

**LAB #3: FLUORESCENCE SPECTROSCOPY AND ELECTRON TRANSFER**

(This lab is adapted from the U of MN Physical Chemistry lab manual)

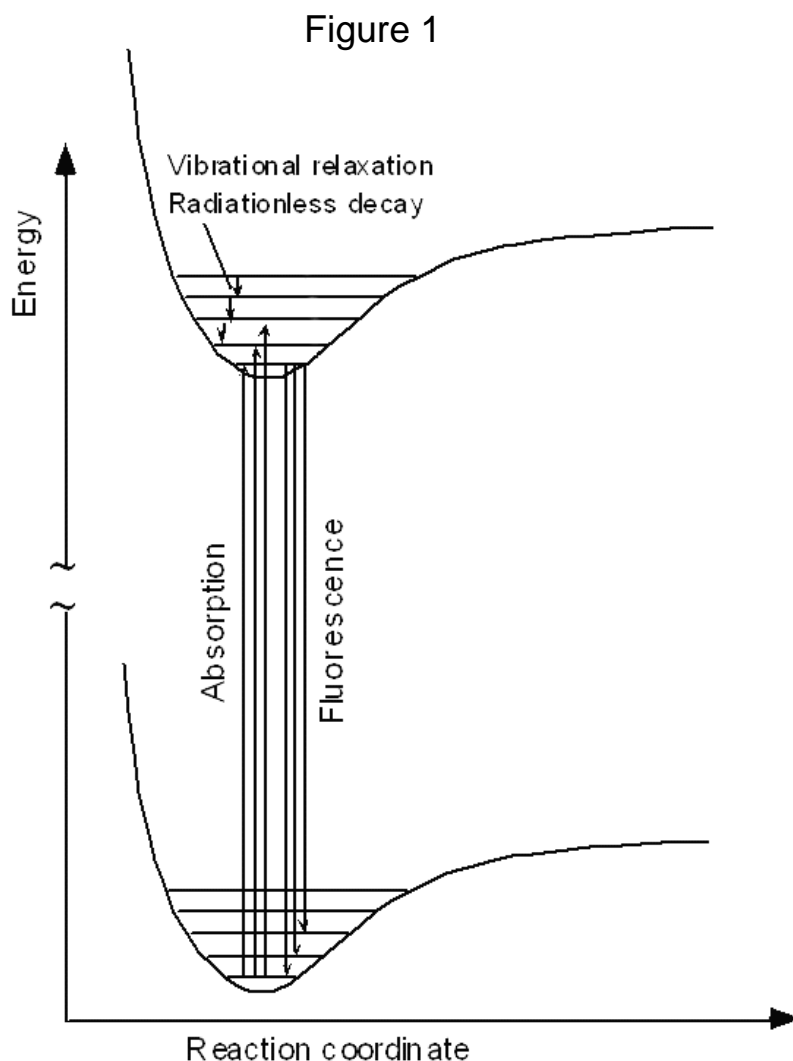
**A. Purpose**

In this experiment, you will observe the quenching of fluorescence emission by  $[\text{Ru}(\text{bipyridyl})_3]^{+2}$  that occurs upon transfer of electrons to ferric and cupric ions. You will determine the rate of electron transfer using a Stern-Volmer plot, and will compare your experimental value to a theoretical value calculated using the Marcus equations.

