7.11 Fluorescence spectra and rate of electron transfer by quenching

(5 points)

Outline of Experiment

In this experiment you will study the fate of excited molecules in which fluorescence competes with quenching, whereby energy is passed on to other molecules in solution. The experiment is in two parts. In part A you will gain experience using a fluorimeter by measuring the fluorescence excitation spectrum and fluorescence emission spectrum of fluorescein; fluorescein's absorption spectrum is also recorded.

Part B of the experiment uses the short lifetime of the excited state of $Ru(bipy)_3^{2+}$ as an internal clock. Under the conditions of the experiment, fluorescence competes with electron transfer to Fe^{3+} or Cu^{2+}, and the kinetics of the reactions are deduced from the degree by which the fluorescence is quenched. From the rate constants for quenching the activation energy for the electron transfer is calculated, and you will also determine whether the electron transfer reaction is diffusion or activation controlled.

Relevant lecture courses and topics

Courses: Molecular energy levels; molecular spectroscopy, valence and electronic spectroscopy; modern liquid kinetics. *Topics*: Electronic transitions, fluorescence, electron transfer reactions, diffusion vs activation control, Born-Oppenheimer separation, fluorescence quenching, Stern-Volmer plot.

Overview of Theory

If a molecule in solution is excited through absorption of radiation, it may dispose of the excess energy in a variety of ways. Energy may be passed on to the solvent or other molecules through collision, the molecule may react or decompose, or it may emit a photon. If emission of a photon takes the molecule between two states of the

same spin multiplicity (usually, but not invariably, two singlets), the process is spin-allowed and is fast; this process is known as *fluorescence*. If the emission occurs between states of different multiplicty the transition is spin-forbidden, and is much slower, with a half-life from microseconds to minutes or even hours; this type of emission is known as *phosphorescence*.

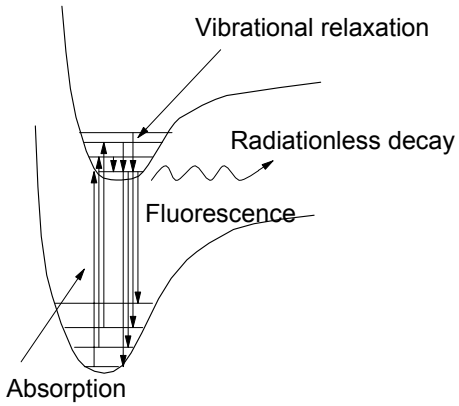
Generally, light emissions is one of several processes that may lead to destruction of the excited state. The amount of light emitted then provides an indication of how many molecules emit light compared to those that takes other routes. If fluorescence, say, is much faster than the other processes, every molecule will take that way of shedding excess energy. If one of the competing processes is much more rapid than fluorescnce, the level of emitted light will be negligible.

Finally, if a process has a similar rate to fluoresence, the extent by which the emitted light is diminished can be used to establish the relative rates of the two processes. This is the approach taken in this experiment, in which, by adding varying amounts of an ion which quenches (prevents) the fluorescence of excited species, you can determine the rate of quenching.

Detailed Theory

Electronically excited molecules dispose of their excess energy in various ways: they may undergo reaction, change oxidation state, change spin multiplicity, pass energy on to other molecules through collision, or lose energy by emitting a photon.

When a molecule in an excited singlet state emits a photon and thereby reaches another singlet state, the process is known as *fluorescence,* this is generally a very rapid process[1]. If the emission takes the molecule from an excited triplet state to a ground singlet state, (in other words, there is a change of multiplicity), the process is much slower because the transition is spin forbidden; this is *phosphorescence*.



