
=\text{SUMXMY2(B1:B250,C1:C250)}
\]

If the predicted curve is very close to the experimental curve then the value for \( \chi^2 \) will be small. The best fit curve is then expected to have the minimum value of \( \chi^2 \). We can use the “Solver” add-in in Excel to find the values of A, c and k that result in the minimum value for \( \chi^2 \) (cell G4). Stepwise instructions are given below.

**Steps for curve fitting**

2. In the box labeled “Set Target Cell” type in \( G4 \).
3. Below this select the “Equal To” section to the “min” function since we are trying to minimize the value in cell G4.
4. In the box labeled “By Changing Cells” type \( G1:G3 \). This allows the solver to vary the values for A, c and k to minimize the sum of chi squared.
5. Now click on “Solve”. The program will alter your initial values to fit the data.
6. A new pop-up window will appear asking if you want to keep the new values or revert to your original values. Select “Keep Solver Solution” and click the “OK” button.

The best fit values for A, c and k will now be in cells G1:G3. To be sure the values make sense, plot the columns B and C vs. column A. The two curves should match very closely. If they do not, then you need to make better guesses for A, c, and k to start with. Enter new guess values and repeat the procedure.
Kinetics Analysis, Determination of $K_4$ and $k_5$

Record the mass of $\text{PPh}_3$, volume of cyclohexene and the observed rate constant for each reaction your group performs. Turn in these values to your TA before you leave the lab. Look up the class experimental data on the course website and construct a plot of $1/k_{\text{obs}}$ (y-axis) vs. $[\text{PPh}_3]/[\text{C}_6\text{H}_{10}]$ as in equation 10 above. In this analysis you are plotting a ratio of concentrations. Since the triphenylphosphine and cyclohexene are in the same volume of solution one does not need to know the total volume of solution in order to calculate the concentration ratio.

$$\frac{[\text{PPh}_3]}{[\text{C}_6\text{H}_{10}]} = \frac{\text{moles of PPh}_3}{\text{moles of C}_6\text{H}_{10}} \quad (12)$$

Report the values for $K_4$ and $k_5$. Compare the class values to those reported in the literature.2

Temperature Dependence of the Rate Limiting Step

The rate limiting step in this reaction has been shown to have a very large activation energy ($E_a$). Wilkinson reported the activation energy to be 22.9 kcal mol$^{-1}$.3 Given that $k_5$ at 298 K was reported by Halpern4 to be $0.2 \pm 0.04 \text{ s}^{-1}$ the Arrhenius factor, $A$ can be found by the familiar equation 13. Once the value for $A$ has been calculated the rate constant at other temperatures can be determined.

$$\ln k_5 = \ln A - \frac{E_a}{RT} \quad (13)$$

An assumption is made in this analysis that the equilibrium constant $K_e$ is relatively insensitive to the temperature near room temperature. A plot of the rate constant as a function of temperature given in figure 4.

---

Figure 4. Plot of the temperature dependence of the rate limiting step in the hydrogenation of cyclohexene. The dashed lines are the upper and lower estimates based on the reported uncertainty in $k_5$. 
**Synthesis and Characterization of CdSe Nanocrystals**

**An Example of a Real Particle in a Box**

**Introduction**

In a semiconductor absorption of light of a sufficient energy causes an electron to move from the valance band to the conduction band. In the process a hole (positive charge) is created in the valence band. This electron-hole pair is called an exciton and is analogous to an excited state in a molecule. The excited electron and hole remain at a fixed distance from each other known as the Bohr exciton radius. In bulk CdSe this distance is 5.4 nm.\(^1\) When the size of a particle of CdSe is less than 11.2 nm in diameter the electron-hole pair cannot reach the Bohr exciton radius and behave as particles trapped in a box. Particles of this size are known as “quantum dots” or “nanocrystals”. Theoretically, the exciton absorbance should correspond to the confinement energy of a particle in a three dimensional box (figure 1). This is indeed the case as shown in figure 2 where the lowest energy exciton absorption shifts to longer wavelength with increasing particle size. The confinement energy of a charged particle in a cubic box is given by the equation below. The potential energy is defined as infinite outside the box and zero inside. This is a three dimensional extension of a particle on a line in an infinite potential well.

\[
E(\psi) = U(x, y, z)\psi - \frac{\hbar^2}{8\pi^2 m} \left[ \frac{\partial^2 \psi}{\partial x^2} + \frac{\partial^2 \psi}{\partial y^2} + \frac{\partial^2 \psi}{\partial z^2} \right] 
\]

\(1\)

**Figure 1.** A cube of CdS 5 nm on edge.

---

Figure 1. Absorbance spectra of various sizes of CdSe nanocrystals.