**B. Background and Theory**

When a molecule absorbs light energy of a certain wavelength, it enters an excited electronic state, and relaxes back to the ground state by emitting light or heat. Fluorescence is a way by which molecules electronically excited at a wavelength relax by emission of light of a longer wavelength (lower energy) than was originally absorbed. Figure 1 presents a schematic for the excitation and relaxation of molecules due to absorbance and emission of light energy. Molecules are promoted from a certain vibrational level within the ground electronic state to various vibrational levels in the excited electronic state. This electronic transition takes about  $10^{-15}$  seconds. Through a mechanism called "radiationless decay," the

molecules relax to the lowest vibrational state in the excited electronic state on a time scale of  $10^{-12}$  to  $10^{-15}$  seconds. This preliminary relaxation occurs without the emission of light (hence, the term "radiationless"), and accounts for the difference in wavelength between the light absorbed and the light emitted. From the lowest vibrational state in the excited electronic state, the molecules relax to various



vibrational states in the ground electronic state by the emission of light, which you can observe using fluorescence spectroscopy. The inverse rate of relaxation from the lowest vibrational level in the excited state to the ground state via fluorescence *and* non-radiative decay is called the fluorescence lifetime. This is an often confused but important point: The fluorescence lifetime is a measure of both the radiative and non-radiative decays to the ground state.

Figure 2 presents an example of a fluorescence spectrum. You can see that the molecule pictured here absorbs light over a range of frequencies, with a maximal absorbance wavelength of around 350 nm (labeled “Excitation”). It also emits light over a range of frequencies, with a maximal emission around 450 nm (labeled “Emission”). The separation, called a “Stokes shift,” between the maximal excitation and emission results from radiationless decay from the excited state. Measuring fluorescence excitation with a fluorimeter is much more sensitive than measuring absorbance with spectrophotometers. The reason for this higher sensitivity stems from the fact that fluorescence is a “zero background” technique. Imagine that you are looking at a bright screen when the brightness suddenly increases by 1%. Could you notice the difference? Now imagine you are seated in a pitch dark room and a light is turned on with an intensity equal to that 1% brightness increase. Chances are that you will see this light with your “eyeball detectors”. This is because it is always easier to see/detect something on a background of nothing than it is to detect the same change on a large background. Another advantage of fluorimetry is that many substances absorb light while far fewer substances fluoresce, giving fluorimetry unique advantages over absorption spectroscopy.

There are mechanisms, other than fluorescence, by which a molecule in the excited state can lose its excitation energy or relax. These alternative mechanisms for relaxation compete with fluorescence. If these mechanisms occur while you are observing the fluorescence of a molecule, you will see a loss of intensity in your fluorescence spectrum.

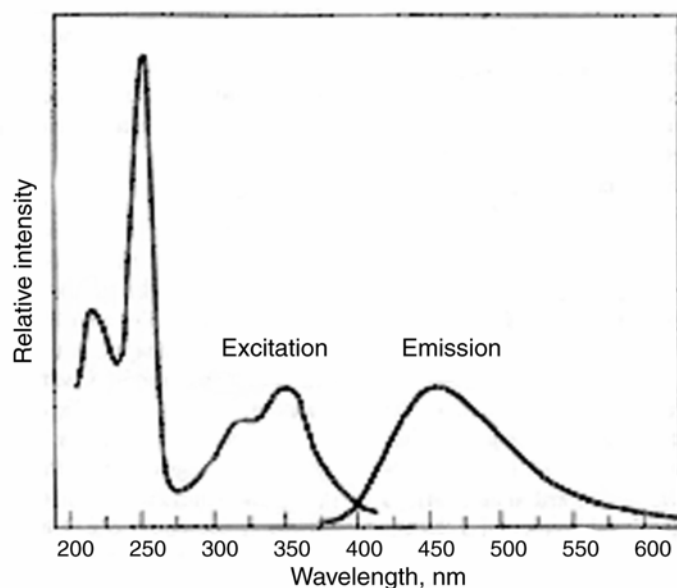
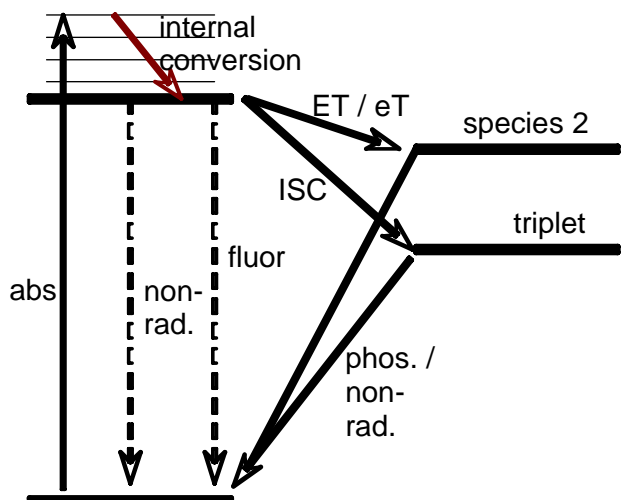


