Fluorescence Spectrophotometry

I. Introduction

Ru(bpy)$_3^{2+}$, where bpy = 2, 2' bipyridine, has been one of the most widely studied metal complexes in recent years. Interest in this compound is due to its very interesting spectroscopic and electrochemical properties, including its use as a sensitizer in solar energy induced conversion of water into useful fuels (hydrogen and oxygen). Absorption of visible light by Ru(bpy)$_3^{2+}$ leads to the formation of the charge transfer excited state $^*\text{Ru(bpy)}_3^{2+}$ (eq. 1).

$$\text{Ru(bpy)}_3^{2+} + \text{hv} \rightarrow ^*\text{Ru(bpy)}_3^{2+} \quad (1)$$

This excited state undergoes radiative decay (primarily phosphorescence) back to the ground state due to its interaction with solvent molecules, as described in eq. 2. Note: The light emitted will be of lower energy (higher wavelength) compared to the light absorbed (shorter wavelength).

$$^*\text{Ru(bpy)}_3^{2+} \rightarrow \text{Ru(bpy)}_3^{2+} + \text{hvN} \quad (\text{emission intensity } I_0) \quad (2)$$

However, if another species called a quencher (in this case molecular oxygen O$_2$) is present, the excited state will decay to the ground state more rapidly because an additional deactivation path (eq. 3) will be available. Consequently, the emission intensity will decrease.

$$^*\text{Ru(bpy)}_3^{2+} + \text{O}_2 \rightarrow \text{Ru(bpy)}_3^{2+} + ^*\text{O}_2 + \text{hvN} \quad (\text{emission intensity } I) \quad (3)$$

Fluorescence spectroscopy will be used to determine the rate constant for the quenching of $^*\text{Ru(bpy)}_3^{2+}$ when molecular oxygen is present as a quencher. The emission intensity will decrease from $I_0$ (no quencher present) to different values ($I$) when quencher is present in varying concentrations. Any decrease in emission intensity with an increase in quencher concentration is related by the following equation.

$$I_0/I = 1 + K_{sv}[O_2] \quad (4)$$

Equation 4 is known as the Stern-Volmer equation, and $K_{sv}$ is called the Stern-Volmer constant. The value of $K_{sv}$ can be obtained from the slope of a $I_0/I$ vs. [O$_2$] plot. The quenching rate constant $k_q$ is related to $K_{sv}$ by the expression $k_q = K_{sv}/\tau^0$. Here $\tau^0$ is the lifetime of the excited state. The value of $\tau^0$ for $^*\text{Ru(bpy)}_3^{2+}$ in 0.1 M NaCl aqueous solution is 600 ns.
II. Equipment

A. Perkin Elmer Model LS-50B Luminescence Spectrophotometer with computer interface
B. Hewlett Packard Model 8453 UV-VIS Diode Array Spectrophotometer with Chemstation computer interface
C. 1-cm² fluorescence cell (check out from the instructor) (Be careful; It is very expensive!)
D. matched pair of quartz cuvets (obtain from the instructor)
E. two 10-mL volumetric flasks
F. 50-mL volumetric flask (check out from the stockroom)
G. 5-mL adjustable autopipet (obtain from the instructor)

III. Reagents

A. NaCl (high purity)
B. 5.0 x 10⁻⁵ M [Ru(bpy)₃²⁺]Cl₂·5H₂O (stock solution) in deionized water
C. high-purity N₂ and O₂ gases

IV. Procedure

A. Sample Preparation

A. To prepare the sample solution, weigh out the necessary mass of NaCl to prepare 50 mL of 0.10 M NaCl solution. Transfer the NaCl to a 50-mL volumetric flask using 5-10 mL of deionized water to effect the transfer. Pipet 5.00 mL of stock Ru(bpy)₃²⁺ solution and transfer it to the 50-mL flask containing the NaCl. Dilute the sample to the mark with deionized water. Calculate the concentration of Ru(bpy)₃²⁺ in the sample solution. Also, calculate the expected absorbance at 450 nm for this solution assuming a cell pathlength of 1.0 cm and ε₄₅₀ = 14,500 M⁻¹cm⁻¹ for Ru(bpy)₃²⁺.

B. Check out a quartz fluorescence cell from the instructor. Carefully clean the cell with deionized water and rinse it with 1-2 mL of the sample solution prepared above. Fill the fluorescence cell with this unpurged solution (~4.00 mL), cap it and gently place it in the sample compartment of the instrument when the instrument is ready. Close the lid of the sample compartment.

B. Solution Purging

1. Rinse two 10-mL volumetric flasks with 2-3 mL of sample solution. Then fill each to the mark with sample solution.

2. Bubble N₂ gas for 20 minutes through the solution in one flask ([O₂] = 0.0). Simultaneously bubble O₂ gas for 20 minutes through the solution in the second flask ([O₂] = 1.30 x 10⁻³ M).