The exciton in a semiconductor nanocrystal can be thought of as a real world example of the particle in a box problem, however there are some significant differences. First the nanocrystal is not a cubic shape. Experiments reveal that the shape is better thought of as ellipsoidal. The equation above can be modified to account for this geometry. Another important difference is that the optical transition we observe as the exciton band results from the creation of two charged particles; an electron(-) and a hole(+). This too can be accounted for and the Hamiltonian can be written for the electron and hole as in equation 1.

\[
\begin{align*}
\mathbf{H} &= -\frac{\hbar^2}{8\pi^2 m_e} \nabla^2 - \frac{\hbar^2}{8\pi^2 m_h} \nabla^2 + U(S_e, S_h) \\
\nabla^2 &= \left[ \frac{\partial^2 \psi}{\partial x^2} + \frac{\partial^2 \psi}{\partial y^2} + \frac{\partial^2 \psi}{\partial z^2} \right]
\end{align*}
\]

where \( m_e \) and \( m_h \) are the effective masses of an electron and a hole in CdSe and \( S_e \) and \( S_h \) are the positions of the electron and hole inside the nanocrystal. Now the potential energy is considered infinite outside of the nanocrystal but inside coulombic attraction between the oppositely charged particles must be taken into account. Including the appropriate terms leads to the following equation known as the effective mass model

\[
E^* \approx E_{\text{gap}} + \frac{\hbar^2}{8R^2} \left[ \frac{1}{m_e} + \frac{1}{m_h} \right] - \frac{1.8e^2}{4\pi\varepsilon R}
\]

where \( E_{\text{gap}} \) is the bulk CdSe bandgap energy and \( \varepsilon \) is the dielectric constant of CdSe. The effective mass model is good for particles above ~7 nm in diameter. For smaller particles the approximations made in reaching the effective mass model are no longer valid. Research is currently underway to develop a more general theory.
Synthesis

Add 75 mg of Li$_4$[Cd$_{10}$Se$_4$(SPh)$_{16}$] and 5 grams of hexadecylamine to a crimp top vial containing a magnetic stir bar. Seal the vial and purge with argon for 5 minutes. Place the vial in an aluminum heating block and raise the temperature to 200°C. Over the course of several hours the solution will change from colorless to yellow. You may remove the vial at this time and allow to cool. If larger diameter particles are desired, the temperature is raised to 220°C and allowed to stir overnight. Raising the temperature further (230°C) and allowing the mixture to stir for up to a week will result in still larger particles. The solution will change from yellow to orange to red as the particles grow. If the solution turns cloudy at any time, decrease the temperature to 190°C until the solution becomes clear. The temperature is raised to the previous point and the growth is allowed to continue.

Periodically remove the vials from the heating block and allow to cool slightly. Remove 5-6 drops of the molten wax and place it in a test tube. Recap the vial, purge the headspace with argon for several minutes, return it to the heating block and continue heating and raising the temperature. Heat the test tube in a hot water bath until the wax has melted. Add 2-3 mL of methanol to the liquid. The CdSe nanocrystals should precipitate at this point. Place the test tube in centrifuge and spin the solutions for ~5 minutes. Note: The centrifuge must be balanced for safe use. Tubes opposite each other in the centrifuge rotor must have the same mass. Check the tubes before your turn on the centrifuge. After spinning, decant the liquid and then add 1-2 mL of toluene to the remaining solid. The result will be a suspension of nanocrystals in toluene suitable for optical characterization.

Record the absorbance spectrum of your nanocrystals in toluene solution. Determine the wavelength ($\lambda_{\text{max}}$) of the exciton peak. The exciton peak is identified in the CdSe optical spectrum shown below in figure 3.

![Optical absorbance spectrum of CdSe. The arrow indicates the $\lambda_{\text{max}}$ for the exciton.](image_url)

**Figure 3.** Optical absorbance spectrum of CdSe. The arrow indicates the $\lambda_{\text{max}}$ for the exciton.
Optical characterization methods can be utilized to determine the size of nanocrystals. Extensive data have been collected to correlate the exciton energy and the particle size. Particle sizes are determined by SEM images and x-ray powder diffraction techniques (see figure 4).