Figure 2



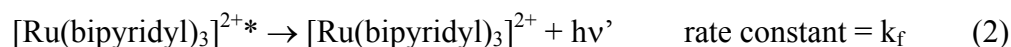
**Figure 3.** Jablonski diagram summarizing the fate of electronic excitation in a molecule.

another molecule, with the loss of excitation energy. The non-excited molecules that facilitate these mechanisms are called “quenchers.” You will observe a loss in the fluorescence intensity of  $[\text{Ru}(\text{bipyridyl})_3]^{2+}$  when it relaxes by transferring an electron to a ferric or cupric ion quencher during that  $10^{-9}$  to  $10^{-7}$  second fluorescence lifetime. The complexity of the fate of excitation energy in a molecule is summarized in the Jablonski diagram in Figure 3.

To determine the rate of electron transfer from  $[\text{Ru}(\text{bipyridyl})_3]^{2+}$  to ferric or cupric ions, we need to build a model that described the mechanism and the rate of reaction. We can use the steady state approximation to relate the concentration of the excited state to the fluorescence intensity. The equations will only be described here for the ferric ion, but they are exactly same for the cupric ion. The series of events starts with the absorption of light by  $[\text{Ru}(\text{bipyridyl})_3]^{2+}$  leading to electronic excitation (denoted by the \*):

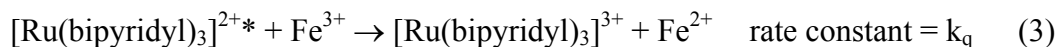


The excited state may relax back to the ground state by fluorescence:



One such mechanism is molecular collisions which occur during the  $10^{-9}$  to  $10^{-7}$  seconds that the molecule spends in the excited electronic state. During a collision, the excited molecule may transfer its excitation energy to another molecule or may cause a reaction to occur. Another such mechanism involves the transfer of an electron from the excited molecule to

Notice that  $h\nu'$  is of a different wavelength than the  $h\nu$  originally absorbed. The excited state may also relax by transferring an electron to a ferric ion:



The reaction rate is given by the sum of all processes forming and destroying the excited state,  $[\text{Ru}(\text{bipyridyl})_3]^{2+*}$ . For simplicity, we can set  $[\text{Ru}(\text{bipyridyl})_3]^{2+*} = R^{2+*}$ .

$$\frac{d[R^{2+*}]}{dt} = k_a [R^{2+}] - k_f [R^{2+*}] - k_q [R^{2+*}][\text{Fe}^{3+}] \quad (4)$$

The steady state approximation states that the concentration of the intermediate species in the reaction (the excited state) is present in concentrations much lower than the reactants or products, and is approximately constant during the reaction. This means that we can set the rate of change of the intermediate,  $\frac{d[R^{2+*}]}{dt}$ , to zero.

$$0 = k_a [R^{2+}] - k_f [R^{2+*}] - k_q [R^{2+*}][\text{Fe}^{3+}] \quad (5)$$

Since the fluorescence intensity that you will observe comes from the excited state intermediate, it is useful to write an equation giving the concentration of the intermediate,  $[R^{2+*}]$ .

