The Synthesis and Characterization of Ruthenium Polypyridyl complexes

Introduction

Photo-induced electron transfer reactions in metal complexes play an important role in inorganic chemistry. 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)]\(^{2+}\), (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)]\(^{2+}\) utilizes Ru(DMSO)\(_4\)Cl\(_2\) as a precursor, since it binds bidentate ligands more efficiently than readily available starting materials, such as RuCl\(_3\)·xH\(_2\)O. Once purified, the Ru(DMSO)\(_4\)Cl\(_2\) is refluxed in ethanol with 2,2'-bipyridine in the proper molar ratio to generate [Ru(bpy)]\(^{2+}\)Cl\(_2\). For this experiment several polypyridyl ligands will be available for you to choose from. Following its synthesis, [Ru(bpy)]\(^{2+}\)Cl\(_2\) will be characterized by UV-vis and fluorescence spectroscopy, as well as cyclic voltammetry.

Experimental

Synthesis of Ru(DMSO)\(_4\)Cl\(_2\). Place 0.50 g RuCl\(_3\)·3H\(_2\)O 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\(_3\))\(_2\)SO) 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. 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 the crystals to dry then weigh to calculate a percent yield. Determine the melting point and obtain an infrared spectrum to locate the absorption frequency of the S-O band.

Synthesis of [Ru(L)]\(_2\)(PF\(_6\))\(_2\). [Ru(L)]\(_2\)(PF\(_6\))\(_2\) is synthesized from Ru(DMSO)\(_4\)Cl\(_2\) by refluxing with a polypyridyl ligand (L), in a stoichiometric ratio slightly less than 1:3, i.e. a slight excess of ligand. The choice of ligands is presented in figure 1 below. Please note that there is a limited amount of certain ligands. Check your calculations with your TA before proceeding.

A 100 mL round-bottom flask is equipped with a magnetic stirring bar, into which 0.5 g of Ru(DMSO)\(_4\)Cl\(_2\) and an appropriate amount of ligand are placed. 25 mL of reagent grade ethanol are then added to the flask. The mixture is heated to reflux, with continuous stirring overnight. The solution changes in color from yellow to dark red.

To isolate the product, the solution is dried by rotary evaporation. The solid is collected by vacuum filtration and washed with toluene. 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)]\(_2\)Cl\(_2\) to its hexafluorophosphate (PF\(_6\)) salt by metathesis. The [Ru(L)]\(_2\)Cl\(_2\) is heated in a beaker with 10 mL of distilled water. In a separate beaker, a five-fold molar excess of ammonium hexafluorophosphate (NH\(_4\))PF\(_6\) is heated in 10 mL of distilled water. Check your calculations with your TA before weighing the NH\(_4\))PF\(_6\). When both solutions are warm (not boiling), the NH\(_4\))PF\(_6\) solution should be
slowly added to the [Ru(L)]Cl₂ solution. The color of the solution changes from red to orange. Continue to gently heat the resulting solution for 5 minutes (be careful not to bring the solution to a boil). Cooled 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.

**Characterization**

**Spectroscopic Characterization.** Obtain a UV-Vis spectrum of Ru(L)₃²⁺ in acetonitrile solution and calculate the extinction coefficient of the MLCT band at λ_{max} (400-500 nm). Knowing 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.

![Figure 2](image2.png)

**Figure 2.** Absorption and emission spectrum of Ru(bpy)₃²⁺ 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.

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).³

![Figure 3](image3.png)

**Figure 3.** Energy level diagram depicting the triplet to singlet phosphorescence transition (E_{00} energy).

In a later experiment we will need to obtain the E_{00} energy for the triplet state in the ruthenium complex. The E_{00} 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”.⁴ That is, the point at which the emission intensity on the high energy side is 10% of that at λ_{max} (see figure 4). Convert this wavelength into eV for use later (Remember that E = hc/λ and 1 eV = 1.6022 × 10⁻¹⁹ J).

![Figure 4](image4.png)

**Figure 4.** Luminescence spectrum of Ru(bpy)₃²⁺ in acetonitrile solution. The E_{00} energy is estimated to be where the emission intensity is 10% that of the emission at λ_{max}.

**Cyclic Voltammetry.** You will be performing the electrochemical measurements in acetonitrile (CH₃CN) 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. Add 15-20 mL of this solution to a clean and dry 100 mL beaker 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 10-15 minutes using a stainless steel needle. Stop the stirring and remove the needle from the liquid but keep it suspended above the solution.

Record a blank cyclic voltammogram of the electrolyte solution over the range of +2.0 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 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 voltammogram of phenothiazine (PTZ) and N,N,N',N'-Tetramethyl-p-phenylenediamine (TMPD). Record the signal 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 below. Record the E_{1/2} value in your notebook for use in a later experiment.