![Figure 4](image_url)

**Figure 4.** Plot of CdSe diameter vs. exciton absorbance.

The line through the data in figure 4 is an empirical fit and can be used to estimate the diameter of CdSe nanocrystals using the following equation

\[
D = \frac{1 - B \lambda}{A + C \lambda}
\]

where \( D \) is the diameter of the nanocrystal (nm), \( \lambda \) is the exciton wavelength (nm) and \( A = -0.79196, B = 3.8121 \times 10^{-3} \) and \( C = 9.5125 \times 10^{-4} \).

Use Eq. 4 to determine the size of your nanocrystals. To explore the idea of particle in a box further we will look at the emission energy as a function of particle size. Record the emission spectrum of your nanocrystal solution. Use the \( \lambda_{\text{max}} \) of the lowest energy exciton as the excitation wavelength. To avoid saturating the detector start collecting data 10 nm to the red (longer wavelength) of your excitation wavelength. Record the emission maximum in your laboratory notebook. Report the diameters and emission wavelengths to your TA. The class data will be posted to the web at the end of the week. The particle in a box model predicts that the emission energy should be proportional to \( 1/R^2 \). Therefore a plot of the emission energy (in eV) vs. \( 1/R^2 \) should result in a straight line. Plot the class data and discuss the results.
Introduction

The photosensitivity of carbon monoxy hemoproteins has been known for over a century. Haldane and Smith discovered in 1896 that carbonmonoxy hemoglobin (HbCO) dissociates carbon monoxide upon photolysis. For sixty years following this observation, it was thought that the CO adducts of hemoproteins were the only complexes of heme proteins that were photolabile. The equilibrium constants and binding rates of hemes with dioxygen were examined with interest in discovering how the oxygen transport system works within the body. A useful tool to study the kinetics of very fast reactions is flash photolysis. It was by using flash photolysis that the photolability was demonstrated with other hemoprotein diatomic adducts. The NO, CO and O₃ adducts of hemoproteins as well as some model systems (free hemes, porphyrins) have been extensively examined by flash photolysis techniques.

The mechanism for reversible binding of ligands to the heme centers in hemoglobin is an important biological process. A useful method for investigating processes of these types is flash photolysis. Bond cleavage may be induced by absorption of light with the appropriate energies allowing for studies of rebinding. In order for bond cleavage to ensue, one of two events must take place. An electron must be removed from a bonding orbital or an electron must be placed in an anti-bonding orbital. In short, absorption of light must lead to some state either by direct excitation or relaxation to a dissociative state.

Spectroscopy of Porphyrins and Metalloporphyrins

The spectroscopy and structure of porphyrins and metalloporphyrins has been the subject of extensive research. An attempt to summarize the complete body of work in this area is beyond the scope of this manual. Instead, the reader is referred to the several volume set entitled “The Porphyrins” edited by Dolphin and the references therein. The aspects of structure and spectroscopy relevant to the areas under investigation in this experiment will be outlined below.

The electronic absorption spectra of porphyrins and metalloporphyrins are dominated by strong $\pi \rightarrow \pi^*$ ligand bands. The observed electronic transitions are explained by Gouterman's four orbital model which consists of two HOMO (highest occupied molecular orbital) ligand $\pi$ orbitals of $a_{1u}$ and $a_{2u}$ symmetry and two degenerate LUMO (lowest unoccupied molecular orbital) ligand $\pi^*$ orbitals of $e_g$ symmetry (see Figure 1). Constructive and destructive

mixing of the HOMO orbitals results in two types of transitions for porphyrin systems. Constructive mixing results in the dominate Soret or B band ranging from ~350 to 450 nm with extinction coefficients >10^5 M^-1 cm^-1. Destructive mixing produces the lower energy Q bands appearing in the 500 to 700 nm range with extinction coefficients >10^4 M^-1 cm^-1. The lower energy Q band (Q_0) is separated by one mode of vibrational excitation from the higher energy Q band (Q_1).