$$[R^{2+*}] = k_a [R^{2+}] / (k_f + k_q [\text{Fe}^{3+}]) \quad (6)$$

The fluorescence intensity is actually proportional to  $[R^{2+*}]$ . If we designate  $I_0$  as the intensity when the quencher ( $\text{Fe}^{3+}$ ) is equal to 0, and  $I$  the intensity at any quencher concentration, we can rewrite equation (6) in terms of fluorescence intensity. This is something you can measure in the lab.

$$I_0/I = \{k_a [R^{2+}] / k_f\} / \{k_a [R^{2+}] / (k_f + k_q [\text{Fe}^{3+}])\} \quad (7)$$

$$I_0/I = 1 + k_q/k_f [\text{Fe}^{3+}] \quad \text{Stern-Volmer relation} \quad (8)$$

In your experiment, you will first measure the fluorescence intensity of the  $[\text{Ru}(\text{bipyridyl})_3]^{2+*}$  with no  $\text{Fe}^{3+}$  present. Then you will measure the fluorescence intensity of the  $[\text{Ru}(\text{bipyridyl})_3]^{2+*}$  with several  $\text{Fe}^{3+}$  concentrations. When you plot the ratio of that initial intensity,  $I_0$ , to the intensity at each  $\text{Fe}^{3+}$  concentration,  $I$ , versus the  $\text{Fe}^{3+}$  concentration, you will get a straight line (a Stern-Volmer plot). The slope of the line is equal to  $k_q/k_f$ , so  $k_q$  can be calculated when  $k_f$  is known. ( $k_f$  is known and is provided in Table 2).

Once you have determined the rate of electron transfer experimentally, you will compare your value to a theoretically calculated rate of electron transfer, using the Marcus equations.

Professor Rudolph A. Marcus of Cal Tech was awarded the Nobel Prize in 1992 for his theoretical work describing electron transfer reactions. The press release from the Swedish Royal Academy of Sciences describes the basis of Marcus' contributions:

#### **“Background**

When two molecules in a solution exchange one or more electrons, there is a reduction/oxidation process (redox process) in which one molecule accepts the electrons (reduction) and the other loses them (oxidation). Several different mechanisms can underlie such reactions. The simplest is the transfer of one single electron from one molecule to another. Changes take place in the structure, both in the reacting molecules and in those of the solution medium. Because of all these changes the energy of the molecular system rises temporarily and enables the electron to jump between the molecules. Energy must thus be supplied for the electron to be able to cross an energy barrier. The size of the energy barrier determines the speed of the reaction. An electron transfer of this kind is the simplest chemical elementary process, and is eminently suitable for theoretical studies.

At the beginning of the 1950s it was possible to determine the speed of a number of electron transfers between inorganic ions. Some of the reactions turned out to be very slow, which was surprising in view of the fact that only one electron changed places. It was considered at the time that such an insignificant change should not give rise to any large energy barrier.

### The prizewinner's contributions

From 1956 to 1965 Marcus published a series of papers on electron transfer reactions. His work led to the solution of the problem of greatly varying reaction rates.

Marcus made two assumptions about the reacting molecules. First, they had to be very loosely bonded to each other during the course of the reaction for classical physical-chemical theory to apply. Secondly, he assumed that it is the solvent molecules in the immediate vicinity that change their positions, thus increasing the energy in the molecular system. The electron can only jump between two states that have the same energy, and this condition can be fulfilled only by increasing the energy for both molecules. Marcus found a simple mathematical formula for calculating this energy change and was thus also able to calculate the size of the energy barrier. Somewhat later he extended the theory to include the energy associated with changes in the bonds of the reacting molecules.”

