Determination of an Excited-State Electron-Transfer Rate Constant by Fluorescence Quenching.

(i) Absorption and Emission of Light:

A given molecule M in the ground state, denoted $^1M_0$, can interact with electromagnetic radiation, absorb a photon, and thus populate an excited electronic state of M. The Franck-Condon allowed vertical transition populates an excited state denoted ^1M_n, where the subscript n indicates the excited electronic level. A schematic representation of such a transition is shown in Figure 1, with different vibrational sublevels, v and v', of a given state.

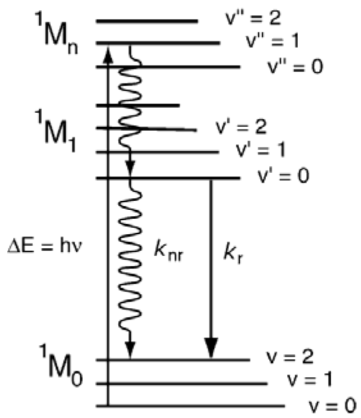
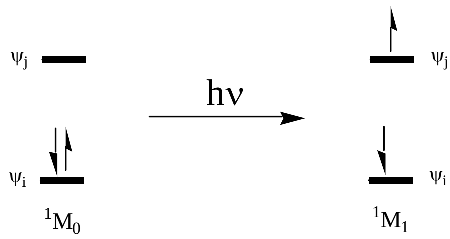


Figure 1. A state energy diagram that illustrates key aspects of light absorption and emission. The rate constants k_{nr} and k_r are defined in the text.

Using the HOMO-LUMO convention, this process can be illustrated as shown in Figure 2.



For most organic molecules, the energies of the $^1M_0 \rightarrow {}^1M_n$ transitions, ΔE, are such that the absorbed light generally falls in the UV or visible regions of the electromagnetic spectrum.

The ^1M_n state initially formed upon light absorption almost always relaxes very quickly to populate the v'=0 level of the lowest excited singlet state, $^1M_1$ (Figure 1). This is called the Kasha rule. Depopulation of the $^1M_1$(v'=0) state can proceed by several competing pathways: (1) as heat in a nonradiative process with rate constant k_{nr}, (2) by emitting a photon in a *radiative* process with rate constant k_r, or (3) a separate molecule Q could interact with M and thus quench the excited state in a bimolecular process. (Discussed below).

In the process of light absorption by a solute M dissolved in a solvent, the transition originates from a state in which the solvent is in equilibrium with the charge distribution of the solute. Using the $^1M_0 \rightarrow {}^1M_1$ transition as an illustration (Figure 3), the $^1M_1$ state created upon light absorption can have a significantly different charge distribution than the $^1M_0$ state. Because the solvent does not immediately respond to this change in solute charge distribution, the $^1M_1$ state initially produced is not in equilibrium with the surrounding solvent. However, within picoseconds (the actual time is solvent dependent), solvent reorientation yields a lower energy, equilibrated $^1M_1$ state. Upon light emission the change in solute charge density, associated with the $^1M_1 \rightarrow {}^1M_0$ transition, is likewise not in equilibrium with the surrounding solvent, and an equilibrated state is again achieved only after solvent reorientation. This sequence of events is illustrated in Figure 3.

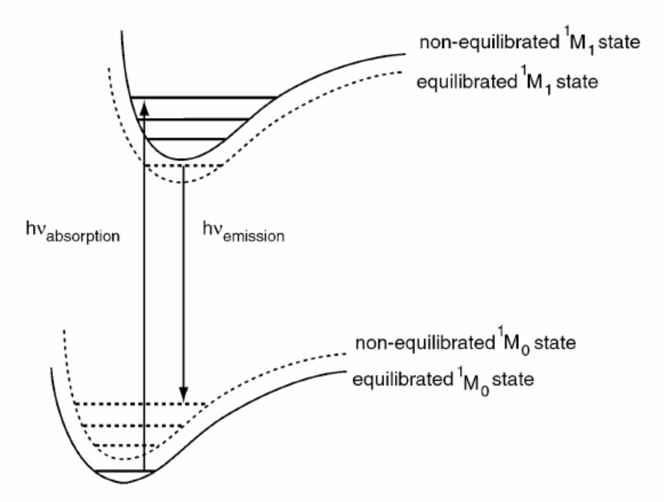


Figure 3. Potential energy curves that illustrate events associated with light absorption and emission in a generic solute/solvent system.