Figure 1. The molecular orbitals used in Gouterman's four orbital model for porphyrin spectroscopy. Figure adapted from Suslick and Watson.4

In the case of hypso and hyper type spectra the metal orbitals interact with the porphyrin π orbitals resulting in spectral shifts and/or additional bands (figure 2). Hypso type spectra are observed for d^6 to d^9 metalloporphyrins. These spectra are characterized by a blue shift of the Q bands due to mixing of the metal LUMO with the porphyrin ligand HOMO causing the π to π* energy gap to increase. Hyper porphyrin spectra are further broken down into two subcategories, p-type and d-type. Both p-type and d-type hyper spectra exhibit additional charge transfer bands, MLCT (metal to ligand charge transfer) or LMCT (ligand to metal charge transfer)

---

respectively. Main group metalloporphyrins have p-type hyper spectra and have an additional absorbance due to charge transfer from metal non-bonding $p_z$ orbitals to ligand $\pi^*$. Metalloporphyrins with d$^1$ to d$^6$ metal electron configurations have d-type hyper spectra. The vacancy in the metal $e_g$ orbital allows for a porphyrin p orbital to metal d orbital transition to occur. For metals such as Cr, Mn and Fe the relative energy of the metal $e_g$ orbitals is such that there is good overlap with the porphyrin $\pi^*$ orbitals of the same symmetry.

Figure 2. Molecular orbital diagrams for metalloporphyrins having hypso or d-type hyper spectra. Figure adapted from Suslick and Watson.4

Figure 3. Molecular structures of TPPS and Fe$^{II}$(TPPS)(CO).
**Synthesis of Fe\textsuperscript{III}(TPPS)**

Place 50 mg (0.05 mmol) of H\textsubscript{2}TPPS and 20 mL of DMF in a 100 mL 3-necked round bottom flask that has been equipped with a magnetic stir bar. Fit the flask with a reflux condenser and rubber septa. Bubble the solution with Argon for 15 minutes then add 70 mg of FeSO\textsubscript{4}·7H\textsubscript{2}O (0.25 mmol) to the flask. Heat the solution at reflux temperature for 3-4 hours under an argon atmosphere. Record the electronic absorbance spectrum of the solution every 30 minutes. Transfer the cooled solution to a roundbottomed flask and bring to dryness by rotary evaporation. Collect and save the solid material.

**Synthesis and spectroscopic characterization of Fe\textsuperscript{II}(TPPS) and Fe\textsuperscript{II}(TPPS)CO**

Add 15 mL of water to a 50 mL roundbottom flask that has been fitted with a rubber septum. Bubble this solution with argon for 10-15 minutes. Add 100 mg of sodium dithionite (a.k.a. sodium hydrosulfite, Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4}) to a second flask. Purge this flask with argon while the water is deoxygenating. Transfer the deoxygenated water to the flask containing sodium dithionite. Dissolve a small amount of Fe\textsuperscript{III}(TPPS) in acidic water so that the Soret band absorbance is approximately 0.5 absorbance units. Record the spectrum of this solution over the range of 300-600 nm. Place the solution in a septum cap cuvette and purge with argon for ~5 minutes. Transfer 1 drop of the dithionite solution to the cuvette using a syringe to reduce the iron(III) to iron(II). Record the absorbance spectrum of the resulting solution. The Soret band should increase in intensity and shift to ~428 nm. See figure 4. If the shift is incomplete add additional dithionite solution one drop at a time, recording the spectrum after each addition. Once you have formed the Fe\textsuperscript{II}(TPPS) compound, bubble the cuvette with carbon monoxide (CO) for 3-5 minutes. Again record the spectrum over the 300-600 nm range. The Soret band should blue shift to ~418 nm (figure 4).

![Absorbance spectra of (A) Fe\textsuperscript{III}(TPPS) (B) Fe\textsuperscript{II}(TPPS) and (C) Fe\textsuperscript{II}(TPPS)CO in aqueous solution.](image)

**Figure 4.** Absorbance spectra of (A) Fe\textsuperscript{III}(TPPS) (B) Fe\textsuperscript{II}(TPPS) and (C) Fe\textsuperscript{II}(TPPS)CO in aqueous solution.

To make “acidic water” add a few drops of 6M HCl to 50 mL of D.I. water.