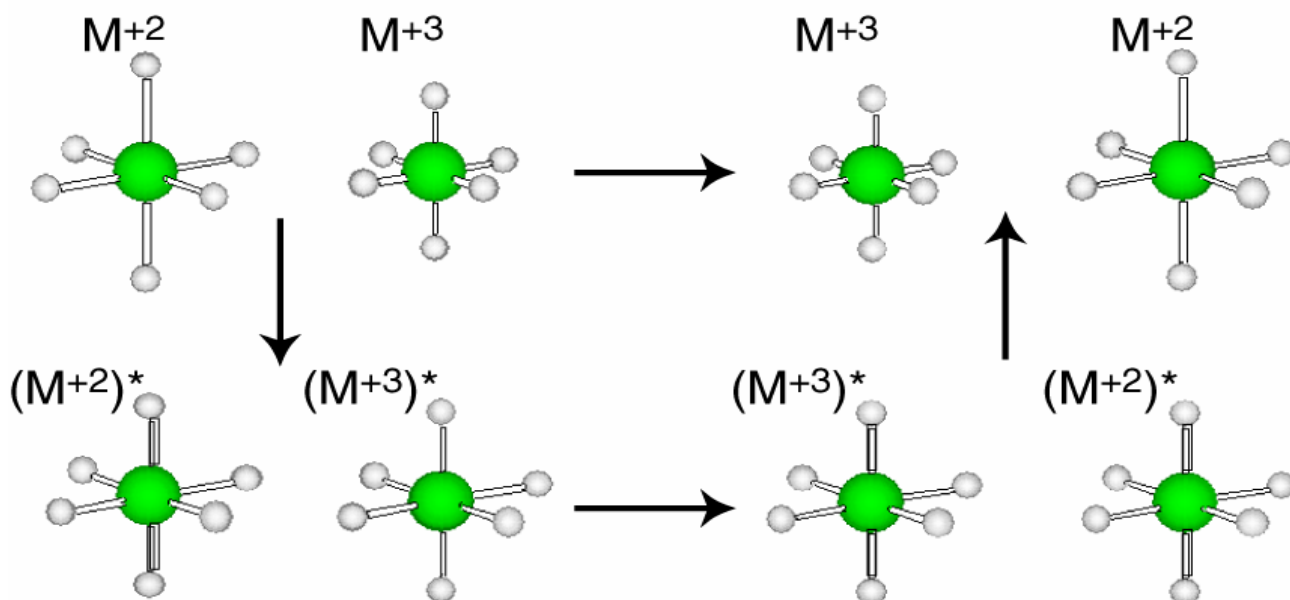
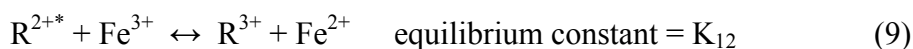


Figure 4.

Figure 4 provides a representation of a Marcus electron transfer between two metal ions. The different energies of the two ionic states are reflected in their different bond lengths (the ions on the top). In order to transfer an electron between them, this energy difference (here, the bond length difference) must be eliminated (the ions on the bottom). Marcus theorized that movement of solvent molecules could raise the energy of the system, which would eliminate the difference in energy between the two ions. In this

experiment, the excitation energy of  $[\text{Ru}(\text{bipyridyl})_3]^{2+}$  will also contribute to the equilibration of the energy of this ion with its quencher ion.

Marcus's equations to describe the rate of electron transfer reactions are highly accurate – in fact, they predict rate constants to within one order of magnitude over range of 12 orders of magnitude. Moreover, while his work was highly complex, his solutions are relatively simple. The electron transfer rate constant is calculated from the equilibrium constant for the overall reaction and the self-exchange rate constants for each species involved. The equations that you will need are summarized below for the ferric ion quencher (again, they are exactly the same for the cupric ion quencher):



The electron transfer rate,  $k_{12}$  ( $= k_q$ ), is given by:

$$k_{12} = (k_{11} k_{22} K_{12} f)^{1/2} \quad (12)$$

where  $f$  is given by:

$$\log f = \frac{(\log K_{12})^2}{4 \log (k_{11} k_{22} / Z^2)} \quad (13)$$

$Z$  is the collision frequency (about  $10^{11} \text{ L mol}^{-1} \text{ s}^{-1}$  in aqueous solutions).  $K_{12}$  can be calculated using the Nernst equation:

$$E = (RT/nF) \ln K_{12} \quad (14)$$

Here, the energy is given by the sum of the standard half-cell potentials of the reactants (given in Table 2),  $R$  is the gas constant,  $T$  is the temperature,  $n$  is the number of electrons transferred in the reaction, and  $F$  is Faraday's constant.