Question 1: *In drawing potential energy curves such as those shown in Figure 3, axes are often omitted. What is the x-axis usually implied in such drawings. Furthermore, the $^1M_1$ potential energy curve is shifted to the right with respect to the $^1M_0$ potential energy curve. What is the justification for this?*

As can be inferred from the diagrams in Figures 1 and 3, the spectral profile of fluorescence is generally shifted to longer wavelength than the spectral profile of absorption. The difference between the peak maxima of these two spectra is called the Stokes shift. A reasonable estimate of the energy gap between the $^1M_1(v'=0)$ level and the $^1M_0(v=0)$ level, $\Delta E_{0,0}$, can be obtained by determining the point at which the fluorescence and absorption profiles cross each other when scaled to the same peak amplitude (see example in Figure 4). In the course of this lab., you wil determine $\Delta E_{0,0}$ in this way for 9-cyanoanthracene (CNA).

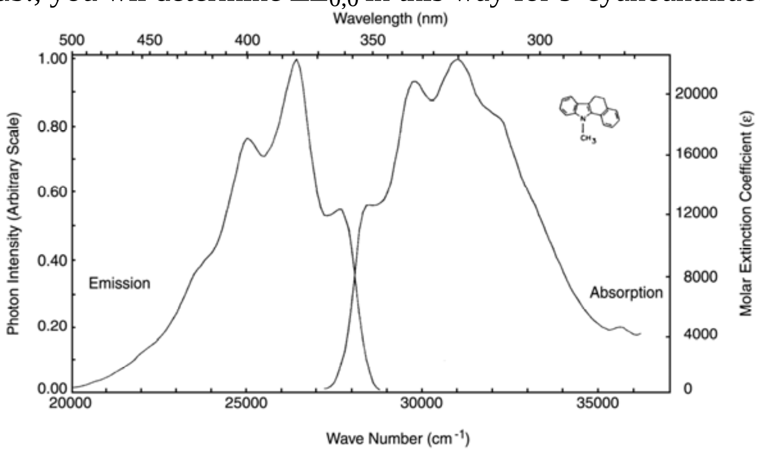


Figure 4. Absorption and emission spectra for a substituted indole.

Question 2: *Provide an explanation for the structure shown in both the absorption and emission spectra of Figure 3. In particular, comment on the spacing between the "bumps"* *on a given spectrum.*

(ii) Decay Kinetics of an Excited Electronic State:

Upon irradiation of compound M with a very short pulse of light, one will create a discrete $^1M_1$ population very quickly. If, for the moment, we limit ourselves to the unimolecular processes of radiative, k_r, and non-radiative, k_{nr}, deactivation paths; the concentration of $^1M_1$ as a function of time can be described as:

$$[^1M_1]_t = [^1M_1]_0 \; e^{-(k_r + k_{nr})\,t} \quad (5)$$

At time t = $(k_r + k_{nr})^{-1}$, the initial population of $^1M_1$, $[^1M_1]_0$, will have been reduced by a factor of 1/e (note that k_r and k_{nr} have the units of s^{-1}). This time is defined as the *lifetime*, τ, of the $^1M_1$ state (equation 6).

$$\tau\,(^1M_1) = \frac{1}{k_r + k_{nr}} \quad (6)$$

In an experiment in which fluorescence is spectroscopically monitored, it is important to recognize that the intensity of fluorescence will always be proportional to the concentration of $^1M_1$ in the system. Thus, the fluorescence decay represents τ, not τ_r the radiative lifetime $^1M_1$ (defined as $1/k_r$)

(iii) Decay Kinetics in the Presence of a Quencher:

For many solute molecules M, the fluorescent state can also be deactivated in a bimolecular reaction with some quencher Q present in solution. The expression for the change in $[^1M_1]$ as a function of time must account for this extra deactivation pathway:

$$\frac{d[^1M_1]}{dt} = -\,(k_r + k_{nr} + k_q[Q])\,[^1M_1] \quad (7)$$

In many common systems, the concentration of Q does not change during the quenching process (i.e., Q is not chemically consumed during the quenching process, or [Q] is sufficiently large that any change in [Q] is sufficiently small). As a consequence, the product k_q[Q] can be considered a constant over the period 0 to t, which, in turn, simplifies the integration of equation 7 to give:

$$[^1M_1]_t = [^1M_1]_0 \; e^{-(k_r + k_{nr} + k_q[Q])\,t} \quad (9)$$

The lifetime of $^1M_1$ is again defined as the reciprocal of the sum of all first-order deactivation rate constants, that now includes the product k_q[Q].