Dip the tip of a Pasteur pipette into the hot solution to obtain a small sample for spectroscopic analysis.
Laser flash photolysis

When Fe$^{ll}$(TPPS)CO absorbs light of sufficient energy the Fe-CO bond cleaves resulting in free CO and Fe$^{ll}$(TPPS). In the presence of an excess of CO, the starting material is rapidly reformed. Using the pump-probe technique of laser flash photolysis, the transient absorbance spectrum of this system can be recorded. In this situation the starting material as well as the expected transient intermediate (Fe$^{ll}$(TPPS)) can be isolated and the spectra recorded independently (figure 4). Upon photolysis one expects a decrease in absorbance due to loss of Fe$^{ll}$(TPPS)CO and an increase in absorbance due to formation of Fe$^{ll}$(TPPS). The expected transient absorbance should look like the difference spectrum of Fe$^{ll}$(TPPS) – Fe$^{ll}$(TPPS)CO as shown in figure 5.

\[\text{Abs} = \log \frac{I_o}{I} \]  

Figure 5. Difference spectrum of Fe$^{ll}$(TPPS) - Fe$^{ll}$(TPPS)CO in the Soret band region.

In a time resolved optical (TRO) pump-probe experiment a sample is perturbed with a laser source (the pump) and the resulting changes are monitored using a light source and photodetector (the probe). By monitoring the probe as a function of time and at various wavelengths the time-resolved absorbance spectrum can be obtained. Additional kinetics information can be obtained if the experiment is repeated using a range of temperatures and CO concentrations. The data is collected as the voltage reading from a photomultiplier tube (PMT). This signal must be converted to absorbance to display the correct units. Absorbance is defined in equation 1 below;

where \( I_o \) is the intensity of light without a sample and \( I \) is the intensity of light with a sample. In this experiment, \( I_o \) is the signal before photolysis and \( I \) is the signal after photolysis. A digital storage oscilloscope (DSO) measures the PMT signal continuously so it is

possible to collect data prior to the laser flash. This “pre-trigger” data is used to define the value for $I_0$.

**WARNING!** The laser you will be using is a high power pulsed Nd:YAG laser. The UV (355 nm) light output can be very dangerous for eyes and skin. Laser safety goggles must be worn at all times in the laboratory. Failure to follow safety guidelines will result in a failing grade. Prepare a solution of Fe$_{II}$(TPPS)CO in a septum topped cuvette as described in the synthesis procedure above. Set the monochromator on the TRO instrument to 430 nm and collect a time response trace using the LabView program “Scope”. Use the two cursors to define the $I_0$ region (see figure 6) and press the “convert to abs” button. The data will now be in units of Δabs. Record the transient absorbance over the range of 400-450 nm. Record data every 2 nm. Be sure to save your data at each wavelength with the “Reduced data set” box checked. This reduces the number of data points from 5120 to 204.

![Figure 6](attachment:figure6.png)

**Figure 6.** Transient signal showing the $I_0$ and $I$ regions used to convert the signal from mV to ΔAbs. units.

**Data Analysis**

Now that all of the data has been collected is must be processed for display. Each data file needs to be combined to generate the three dimensional data set. The data should be arranged with the time data in the first column and the wavelength data in columns after.

<table>
<thead>
<tr>
<th>time</th>
<th>400nm</th>
<th>402nm</th>
<th>404nm</th>
<th>...</th>
<th>450nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1.12E-04</td>
<td>1.17E-02</td>
<td>1.47E-02</td>
<td>1.16E-02</td>
<td>1.57E-02</td>
<td>1.69E-02</td>
</tr>
<tr>
<td>-1.09E-04</td>
<td>-7.01E-03</td>
<td>-6.01E-03</td>
<td>7.41E-03</td>
<td>-7.01E-03</td>
<td>5.9E-03</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>5.00E-07</td>
<td>1.09E-02</td>
<td>1.11E-02</td>
<td>1.19E-02</td>
<td>1.29E-02</td>
<td>1.58E-02</td>
</tr>
</tbody>
</table>
You should eliminate the pretrigger data by deleting all of the rows in which the time values are negative. Insert a new row above all of your data and label each column with the appropriate wavelength. In the end your data should be arranged like the schematic below. Note: This arrangement may need to be altered depending upon your data analysis software. The described arrangement is suitable for Excel.

<table>
<thead>
<tr>
<th>empty cell</th>
<th>wavelength data</th>
</tr>
</thead>
<tbody>
<tr>
<td>time data</td>
<td>Absorbance data</td>
</tr>
</tbody>
</table>

Using a graphical analysis program of your choice construct the 3D surface plot with this data set. Excel, Mathematica, SigmaPlot are a few programs that can plot this type of data. Refer to the manual or help files for each program for more specific information. An example 3D plot is shown in Figure 7. The plot should be rotated so that data on the wavelength and time axis are visible.