Fluorescence spectroscopy, (in which, a little confusingly, we may study both fluorescent and phosphorescent processes), is a technique of considerable practical importance. Its analytical sensitivity approaches that of electrochemical methods, since in most instruments fluorescence from a sample is compared electronically with a reference emission of zero. (This contrasts with conventional UV/visible absorption spectroscopy, in which weakly-absorbing samples are detected as a small difference in

[1] Strictly, the term fluorescence covers a broader range of transitions than this, referring to any radiative electronic transition *between states of the same multiplicity*. Transitions between singlet states are, naturally enough, by far the most common.

the intensity of two quite high energy beams; the weak absorption signal may be lost in instrument noise.)

The sensitivity of fluorescence also leads to application in crime investigation. Argon-ion lasers are used to flood an area with intense blue light. Fingerprints are then revealed by their yellow fluorescence, which can be seen by a viewer wearing blue-filter goggles (to both remove the blue light and to provide protection from the laser radiation). Prints can be seen on porous materials, such as fabrics, which would not be apparent using more conventional methods of detection. Since lasers are expensive, their use in this fashion is normally limited to major crime.

Absorption and fluorescence [1]

The molecular potential energy curve of the singlet ground state S_0 and of an excited state S_1 of a typical organic molecule in solution are shown in the diagram above. The assumptions on which this type of diagram is based are discussed in the theory section of experiment 7.08. Since the excitation of an electron in a large molecule has little effect on the nuclear framework, the potential energy curves of the S_0 and S_1 states are usually similar, as are the spacings of their vibrational levels.

The energy gaps between vibrational levels are quite large, so at room temperature most molecules are in the vibrational ground state, v" = 0. The absorption spectrum thus arises from transitions from this state to different vibrational levels v' of the S_1 state. Although transitions within a single potential energy well (which give rise to IR spectra) are subject to the selection rule $\Delta v = \pm 1$, no such rule applies when the electronic state changes. We therefore can - in principle - observe transitions from the ground state to any of the large number of vibrational states in the excited electronic state[2].

Collisions with solvent rapidly remove excess vibrational energy from the molecules, bringing them down to the lowest vibrational level, v' = 0. Frequently, electronic energy is also lost through radiationless processes, but fluorescent molecules may emit a photon, and in this way return to one of the vibrational levels in the ground state.

Although we might expect that the frequencies of the $v' = 0 \rightarrow v'' = 0$ and $v'' = 0 \rightarrow v' = 0$ transitions in the fluorescence and absorption spectra would be identical, this is not always the case. Since the ground and excited states have different electronic structures, their interactions with solvent molecules are generally different, and the effect of the solvent may be to alter the energy of the excited state once it has been formed. For most complex molecules in solution, the fluorescence efficiency (the

[2] In practice the intensities of the transitions to the various excited vibrational levels differ, and can be related to the overlap of the vibrational wavefunctions in the two states concerned, through the Franck-Condon factor.

ratio of the amount of light emitted to that absorbed) is almost independent of the wavelength of the exciting light.

Phosphorescence may occur if an electronically excited molecule converts to a triplet state T_1 (inter-system crossing). Although the transition from $T_1 \rightarrow S_0$ which returns the molecule to the ground state is spin forbidden, the transition does take place in some molecules, (through spin-orbit coupling), but only after a period of 10^{-4} - 1 s. Thus phosphorescence is very much slower than fluorescence.

Determination of the electron transfer rate constant [2,3]

In the kinetics section of the experiment you will be using $Ru(bipy)_3$ provided by the compound tris(2,2'-bipyridine)ruthenium (II) chloride. When this compound is excited by absorption of a photon, the species $[R^{2+}]^*$, where R = $Ru(bipy)_3$, is left in a charge transfer excited state - an electron has been partially transferred from the metal atom to the antibonding orbitals of the ligand. The excited state, which lies 202 kJ mol^{-1} above the ground state, may undergo a variety of processes, as shown below.