$$\tau\,(^1M_1) = \frac{1}{k_r + k_{nr} + k_q[Q]} \quad (10)$$

Many light-absorbing systems of importance in our daily life are in equilibrium with the ambient atmosphere, and one common quencher of fluorescence of which one must constantly be aware of is molecular oxygen. For many organic molecules, quenching of the $^1M_1$ state by oxygen is so efficient that it is often limited by the rate at which $^1M_1$ and O_2 diffuse together. For most organic solvents in equilibrium with our atmosphere, the product $k_{O2}[O_2]$ can be large enough to affect the $^1M_1$ lifetime (equation 11):

$$\tau\,(^1M_1) = \frac{1}{k_r + k_{nr} + k_{O2}[O_2]} \quad (11)$$

***Question 3**: In the absence of oxygen, some arbitrary molecule M has a $^1M_1$ lifetime of 16 ns. What will the $^1M_1$ lifetime be in an air-saturated solution where $[O_2] = 2 \times 10^{-3}$ mol L^{-1}*

and k_{O2} = 3 x 10^{10} mol^{-1} L s^{-1} ? What percentage of $^1M_1$ states will be quenched by oxygen under these conditions?

(iv) The Stern-Volmer Treatment:

In a solution of M, the intensity of $^1M_1$ fluorescence, I^0, will be proportional to the fraction of $^1M_1$ states that decay via the radiative channel (equation 13).

$$I^o = \beta \frac{k_r}{k_r + k_{nr} + k_{O2}[O_2]} \qquad (13)$$

Included in the proportionality constant β are a variety of experimental parameters (e.g., collection efficiency of the emitted light). For an analogous solution containing the quencher Q, the fluorescence intensity, I, will likewise be proportional to the fraction of $^1M_1$ states that decay via the radiative channel (equation 14).

$$I = \beta \frac{k_r}{k_r + k_{nr} + k_{O2}[O_2] + k_q[Q]} \qquad (14)$$

If the fluorescence intensity I is recorded under the identical conditions used to record I^0, then the proportionality constant β will be the same in both experiments. Thus, in taking the ratio I^0/I, the constants β and k_r cancel.

$$\frac{I^o}{I} = \frac{k_r + k_{nr} + k_{O2}[O_2] + k_q[Q]}{k_r + k_{nr} + k_{O2}[O_2]} = 1 + \frac{k_q[Q]}{k_r + k_{nr} + k_{O2}[O_2]}$$

$$\frac{I^o}{I} = 1 + k_q\tau[Q] \qquad (15)$$

As seen in equation 15, the Stern-Volmer equation, a plot of I^0/I against [Q] should be linear and yield an intercept of 1 and a slope of k_q τ.

Experimental Section

The fluorescence quenching of 9-anthracenecarbonitrile (aka 9-cyanoanthracene) (CNA) will be studied using two quenchers: 1,4-dimethoxybenzene (DMB), and naphthalene.

i) Weigh about 20 mg of CNA and dissolve them in acetonitrile using a 100 ml volumetric flask (~10^{-3} M). Dilute a small volume of this solution to ~5*10^{-5} M (0.5 ml to 10 ml for example) and record the absorption spectrum with the UV/vis instrument used for the S3 experiment. You need to know the precise concentration of CNA of both solutions.

ii) Prepare 25 ml solutions of each quencher in acetonitrile and measure the corresponding UV/vis spectra. The concentrations for these spectra are not relevant, although it is recommended that the maximum absorption is lower than 1.5 (~10^{-4}M)

iii) Dilute again the CNA solution to lower the maximum absorbance measured in i) to about 0.1 and record the *emission* spectrum of this solution using the Ocean Optics fluorimeter following the procedure below:

1) Open the OOIBase32 software. Make sure the teflon scatterer is inside the sample holder, cap the sample holder and turn the spectrometer on from the back of the white box and turn the flash mode to single.
2) Click on Strobe/Lamp enable on the software; after a few seconds you should see a peak on the spectrum. Move the slider (between sample and lamp) to select the excitation wavelength at about 400 nm (blue enough to excite CNA, but no so

blue to avoid exciting the quenchers). Click on the camera icon, and save the spectrum: File/Save/Sample