![3D plot](image)

**Figure 7.** Plot of the transient absorbance spectrum of FeII(TPPS)CO in the Soret band region.

An alternative way to present this data is in the form of a stacked plot as shown in Figure 8. Both plots present the data in a similar fashion. Use whichever format you judge best.
Figure 7. Stacked plot of the transient absorbance spectrum of Fe(TPPS)CO. Spectra are reported every 4 μsec from time = 0 to 160 μsec.
Introduction

Becoming proficient with a computer assisted data analysis program is a very important skill to acquire. There are a wide variety of programs available including Mathematica, MathCad, Origin, SigmaPlot, Excel etc... An introduction to using Excel is provided here because this program is widely available and installed on all of the computers in the upper division laboratory.

Excel is a spreadsheet program used for the analysis of large (or small) data sets. It is primarily targeted towards business users but can be used effectively in the chemistry laboratory. This appendix will present a very brief tutorial on the basic capabilities of Excel required for the Chemistry 116 Lab class. Refer to the Excel help files for detailed information.

Working with Data

Before entering data into a spreadsheet a little thought should be given as to the arrangement of the data. A typical use of Excel in lab is to store xy data sets (time vs. absorbance, absorbance vs. wavelength etc...) This type of data is best arranged in columns. For example, date representing a UV-Visible spectrum would have wavelenth in the first column and absorbance in the second. The formatting of data (text, numbers, dates, currency etc..) can be set to your personal preferences. Select the data you wish to format and under the Format menu select Cells. The dialog box that comes up lists many different available formats. For instance you can choose to show only one decimal place for all values. Customizing the formatting in this way can make it easier to view data on the screen. Individual cells are named according to which row and column they are in. By default excel labels columns with letters and rows with numbers. The data in the first column and first row is referred to as A1.

Formulas

The power of Excel comes from it's ability to perform complex calculations on an active spreadsheet. This means that you can enter formulas for the manipulation of experimental data that update when the data changes. In order for excel to differentiate between a formula and text, the equal sign, =, is used to let the software know that what follows is a formula. All of the normal math functions are available. For example, if you wish to add 5 and 9 you would simple click on any cell and type =5+9 , the cell would then display 14. Or if you would like to multiply the value in cell A10 by the value in cell B12 you would enter =A10*B12. Parenthesis are used the same way you would use them in algebra. For example, =(A10*B12)+C2 is different from =A10*(B12+C2). The following table lists some common functions and how to enter them in a spreadsheet.
### Arithmetic Operator

<table>
<thead>
<tr>
<th>Operator</th>
<th>Meaning</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Addition</td>
<td>=3+3</td>
</tr>
<tr>
<td>-</td>
<td>Subtraction</td>
<td>=3-1</td>
</tr>
<tr>
<td>-</td>
<td>Negation</td>
<td>=-1</td>
</tr>
<tr>
<td>*</td>
<td>Multiplication</td>
<td>=5*8</td>
</tr>
<tr>
<td>/</td>
<td>Division</td>
<td>=7/3</td>
</tr>
<tr>
<td>^</td>
<td>Exponentiation</td>
<td>=4^3</td>
</tr>
<tr>
<td>E or e</td>
<td>Scientific Notation</td>
<td>=3.1E-3</td>
</tr>
<tr>
<td>Sqrt()</td>
<td>Square root</td>
<td>=sqrt(9.1)</td>
</tr>
<tr>
<td>π</td>
<td>Value of π</td>
<td>=PI()</td>
</tr>
<tr>
<td>sin(), cos(), tan(), etc.</td>
<td>Trigonometry functions*</td>
<td>=sin(2*PI())</td>
</tr>
</tbody>
</table>

*Note: The default angles are in radians.

### Selecting Data

A basic task is selecting individual data points or data ranges listed in other cells. Cells are referenced in formulas according to the table below.