### C. Specific Aims

You will measure the fluorescence intensity of  $[\text{Ru}(\text{bipyridyl})_3]^{2+*}$  in the presence of varying concentrations of ferric or cupric ion quenchers. You will then calculate the rate

of electron transfer using a Stern-Volmer plot. You will finally compare your experimental value to a theoretical value calculating using the Marcus equations. In order to accomplish these purposes, you must accomplish these specific aims:

- Measure the fluorescence intensity of  $[\text{Ru}(\text{bipyridyl})_3]^{2+*}$  in the presence of several concentrations of ferric and cupric ions, as described in Table 1.
- Plot the ratio  $I_0/I$  versus quencher concentration in a Stern-Volmer plot.
- Calculate the rates of electron transfer for both ferric and cupric ions from the slope of the Stern-Volmer plot.
- Calculate the theoretical rates of electron transfer for both ferric and cupric ions using the Marcus equations.

### **E. Apparatus**

All measurements will be taken on the Perkin-Elmer 204 Fluorescence Spectrometer (fluorimeter). There is a shutter on the monochromator that protects the light source (the excitation shutter) and protects the detector from stray light (the emission shutter). The shutters **MUST BE CLOSED** whenever you open the sample compartment to change the sample. If you can see light when you open the compartment – you are not closing the shutters!!

### **Safety!**

The solutions you will use to prepare your samples are acidic. Take care when preparing and handling these solutions. If the solutions come in contact with your skin, wash your skin immediately with water.

### **F. Procedure**

- Prepare 10 mL of each solution listed in Table 1 using the stock solutions of  $10^{-4}$  M  $[\text{Ru}(\text{bipyridyl})_3]^{2+}$ , 0.002 M ferric ammonium sulfate, and 0.2 M cupric sulfate. Dilute them to the indicated concentrations using 0.5 M  $\text{H}_2\text{SO}_4$ .



**Table 1 --** All solutions should have  $10^{-5}$  M  $[\text{Ru}(\text{bipyridyl})_3]^{2+}$ .

Ferric ion solutions (mM):

a.	0.0
b.	0.2
c.	0.4
d.	0.8
e.	1.2
f.	1.6
g.	1.8

Cupric ion solutions (M):

a.	0.00 (Reuse solution a.)
h.	0.02
i.	0.04
j.	0.08
k.	0.12
l.	0.16
m.	0.18

- You will again use your pipet to transfer solutions into your sample cuvette.
- Set the excitation wavelength to 460 nm and the emission wavelength to 590 nm. You will not be able to see an entire spectrum, but can read the intensity of emission from the instrument.
- After your blank solution (0.5 M  $\text{H}_2\text{SO}_4$ ) has been placed in the sample compartment, you can open the excitation and emission shutters. **Make sure you go back and close the shutters prior to changing the sample!** You must do this each time you change samples.
- Find a sensitivity level such that the intensities of the solutions with the highest and lowest concentrations of quencher (e.g., solutions a and g) are on the same scale.
- Measure the intensity of the blank (0.5 M  $\text{H}_2\text{SO}_4$ ). Do NOT use Solution A as the blank.
- Close the shutters and change to the first sample solution. Re-open the shutters. Read the peak intensity at 590 nm from the instrument. Repeat the measurement 3 times for error estimation. Repeat for each of the sample solutions. At high quencher concentrations, it may become difficult to measure, so more than 3 repetitions may be required.
- Record the table of values in your lab notebook, so you have a hard copy of the data.
- Before removing the final sample, close the shutters. If you are the last group, turn off the fluorimeter.
- Dispose of your sample solutions to the appropriate waste bottle(s) and clean up.