3) Replace the teflon scatterer with your sample and switch the flash mode to multiple on the back of the white box. You might see already the fluorescence from CNA.
4) Maximize the signal/noise by playing with the strobe frequency, integration time, and average. Also, subtracting a Dark Spectrum will help a lot to reduce the noise: unclick Strobe/Lamp Enable, wait a few seconds, click on Store Dark (grey lamp icon), and then click on Subtract Dark Spectrum (grey lamp icon with a minus sign in front). Finally turn the Strobe/Lamp Enable back on. Keep in mind that every time you change either the integration time or the average a new Dark Spectrum should be acquired and subtracted. To save the spectrum with the subtracted background do File/Save/Processed (after clicking on the camera icon).

iv) Following the same procedure as in iii) measure the fluorescence of at least 5 different solutions of CNA (at about the same concentration used for iii)) and each of the two quenchers. The concentration ranges that you should explore for the quenchers are:

1,4-dimethoxybenzene	~ (0.1 – 4.0) x 10^{-2} mol L^{-1}
naphthalene	~ 0.1 – 0.8 mol L^{-1}

Prepare these solutions in 25 ml volummetric flasks keeping good records of the dilutions you make since you will need to know the exact concentration of both quencher and fluorophor for the Stern-Volmer analysis. Make sure you use the same experimental conditions in iii) and iv) (integration time, average, frequency)

v) Calculate I_0 and I by either integrating the different emission spectra or just consider the intensity at a specific wavelength (which method has the largest error?). Keep in mind that I should be scaled by the dilution factor of CNA (for example, if the CNA solution from iii) was $2*10^{-5}$ M and one of the quenched solutions is $1.5*10^{-5}$ in CNA then I should be multiplied by 1.333)

vi) Plot I_0/I vs [Q] and run a linear regression to calculate kq for the two different quenchers, consider τ=11.6 +/- 0.1 ns.

vii) If you need to store solutions to measure on the next lab period, do so in the S6 drawer. After you are done dispose of the solutions in the appropriate waste disposal bottle.

Turn Fluorimeter Lamp OFF after you are done!!!

Quenching via Electron Transfer:

One possible mechanism by which a quencher Q can deactivate an excited electronic state is via a process of electron transfer. Key steps in such a process are illustrated below:

Scheme 1:

$$^{1}M_1 + Q \underset{k_{-diff}}{\overset{k_{diff}}{\rightleftharpoons}} \underset{\text{encounter pair}}{^{1}M_1 \cdots Q} \underset{k_{-et}}{\overset{k_{et}}{\rightleftharpoons}} \underset{\text{radical ion pair}}{M^{-\cdot}\, Q^{+\cdot}} \xrightarrow{k_{decay}} {}^{1}M_0 + Q$$

Briefly, $^1M_1$ and Q must first diffuse together to form an *encounter pair* in which the distance between $^1M_1$ and Q, the so-called *interaction distance*, is appropriate for the transfer of an electron. The rate of this diffusion-dependent encounter is related to the magnitude of the bimolecular rate constant k_{diff}. Of course, once formed, the encounter pair can diffuse apart to regenerate $^1M_1$ and Q. The rate of this process is related to the unimolecular rate constant k_{-diff}. Once the encounter pair has formed, electron transfer can occur with the rate constant k_{et} to generate the radical ion pair. A simplistic molecular orbital diagram illustrating this process is shown in Figure 5. (In scheme 1, we show electron transfer from Q to $^1M_1$. However, depending on the redox potentials of both M and Q, it is also reasonable to consider electron transfer from $^1M_1$ to Q.)

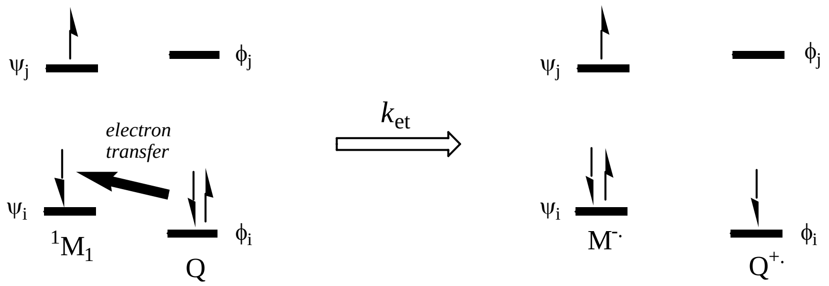


Figure 5. Simplistic molecular orbital diagram that illustrates the process of electron transfer in the $^1M_1$-Q encounter pair to produce a radical ion pair.