3. While you are bubbling N₂ and O₂ gases through the two solutions, turn on the luminescence spectrophotometer computer and the printer to let them warm up for 15 minutes. When the computer boots up, double click on the FL WinLab icon.
C. Emission Intensity Measurements

1. Select 422l.mth from the Methods Selection list and double click. Select the SINGLE SCAN MODE (next to the Traffic Lights icon) and click on the SET PARAMETERS tab. Verify that the excitation wavelength scan range is 400 to 500 nm and the slit width is 5.0. Verify that the emission wavelength scan range is from 550 to 650 nm and the slit width is 12.0. Set the scan rate to 240 nm/min. In the RESULT FILENAME box, the default filename is xxfluor.sp. Replace the “xx” in the default filename with your initials and the “fluor” with the unique name you wish for this sample. Data are saved automatically under the file name you specify here. Use an unique filename for each run and one that is indicative of the sample measured.

2. Click on the Green Traffic Light icon to initiate the Prescan. After the Prescan is complete, record the excitation and emission maxima in your notebook. (The value is typically between 455-460 nm for excitation and 590-595 nm for emission.)

3. Remove the sample from the instrument, empty the cell, clean with deionized water and rinse with 1-2 mL of the nitrogen-purged sample. Completely fill the fluorescence cell with this solution (no air bubbles should remain), cap it and gently place it in the sample compartment of the instrument. Close the lid of the sample compartment. Click on the SETUP PARAMETERS tab, then click on the EMISSION BUTTON and ensure that the following parameters are set.

   Wavelength scan range: 550 to 650 nm
   Excitation wavelength: use value found in Prescan
   Scan speed: 240
   Excitation slit width: 5.0   Emission slit width: 12.0
   Result filename: xxfluor.sp where xx are your initials and fluor is the unique name given this sample.

When these parameters are set, click on the Green Traffic Light icon to initiate the scan. At this point the prescan excitation spectrum will be displayed on the left of the screen and the prescan emission spectrum will be displayed at longer wavelengths on the right side. The fluorescence spectrum for each successive sample that is run will be displayed as well. Each spectrum is color coded and linked to the unique filename (located in the lower left corner of the screen) used to save the data.

4. When the scan is complete, determine the point of maximum intensity (ordinate max) for your sample fluorescence spectrum by clicking on the icon next to the sample filename. Record this intensity.

   The maximum intensity value in the emission spectrum of the N₂-saturated solution is I₀. The values of I are determined relative to I₀ using solutions containing different concentrations of oxygen by following the procedure given below.
5. Repeat steps IV.C.3 and IV.C.4 for your original sample (air-saturated solution) and the oxygen-saturated solution.

6. Prepare two mixtures as follows.
   a. Mixture 1 \((N_2 + O_2)\)
      Transfer the remaining \(N_2\)-saturated and \(O_2\)-saturated \(\text{Ru(bpy)}_3^{2+}\) solutions to separate, clean 6-mL vials and continue to bubble the appropriate gas through each solution for another five minutes using parafilm to keep the vial partially closed. Then quickly mix 2.2 mL of each solution in another clean 6-mL vial and rapidly fill the fluorescence cell, quickly close and cap the cell. Record the emission spectrum and determine the maximum emission intensity as described earlier.
   
   b. Mixture 2 \((\text{Air} + O_2)\)
      Prepare this solution in the same fashion as was done for Mixture 1 using the \(\text{air-saturated}\) and \(O_2\)-saturated solutions. Follow the procedure described earlier for making the emission intensity measurements.

8. When all of the data have been collected, select NEW GRAPH WINDOW from the VIEW menu. Select ADD CURVE from the VIEW menu. Overlay all of your scans by clicking on the filenames while pressing the CTRL key. Print a copy of your overlay graph. Make sure each curve is labeled.

9. Use the Hewlett Packard Model 8453 UV-VIS diode array spectrophotometer to obtain the visible absorption spectrum of your air-saturated \(\text{Ru(bpy)}_3^{2+}\) solution in the range 400-700 nm. Identify the wavelength of maximum absorption.

V. Treatment of Data

A. Calculate the concentration of oxygen (to at least 3 significant figures) in each solution. You will need the exact oxygen content of air. Assume that the air concentration in the air-saturated solution equals \(1.30 \times 10^{-3}\) M.

B. Plot \(I/I_0\) vs \([O_2]\) and determine the equation for a linear least-squares fit to the data. Report the values of \(K_{sv}\), the y-intercept and the second-order rate constant \(k_q\), in appropriate units.

VI. Questions

1. How does the value obtained for the excitation maximum obtained in the Prescan in IV.C.1 compare to the wavelength of maximum absorption found in IV.C.9?

2. How does the observed absorbance at 450 nm compare to that calculated in step IV.A?

3. Why must the fluorescence cell be completely filled with solution for all samples but the original solution?
Lab Cautions for Fluorescence Spectrophotometry

1. HANDLE THE FLUORESCENCE CELL WITH EXTREME CARE AT ALL TIMES. When you are finished with the cell, thoroughly rinse the cell and return it to your lab instructor.

2. Do not save any modifications to 322I method under the filename 322I.mth.

3. Before you leave the lab, turn off the luminescence spectrophotometer.

4. Close the valves to the N₂ and O₂ purge gas tanks before you leave.