Figure 5. Cyclic voltammogram of Ru(phen)$_3^{2+}$ in acetonitrile solution with 0.1 M TBAH supporting electrolyte. The arrow indicates the direction of the scan.

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 below in figure 6.

Figure 6. Cyclic voltammogram of phenothiazine in acetonitrile solution.

**Differential Pulse Voltammetry.** Reduction and oxidation potentials can also be measured using differential pulse voltammetry. In this method a saw-tooth potential is applied giving rise to greater sensitivity. Refer to your analytical text for more information regarding electrochemical measurements. A 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 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}).
Figure 8. Differential pulse polarogram of Ru(phen)$_3^{2+}$ in acetonitrile solution with 0.1 M TBAH supporting electrolyte. The arrow indicates the direction of the scan.

References and Notes


2. A ratio of 3.6 moles of ligand per 1 mole of Ru(DMSO)$_4$Cl$_2$ should be used.


5. Working electrode (green), reference electrode (white), counter electrode (red). The working electrode is glassy carbon and the reference is Ag/AgCl.

6. The gas from the needle should not disturb the surface of the solution. A slow flow should gently blanket the cell with inert gas.

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)]²⁺. This process is diffusion controlled (k_{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.

Theory

Intensity measurements, time resolved emission and luminescence quenching

The 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.

\[
\begin{align*}
A + \text{hv} &\rightarrow A^* \quad \text{(excitation process)} \\
A^* &\rightarrow A + \text{hv} \quad \text{(radiative)} \\
A^* &\rightarrow A + \text{heat} \quad \text{(non-radiative)} \\
A^* + Q &\rightarrow A + Q^* \quad \text{(quenching)} \\
A^* + Q &\rightarrow A^- + Q^+ \quad \text{(reductive quenching)}
\end{align*}
\]

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.

Steady-state approximations assume 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:

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

[1]

Where \( I_0 \) is the integrated intensity of emission without a quencher present, \( J \) is the integrated 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, \( k_D \sim 10^{10} \text{s}^{-1} \).

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.

\[
\frac{\tau_0}{\tau} = 1 + k_{SV}[Q]
\]

[2]

Notice that in each case [1] and [2] the equation is in the form of straight line. If the ratio \( I_0/J \) vs. \( Q \) 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 in which two overlapping potential energy surfaces cross as shown in figure 1 below. The electron transfer reaction can occur through an inner sphere or outer sphere mechanism as in equations 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. \( \Delta G = 0 \).
Figure 1. Reaction coordinate diagrams for electron transfer when A) $\Delta G = 0$ and B) $\Delta G < 0$.

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

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

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 above. In such a case the electron transfer rate, equation 5, 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.

$$k_{ET} = A \exp \left[ \frac{(-\Delta G^0 + \lambda)^2}{4\lambda k_BT} \right]$$

As with a symmetrical electron transfer, the frequency factor, $A$, is related to frequency of the system reaching the crossing point. The value for $\lambda$ 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.

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

Experimental

All measurements will be conducted in acetonitrile solutions under oxygen free conditions (oxygen acts as a quencher molecule). The samples are sparged by inserting a needles into the septa of the cell and slowly bubbling $N_2$ through the samples. Sparging times of 3-5 min are sufficient for 2-3 mL of solution.

Prepare 100 mL stock solutions of $\sim$1.0 mM phenothiazine (PTZ) and $N,N,N',N'$-Tetramethyl-p-phenylenediamine (TMPD). Be sure to record the exact concentration 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 $\sim$0.1 mM after dilution. 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. Use the following table as a guide to prepare these solutions.
Table 1. Solutions for Stern-Volmer kinetics analysis.

<table>
<thead>
<tr>
<th>Flask</th>
<th>Ru(L)$_2^{2+}$ (mM)*</th>
<th>[PTZ] (mM)</th>
<th>[TMPD] (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>0</td>
<td>0.100</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>0</td>
<td>0.250</td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
<td>0</td>
<td>0.500</td>
</tr>
<tr>
<td>5</td>
<td>0.2</td>
<td>0</td>
<td>1.000</td>
</tr>
<tr>
<td>6</td>
<td>0.2</td>
<td>0.100</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0.2</td>
<td>0.250</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0.2</td>
<td>0.500</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0.2</td>
<td>1.000</td>
<td>0</td>
</tr>
</tbody>
</table>

* approximate concentration. Use an amount of ruthenium complex to give an appropriate absorbance.

Table 1: Solutions for Stern-Volmer kinetics analysis.

Please note: There are a limited number of volumetric flasks. 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. 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 $\lambda_{\text{max}}$ 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 always 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.