If one were to monitor the quenching of $^1M_1$ fluorescence by some molecule Q, and if the mechanism of quenching proceeded via the events shown in Scheme 1, then the overall quenching rate constant, k_q, could be expressed as a function of the individual rate constants shown in Scheme 1. For the system as shown, this expression is given in equation 16, where the equilibrium constant for electron transfer, K_{et}, is equal to the ratio k_{et}/k_{-et} of the respective rate constants for electron transfer.

$$k_q = \frac{k_{diff}}{1 + \frac{k_{-diff}}{k_{et}} + \frac{k_{-diff}}{k_{decay}} \frac{1}{K_{et}}} \qquad (16)$$

You will use equation 16 to calculate values of k_q for the two quenchers used for the Stern-Volmer experiment to confirm that the fluorescence quenching proceeds via electron transfer. The necessary theory and experimental values for the different rate constants is presented below:

(i) Diffusion-controlled rate constants, k_{diff} and k_{-diff}.

In the *Smoluchowski equations*, the rate constants for diffusion-limited processes are expressed as a function of the solvent-dependent diffusion coefficients, D, of the respective reaction partners (equations 17 and 18). Thus, in this case, the coefficients of concern are D_M and D_Q, both expressed using the standard units of cm^2/s. The other parameter that must be considered is the distance at which M and Q are said to interact. This interaction distance is sometimes expressed as the sum of the interaction radii for M and Q, r_M and r_Q, respectively.

The bimolecular rate constant k_{diff} for diffusion-limited encounter of two solvated species M and Q is shown in equation 17 where N is Avogadro's number. (Note: In the present context, k_{diff} should be expressed with the units of $mol^{-1}\ L\ s^{-1}$).

$$k_{diff} = 4\pi N \ (r_M + r_Q)(D_M + D_Q) \quad (17)$$

By the same token, the unimolecular rate constant for diffusion dependent dissociation of the encounter pair can be expressed by equation 18.

$$k_{-diff} = \frac{3\,(D_M + D_Q)}{(r_M + r_Q)^2} \quad (18)$$

In this study, the molecules M and Q are approximately the same size and thus have approximately the same diffusion coefficient in the solvent acetonitrile: $D_M \sim D_Q \sim$ 2-3 x 10^{-5} cm^2/s. A reasonable estimate for the M-Q interaction distance is 6 Å.

(ii) Equilibrium constant for electron transfer, K_{et}.

As shown in equation 19, the equilibrium constant for electron transfer, K_{et}, can be expressed as a function of the Gibbs energy difference, ΔG_{et}, between the encounter pair and the radical ion pair.

$$K_{et} = \frac{k_{et}}{k_{-et}} = e^{-\Delta G_{et}/RT} \quad (19)$$

This Gibbs energy for electron transfer can be illustrated as the difference between the minima of potential curves for the encounter pair and the radical ion pair (Figure 7).

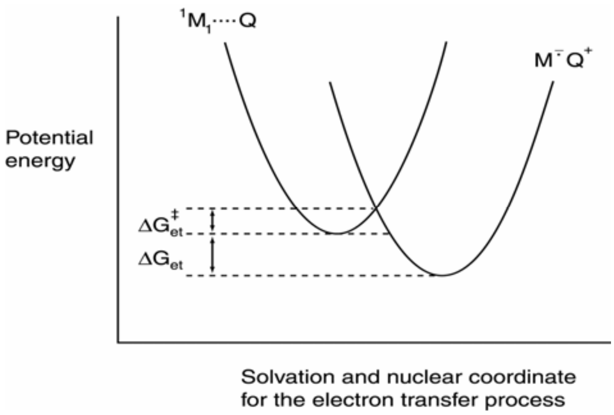


Figure 7. Potential curves that illustrate an exothermic electron transfer process. The x-axis represents changes that occur upon electron transfer in (1) the solvation shell surrounding the M-Q pair, and (2) the configuration of nuclei in the M-Q pair.

With the simplistic molecular orbital diagram of Figure 5 in mind, it can be seen that one can estimate ΔG_{et} for electron transfer from Q to M using the oxidation potential of Q, $E(Q/Q^{+\cdot})$, and the reduction potential of M, $E(M^{-\cdot}/M)$. Specifically, one must consider the energy that must be put into the system to remove an electron from Q, and the energy that will be gained when M acquires an electron. However, since M in this system is in an excited state, $^1M_1$, one must account for the energy of M excitation, $\Delta E_{0,0}$ (equation 20).

$$\Delta G_{et} \cong E(Q/Q^{+\cdot}) - E(M^{-\cdot}/M) - \Delta E_{0,0} + E_{solv} \quad (20)$$

When writing equation 20, it is important to recognize that we have included a term, E_{solv}, to account for the energy associated with the Coulombic attraction of the positive and negative charge in the radical ion pair. In the polar solvent acetonitrile, the medium in which our experiments will be done, this energy is ~ -5 kJ/mol.