$R^{2+} + h\nu$	$\rightarrow R^{2+*}$	Absorption (rate: I_{abs})
R^{2+*}	$\rightarrow R^{2+}$	Fluorescence, ISC, and non-radiative decay (rate constant: k_s)
$R^{2+*} + M^{n+}$	$\rightarrow R^{3+} + M^{(n-1)+}$	Quenching (rate constant: k_q)

The first step is the excitation process; the second represents all processes other than quenching which lead to the excited molecule returning to the ground state. The final step represents the loss of energy by quenching, which can occur through a variety of mechanisms, depending upon the identity of the quenching species.

In the reactions studied in this experiment the quenching involves oxidation of the ruthenium moiety, but other quenchers are capable of reducing the ruthenium, or removing the excess energy without affecting the oxidation state. In many cases, such as quenching by methyl viologen or ferric ion, this step is believed to be an outer sphere electron transfer, during which the inner co-ordination spheres of the reactant remain intact. Electron transfer, which often involves a contribution from tunnelling, is much faster than ligand substitution, typically by several orders of magnitude (the time scales for electron transfer and for nuclear motion are roughly 10^{-15}s and 10^{-12}s respectively). The quenching rate constant then approaches the diffusion-controlled limit of approximately 10^{10} M^{-1} s^{-1}.

$k_s + k_q [Mn^+] \gg I_{abs}$, so we can apply the steady state approximation to $[R^{2+}]^*$

$$[R^{2+*}] = \frac{I_{abs}}{k_s + k_q[M^{n+}]} \qquad (1)$$

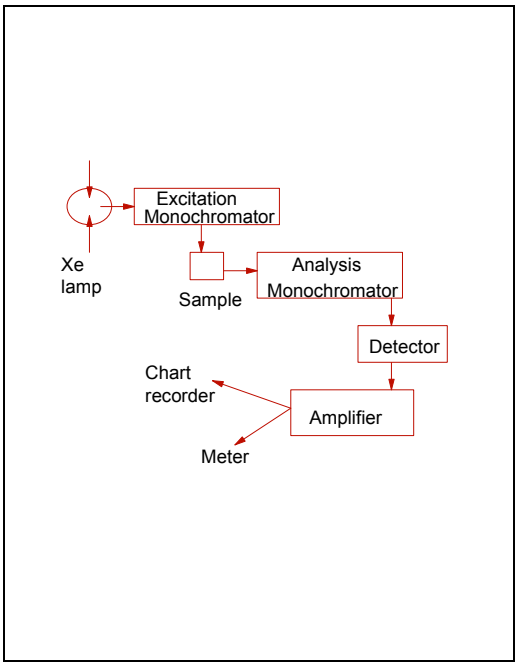
I_0 is the intensity of fluorescence when no quencher is present, and I the intensity in the presence of quencher. Then

$$I_0/I = 1 + [M^{n+}]\, k_q/k_s \qquad (2)$$

and a graph of I_0/I vs $[M^{n+}]$ (Stern-Volmer plot) yields a line of slope k_q/k_s. k_q may then be found if k_s is known. From k_q and k_s the lifetime of the excited state, and the activation energy for the quenching reaction can be found.

Calculation of rate constant by Marcus theory [7]

In an outer sphere redox reaction there is little interaction between the oxidant and reductant at the moment of electron transfer [4]. Marcus has developed a theory by which the electron transfer rate constant k_q may be calculated from the equilibrium constant of the overall reaction and the self exchange rate constants, which relate to the transfer of an electron between oxidized and reduced forms of the same species. The theory is outside the scope of this experiment, but comparison of theory with experiment allows one to determine whether the quenching reaction is diffusion-controlled, and this is a calculation which forms part of the experiment.