Data Analysis

Using EXCEL or another data analysis program (IGOR, SIGMA PLOT, KALEIDAGRAPH etc...) plot $I_0/I$ and $\tau_0/\tau$ vs. [PTZ] and [TMPD]. For each experiment fit the data to a linear least squares regression and record the slope of each line. From these results determine the second order bimolecular quenching constant $k_q$.

Include in your report the plots generated above, the values for the $k_q$ from each experiment and answer the following questions:

Are the two results in agreement?

Which is the more accurate measurement. Why?

Compare your results to literature values. Quenching rate constants can be found at The Radiation Chemistry Data Center of the Notre Dame Radiation Laboratory

www.rcdc.nd.edu/compilations/Quench/intro.htm

Not all complexes will have data online.

Class data will be compiled and posted on the course website for analysis. Send Dr. Laverman your group results, include the name of the complex you synthesized, the value for $\tau_0$, and $\Delta G_{el}$ and $k_q$ for each reaction (PTZ and TMPD).

Calculating the free energy change for electron transfer - $\Delta G_{el}$

The following table contains example values for calculations. Use the values you measured in the laboratory. The red 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>Triplet Energy (from emission data)</th>
<th>$E^{00}$</th>
<th>2.16 eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground State reduction potential (Ru$^{2+/+}$)</td>
<td>GS</td>
<td>-1.38 V</td>
</tr>
<tr>
<td>Excited State reduction potential (*Ru$^{2+}$)</td>
<td>ES</td>
<td>+0.78 V</td>
</tr>
<tr>
<td>Quencher reduction potential (PTZ$^{0+}$)</td>
<td>Q</td>
<td>+0.53 V</td>
</tr>
<tr>
<td>Potential for electron transfer $ES - Q$</td>
<td>$E_d$</td>
<td>+0.25 V</td>
</tr>
<tr>
<td>Free energy change for electron transfer $-nF \Delta G_{el}$</td>
<td>$\Delta G_{el}$</td>
<td>-24.1 kJ mol$^{-1}$</td>
</tr>
</tbody>
</table>

One step beyond

There is some work that takes place during the electron transfer process. 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

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

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

**Calculating diffusion rate constants**

The Debye equation can be used to estimate the diffusion rate in solutions when one or more of the reacting species is uncharged.

\[
k_d = \frac{8RT}{3000\eta}
\]

where \( \eta \) is the viscosity of the solvent.

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 lose 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_f), 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

\[ A^* + Q \xrightarrow{k_q} A + Q^* \] (quenching)

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, \( k_D \sim 10^{10} \text{ s}^{-1} \). This relations can be used to quantitatively determine the concentration of a quencher molecule if \( k_{SV} \) has been determined previously.

Lock-in amplification is a technique that can be used to isolate signals in the presence of (potentially) high levels of noise. The method involves modulating the signal by some reference frequency (\( \omega_{ref} \)) then detecting and amplifying the signal (\( \omega_{sig} \)). 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 2.
Figure 2. The product of two sinusoidal waves with 20 Hz and 18 Hz frequencies.

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. \(^1\)

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

A plot of these the sum and difference frequencies is shown in figure 3. 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 3. The difference (blue) and sum (red) frequencies for 20 Hz and 18 Hz input signals.

The time constant determines the frequencies which are allowed to pass through the filter unattenuated. 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 4.

Figure 4. Low pass filter (RC = 1 second) applied to the signals given in figure 2. 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 5 using a 1 second time constant.
Figure 5. 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 6, 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 6. 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.

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.

Experimental²

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 an 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 it’s 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 7.
Figure 7. Oxygen sensing apparatus constructed with a screwcap cuvette. F1 = highpass filter, F2 = lowpass filter.

Assemble the apparatus shown in figure 7 as instructed by your TA. Pass a stream of N\textsubscript{2} over the film and observe the changes in emission intensity. Pass an air (vacuum) stream over the sample after the signal stabilizes. You may also pass O\textsubscript{2} over the film and observe the changes. The response should resemble that shown in figure 8 below.

Figure 8. Response of a thin film oxygen sensor upon exposure to nitrogen and oxygen gas.

Sensor films may be calibrated by passing known mixtures of N\textsubscript{2} and O\textsubscript{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\textsubscript{2}. Plot the ratio of the signal under pure nitrogen to that under N\textsubscript{2}/O\textsubscript{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\textsubscript{2} and will be lower than expected if the overall pressure in the cell is low. A typical calibration curve is shown in Figure 9.

Figure 9. Calibration curve for a thin film oxygen sensor.

Data Analysis

1. 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.
2. Does the response time change with film thickness?
3. Do different ruthenium dyes have different response times?
4. Are the responses similar for each dye? If there are differences propose an explanation.
5. Suggest ways to quantify the amount of oxygen using your sensor film.
6. Why is the calibration curve nonlinear?

\begin{equation}
\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}}))
\end{equation}

1. This expression does not take into account differences in phase. The complete expression is below