<table>
<thead>
<tr>
<th>To refer to...</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>The cell in column A and row 10</td>
<td>A10</td>
</tr>
<tr>
<td>The range of cells in column A and rows 10 through 20</td>
<td>A10:A20</td>
</tr>
<tr>
<td>The range of cells in row 15 and columns B through E</td>
<td>B15:E15</td>
</tr>
<tr>
<td>All cells in row 5</td>
<td>5:5</td>
</tr>
<tr>
<td>All cells in rows 5 through 10</td>
<td>5:10</td>
</tr>
<tr>
<td>All cells in column H</td>
<td>H:H</td>
</tr>
<tr>
<td>All cells in columns H through J</td>
<td>H:J</td>
</tr>
</tbody>
</table>

The above reference formats are all relative. That is, if you copy a formula in column C containing a reference to cell A10 to column D the reference in the formula is changed to B10. If you want a reference to be absolute (i.e. one that doesn’t change when you copy the formula to another location) use the reference form $A$S10. This is useful when a constant number is required in a series of calculations.
Statistical analysis of experimental data sets can provide useful information. For example, if we have a data set in column A that consists of 15 measurements. We can obtain the following values:

<table>
<thead>
<tr>
<th>Value desired</th>
<th>Formula to enter</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>=AVERAGE(A1:A15)</td>
</tr>
<tr>
<td>standard deviation</td>
<td>=STDEV(A1:A15)</td>
</tr>
<tr>
<td>range</td>
<td>=MAX(A1:A15)-MIN(A1:A15)</td>
</tr>
<tr>
<td>median</td>
<td>=MEDIAN(A1:A15)</td>
</tr>
<tr>
<td>student’s t*</td>
<td>=TINV(alpha, DOF)</td>
</tr>
<tr>
<td>number of data points</td>
<td>=COUNT(A1:A15)</td>
</tr>
<tr>
<td>highest value</td>
<td>=MAX(A1:A15)</td>
</tr>
<tr>
<td>lowest value</td>
<td>=MIN(A1:A15)</td>
</tr>
</tbody>
</table>

* To return the appropriate student’s t value “alpha” is equal to 0.05 for t at 95% confidence, 0.1 for a 90% confidence and so on. DOF is the appropriate integer representing the degrees of freedom for a given data set. For example, if you are calculating the confidence interval for a data set of five values, the value for student’s t at 95% confidence is found by entering = TINV(0.05,4). The resulting cell will display 2.776. The value in this cell can now be used to calculate the confidence interval.

Using these basic functions we can calculate the relative standard deviation (RSD) (standard deviation / mean) and the coefficient of variance (COV) (RSD * 100).

### Basic Plotting

Arrange your data so that the x values are in one column and your y values are in another. For example x values are in column A1:A50 and the y values are in column B1:B50. Select this data range, i.e. click and drag your mouse to select the range A1:B50. Perform the following to create a basic plot.

1. From the Insert tab, click on the Scatter icon (Choose the type of chart you’d like to create, i.e. symbols only, lines only etc...)

2. To add axis labels, click on the chart and then click on the Layout tab. There will be an icon for axis titles. Click on this and select the Primary Horizontal and Primary Vertical axis styles you prefer.
Many items can be customized on the plot, including the marker sizes, shapes and colors and line styles, widths and colors. If you right click on one of your data points and select Format Data Series, a dialog box will come up with options to change all these attributes. Alternatively you can click on one of your data points to select them and from the Format menu select Selected Data Series to bring up the same options box.

Adding a Least Squares Fit to your data

Often times a best fit to a line is needed for data analysis. This can be accomplished with Excel as well. For example we have the above data plotted and we wish to fit this data to a straight line. Right click on a data point and select Add Trendline. A dialog box will come up asking what type of fit you would like. Several options are available, choose Linear. Uncheck the Display Equation on Chart check box. It is good practice to leave this information off of the actual plot.

You can forecast the trendline forward or backward if you would like to extend the best fit line past the range of your data. For example, the above chart is forecast forward and backward by 5 units.
Often times the slope and/or intercept value are needed in other calculations. These values can be obtained as a spreadsheet function for use in other calculations. As above let's assume we have our \( x \) values in column A1:A50 and our \( y \) values in column B1:B50.

<table>
<thead>
<tr>
<th>Desired Value</th>
<th>Enter</th>
</tr>
</thead>
<tbody>
<tr>
<td>slope</td>
<td>( =\text{slope}(B1:B50, \text{A1:A50}) )</td>
</tr>
<tr>
<td>( y )-intercept</td>
<td>( =\text{intercept}(B1:B50, \text{A1:A50}) )</td>
</tr>
</tbody>
</table>

Note the format in each case is function(\( y \) values, \( x \) values)

The resulting values can now be used in further calculations as required.