Procedure [6]

Safety

Wear safety glasses when handling solutions containing acid. Unlike most fluorimeters, whose photomultipliers may be damaged by room light, the Varian fluorimeter can be operated with the lid of the sample compartment open. However, UV radiation may still be scattered from the sample or its container, so keep the lid closed during operation. Safety data on the chemicals used in this experiment can be found through the web site for the experiment, at http://ptcl.chem.ox.ac.uk/~hmc/tlab/experiments/711.html

The optical system of a typical fluorimeter is shown in the figure to the left. Light from a continuous or pulsed xenon source (as used in this experiment) is dispersed by the excitation monochromator, and radiation of the selected wavelength is directed onto the sample. Emission from the sample passes through the analysis monochromator, and then onto a detector which creates a signal for driving a recorder or display on a computer. Controls on the monochromators allow selection of excitation and analysis wavelengths, and automatic scanning by a single

monochromator (to yield excitation or emission spectra), or by both monochromators simultaneously (to yield synchronous spectra).

Excitation and emission spectra of fluorescein

Your first task is to record the emission and excitation spectra of fluorescein. The *emission spectrum* shows the intensity of light emitted as a function of the emission wavelength, for a selected excitation wavelength; the *excitation spectrum* shows how the amount of emitted light at a chosen wavelength varies as the wavelength of the exciting radiation is changed.

Preparation

Turn on power to the fluorimeter, inkjet plotter, and computer. Wait for one minute for the fluorimeter to complete any initialisation, then in the Cary Eclipse window on the computer double-click on the **Scan** application icon; this will start the first application you need.
Half fill a UV plastic sample cell with the solution of fluorescein in NaOH supplied; do not fill to the top, or you may spill solution in the sample compartment. Hold the cell near the top; remember that fingerprints on the cell faces will fluoresce. Note that you must use the sample cells with four clear sides, not those with two clear sides.

Slide back the sample compartment lid of the spectrometer and place the sample in the black sample holder. Close the lid.

Finding emission and excitation wavelengths for maximum emission

The majority of your interaction with the spectrometer will be through the **Setup** menu, but it is useful to use **Prescan** to start with. **Prescan** will automatically locate the emission and excitation wavelengths which give the maximum emission intensity. Click on **Prescan** and then **OK**.

Once **Prescan** has been started the process is entirely automatic. The instrument will scan repeatedly as it tries to find suitable wavelengths, and adjusts the power supplied to the photomultiplier detector in order to keep the signal on scale. You will notice that the screen flickers as this process occurs; this is because every time the software needs to rescale the plot the screen redraws - this is entirely normal. The process will halt once the optimum wavelengths have been found, and they and the PMT voltage will be reported; record these in your data book. (If the process continues indefinitely, the sample probably has such a feeble emission that the instrument is unable to find it. Press the **Stop** button and call a demonstrator.)

When the **Prescan** is complete, print the spectra using the **Print** function in the **File** menu. The plot will show the excitation spectrum, which will cover the entire scanned range, and the emission spectrum, which will cover the range from the optimum

excitation wavelength upwards to higher wavelengths, since no emission occurs to shorter wavelength of the exciting line.

You can delete unwanted spectra using the **remove graph** option in the **Graph** menu, and can expand one spectrum to fill the entire display window by using the **Multi-graph** option in the same menu.

Gathering the complete emission spectrum

Select **Setup** and check that the excitation wavelength is set to that previously found. Ensure that the **Data Mode** is set to Fluorescence and in the **Scan Setup** the Emission button is depressed. Choose emission between 300 nm and 800 nm, a medium scan rate, click on **OK** and press the **Start** button. Print the resulting spectrum, after re-scaling if necessary.

The light emitted during fluorescence necessarily has a wavelength equal to or longer than the wavelength of the exciting light, since some of the energy of the absorbed photon is degraded to heat. It follows that emission will only be seen at wavelengths greater than the excitation wavelength. However, some scattered light may be found when excitation and emission wavelengths are equal, due to Rayleigh scattering and scattering of incident light by dust suspended in the solution.

Dilute the fluorescein solution with 0.1M NaOH if the fluorescence intensity is high, since dilution will reduce distortion of the fluorescence spectrum by self-absorption.

Now set the excitation wavelength at the maximum near 320 nm and run and print an emission spectrum over the wavelength range 200-800 nm. In your report comment on any features of interest in this spectrum.