## G. Before you leave the lab

Make sure you have completed the following items:

- You have recorded your table of intensities in your lab notebook.
- You have turned off the fluorimeter (if you are last group).
- You have disposed of your sample solutions to the appropriate waste bottles and cleaned up.

## H. Data Analysis

All aspects of the data analysis are described in the Background and Theory section. Review the equations to make sure you understand how they are derived. Here is a summary of the data analysis:

Use Excel or SigmaPlot to prepare a Stern-Volmer plot of the ratio  $I_0/I$  versus quencher concentration for both  $\text{Fe}^{+3}$  and  $\text{Cu}^{+2}$  ions. (Your initial intensity,  $I_0$ , comes from solution a. The experimental intensity,  $I$ , comes from each of your sample solutions containing ferric or cupric ions.) Determine the slope of the line using a least-squares linear regression. In your fitting, set the y-intercept to 1 (refer to equation (8) to see why you must do this step).

According to equation (8), the slope of your plot is  $k_q/k_f$ . You can calculate  $k_q$  for each quencher using the value of  $k_f$  supplied in Table 2. Pay careful attention to the units on  $k_q$ !

Theoretical calculations:

You will use equations (12), (13), and (14) to calculate the theoretical  $k_{12}$  for each quencher, which you will compare with your experimental value of  $k_q$ . The first step is to use equation (14) to determine  $K_{12}$  for each quencher. Equation (14) is the Nernst equation, which you may have studied in a general chemistry course. Please refer to your general chemistry text (if you don't have one, the Internet is always a good source) if you need to review the usage of the Nernst equation. Values of  $R$  and  $F$  are supplied in Table 2. The energies of electron transfer between  $\text{Fe}^{+3}$  (or  $\text{Cu}^{+2}$ ) and  $\text{Ru}[\text{bipyridyl}]^{+2}$  can be

calculated using the standard half-cell potentials supplied in Table 2. Again, refer to a general chemistry text to review calculations using half-cell potentials.

Once you have calculated  $K_{12}$  for each quencher, solve equation (13) and determine  $f$ . Values for  $k_{11}$ ,  $k_{22}$ , and  $Z$  are provided in Table 2.

Finally, solve for  $k_{12}$  using equation (12) for each quencher.

Helpful hints:

Watch units carefully! Because of the use of logarithms in equations (13) and (14), some of your values will be unitless. Make sure your units on  $k_{12}$  match your units on  $k_q$ .

Compare  $k_q$  and  $k_{12}$  in your report.

You may have heard or read before that the value of  $f$  is approximately 1 in many cases. While this may hold true in many cases, it does not in this experiment, particularly for the ferric ion quencher. Be sure to calculate  $f$  using equation (13).

### **Table 2 – Values and constants**

$$k_f = 1.74 \times 10^6 \text{ s}^{-1} \text{ (rate constant for de-excitation } \text{Ru}^{+2*} \rightarrow \text{Ru}^{+3}\text{)}$$

$$R = 8.314 \text{ J/Kmol (the gas constant)}$$

$$F = 96484.6 \text{ C/mol (Faraday's constant)}$$

#### Standard half-cell potentials

<u>Half-cell</u>	<u><math>E^0</math> (Volts)</u>
$\text{Ru}^{+3} + \text{e}^-$	1.24
$\text{Cu}^{+2} + \text{e}^-$	0.16
$\text{Fe}^{+3} + \text{e}^-$	0.77

#### Self-exchange rate constants

$\text{Ru}^{+2*} / \text{Ru}^{+2}$	$k_{11} = 1 \times 10^8 \text{ L/mol s}$
$\text{Cu}^{+2} / \text{Cu}^{+}$	$k_{22} = 1 \times 10^{-5} \text{ L/mol s}$
$\text{Fe}^{+3} / \text{Fe}^{+2}$	$k_{22} = 4 \text{ L/mol s}$