Absorption spectrum of fluorescein

Record on the Lambda 5 or another UV/visible spectrometer the visible absorption spectrum of the fluorescein solution. Since a reference solution for the spectrum (0.1M NaOH) would be colourless, no reference is needed. (The spectrometers may be in use, in which case a little diplomacy will be required! Your spectrum should take only 10 minutes to gather).

Fluorescence quenching

You will now measure the fluorescence intensity at selected wavelengths for a series of solutions containing ruthenium ion, using these readings to determine the extent to which the fluorescence is quenched by added ions.

Concentration of sample solutions

	$[Fe^{3+}]$	$[Cu^{2+}]$	$[Ru(bipy)_3^{2+}]$	H_2SO_4

a	0	0	1×10^{-5} M	0.5 M
b	0.2×10^{-3} M	0	1×10^{-5} M	0.5 M
c	0.4×10^{-3} M	0	1×10^{-5} M	0.5 M
d	0.8×10^{-3} M	0	1×10^{-5} M	0.5 M
e	1.2×10^{-3} M	0	1×10^{-5} M	0.5 M
f	1.6×10^{-3} M	0	1×10^{-5} M	0.5 M
g	1.8×10^{-3} M	0	1×10^{-5} M	0.5 M
h	0	0.02 M	1×10^{-5} M	0.5 M
i	0	0.04 M	1×10^{-5} M	0.5 M
j	0	0.08 M	1×10^{-5} M	0.5 M
k	0	0.12 M	1×10^{-5} M	0.5 M
l	0	0.16 M	1×10^{-5} M	0.5 M
m	0	0.18 M	1×10^{-5} M	0.5 M

Preparation

Make up in 10 cm^3 volumetric flasks the solutions listed in the table. You are provided with stock 1×10^{-4} M Ruthenium, 2×10^{-3} M Fe^{3+}, 0.2 M Cu^{2+} (all in 0.5 M H_2SO_4), and 0.5 M H_2SO_4. Note that the final concentration of acid in each solution is the same, so you should be using the dilute acid to make solutions up to the mark, not water.

Finding optimum emission and excitation wavelengths

Sulphuric acid fluoresces in the ultraviolet, so to avoid your data being affected by fluorescence from the solvent, your excitation and emission wavelengths for the Ruthenium compound must be well removed from the wavelengths associated with fluorescence from the acid. A convenient way to prevent sulfuric acid fluorescence from interfering with the experiment is to use plastic UV cells which, while they are transparent to low energy UV wavelengths, do not pass the very short wavelengths required to excite sulfuric acid.

Since different types of plastic transmit at different wavelengths, you must first record the uv/visible absorption spectrum of an empty plastic cell to determine the wavelengths over which the cell absorbs; use the lambda5 or a UV2 spectrometer for this. Sulfuric acid absorbs strongly around 235 nm. If your cell is *transparent* at this wavelength, ask the technician for replacement cells, otherwise you may proceed with the remainder of the experiment.

Half fill a cell with sample (a), and use the **Prescan** option to determine the emission and excitation wavelengths that correspond to maximum fluorescence. Print out the combined emission/excitation spectrum which **Prescan** generates.

IMPORTANT NOTE! You need now to check that these emission and excitation wavelengths are appropriate. Even using a plastic cell, it is possible that the excitation wavelength which the instrument has found might be in or close to the UV region of the spectrum. If this is so, the experiment will not work correctly. You should now gather a quick UV/visible spectrum, using any of the UV/visible spectrometers in the lab, of either the copper or the iron solution and inspect this to try to determine why using an excitation wavelength in the UV would be a bad idea.

If the fluorimeter has chosen an excitation wavelength below 400nm, the value is too low for the quenching experiment to give good results. You should in this case conduct a pre-scan to find an excitation wavelength above 400nm, and a suitable emission wavelength (which will be at least 25 nm greater than the excitation wavelength) to go with it. You may then proceed.

To acquire quantitative data, it is desirable to set the slit widths for emission and excitation to the smallest values consistent with a good signal-to-noise ratio. These slit widths may have been set by the software to 5nm. After setting your chosen emission wavelength, reduce both slits to 1 or 2.5nm and record the fluorescence spectrum to check whether the signal-to-noise ratio is adequate with this setting of slit width. A smooth emission curve with little visible irregularity is required.

If necessary, make further changes to the instrument parameters to give the most suitable emission curve for analysis.

Record the emission and excitation wavelengths you intend to use for the final part of the experiment.

Measuring fluorescence intensity in the presence of a quencher

You need to use a different computer program for this part of the experiment, so exit from the **Scan** application and double-click on the **Concentration** application.

Remove the sample cell from the fluorimeter. Choose **Setup** and click on the **Standards** tab. Enter 2 as the number of standards, and give their concentrations as 0.0 (Std1) and 1.0 (Std2). The first standard will be a sulfuric acid blank (the 0.5M acid used in all solutions), the second will be your solution (a).

Now select the **Samples** tab, set the number of samples to 12 and enter suitable labels for your samples (b) - (m). Click **OK**. Select the Cary tab, and set the emission and excitation wavelengths which you determined in the last step.

Remove any plots on screen and clear the report box, then press **Start**.

Select for analysis Std1, Std2 and your six iron-containing samples. When the dialogue box appears, highlight Std1, insert a cell containing the sulfuric acid blank into the spectrometer and click **OK** to request the instrument to record the fluorescence (which will be close to zero, since the acid solution should not give a significant signal if you have chosen the emission and excitation wavelengths wisely). When requested, place the cell containing solution (a) (your second standard) into the cell holder, record the fluorescence, then enter samples (b) - (g) as requested. Once all your Fe^{3+}-containing solutions have been entered, do not feed in any more solutions, but print out the plot and the report.

After printing the report, clear it and repeat the procedure using your sulfuric acid blank, the standard solution (a) and samples (h) - (m).

When you have taken all the measurements, remove any sample, exit from the Cary control program and turn the fluorimeter off. Unscrew the knurled wheel in the centre left of the black sample holder, and carefully lift it out. Wipe the holder clean with a tissue. If there appears to be any liquid spilt into the body of the holder, give it to the technician to clean. Check that no waste has spilled into the fluorimeter sample area, then close the lid.

Calculation

On one graph draw the absorption, excitation and emission spectra for fluorescein after normalizing the peak of each spectrum to unity. Explain the features of the spectra.

Comment on the spectrum gathered using excitation at 320 nm.

Use Stern-Volmer plots to find k_q for the quenching of $Ru(bipy)_3^{2+}$ by Fe^{3+} and Cu^{2+}. k_s, the rate constant for spontaneous decay of ($Ru(bipy)_3^{2+}$), which includes contributions for all processes other than quenching, is $1.67 \times 10^6\ s^{-1}$.

From the rate constant for quenching the activation energy for this process, ΔE, can be found, using the equation $k_q = z_{12} \exp(-\Delta E/RT)$, in which z_{12} is a collision number, and for a solution is approximately $10^{11}\ M^{-1}\ s^{-1}$. Use this equation to determine values for the activation energy for the quenching reaction with iron and with copper ions.

For diffusion-controlled reactions (those in which the rate-determining step is diffusion together of the reactant species, rather than the acquisition of activation energy), ΔE is approximately 10 to 14 kJ mol^{-1}. (This figure, representing the energy released as the activated complex is formed, balances a roughly equivalent amount of energy required to bring the reactants together against the resistance provided by the solvation shells; the reaction forming the activated complex is then energetically neutral). Comment on the extent to which the quenching reactions involving iron or copper ions approach the diffusion-controlled limit.

Finally, calculate the half-life of the excited ruthenium complex in the presence of each quencher, and suggest a method which could be used to determine the fluorescence half-life of the complex in the absence of quencher.

References

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