(iii) Rate constant for ion pair decay to generate ground states, k_{decay}.

In general, the rate constant for a given process from i to j, k_{ij}, can be expressed as a function of the activation barrier, $\Delta G^{\ddagger}$, for this process (equation 21).

$$k_{ij} \cong k^{\ddagger} e^{-\Delta G^{\ddagger}/RT} \qquad (21)$$

We now apply equation 21 to obtain an estimate for k_{decay}. With respect to Scheme 1, we assume that the process of electron transfer is sufficiently exothermic so that $\Delta G^{\ddagger}$ will be very small. Under these conditions, the overall rate constant k_{ij} will thus be defined by the frequency factor $k^{\ddagger}$. For a process in acetonitrile, this will be on the order of 1-4 x 10^{11} s^{-1}.

(iv) Rate constant for electron transfer from Q to $^1M_1$, k_{et}.

As shown in equation 21, the rate constant for this process of electron transfer, k_{et}, can be expressed as a function of the activation barrier for electron transfer, $\Delta G_{et}^{\ddagger}$. It thus becomes necessary to find an approach by which $\Delta G_{et}^{\ddagger}$ can be estimated.

As illustrated in Figure 7, $\Delta G_{et}^{\ddagger}$ can be represented by considering the point at which the $^1M_1$-Q and $M^{\cdot -}$-$Q^{\cdot +}$ potential surfaces intersect. Upon further examination of Figure 7, it should become apparent that the magnitude of this activation energy will depend on the energy difference between the minima of the respective potential surfaces (i.e., $\Delta G_{et}^{\ddagger}$ will depend on ΔG_{et}). In a landmark study on electron-transfer reactions, R. Marcus used parabolas to represent the $^1M_1$-Q and $M^{\cdot -}$-$Q^{\cdot +}$ potential surfaces and, from this, was able to write an expression showing the dependence of $\Delta G_{et}^{\ddagger}$ on ΔG_{et} (as an aside, Marcus was awarded the Nobel prize in chemistry for this seminal work on electron transfer reactions). Over the years, a variety of different expressions have evolved showing how $\Delta G_{et}^{\ddagger}$ can depend on ΔG_{et}. One such expression, derived by Rehm and Weller, accounts for the diffusion-controlled limit of reactions in solution and is given in equation 22.

$$\Delta G_{et}^{\ddagger} = \left[\left(\frac{\Delta G_{et}}{2}\right)^2 + \left(\Delta G_{et}^{\ddagger}(0)\right)^2\right]^{1/2} + \frac{\Delta G_{et}}{2} \qquad (22)$$

By using this expression, Rehm and Weller were able to model the experimental observation that, for sufficiently negative values of ΔG_{et}, the reaction proceeds at the diffusion-controlled limit (i.e., $k_{ij} = k_{diff}$). In equation 22, $\Delta G_{et}^{\ddagger}(0)$ represents the activation energy for electron transfer in a thermoneutral reaction where $\Delta G_{et} = 0$, and for our present study we will assume that $\Delta G_{et}^{\ddagger}(0) = 10$ kJ/mol.

Thus, using equation 22, one can obtain a value for $\Delta G_{et}^{\ddagger}$ which, in turn, can be used to obtain k_{et} via equation 21.

In conclusion, you will use equation 16 to calculate the values of k_q that would be expected if $^1M_1$ quenching proceded via an electron transfer process. If these values of k_q, for the two different quenchers, were equivalent to the corresponding values experimentally obtained in the Stern-Volmer study, you will argue that, for the system in question, quenching indeed proceeds via an electron transfer mechanism.

Additional experimental results needed to solve equation 20:

$E(Q/Q^+)-E(M^-/M)=$ 2.92 +/- 0.04 V for dimethoxybenzene
3.22 +/- 0.04 V for naphthalene

Written Laboratory Report:

Your report should NOT exceed 20 pages and contain the following material:

(1) A summary of all the recorded data (e.g., absorption and emission spectra, Stern-Volmer plots, quenching rate constants obtained from the Stern-Volmer plots and the calculation results for the theoretical quenching constant assuming electron transfer).

(2) A discussion of your error (i.e., ± uncertainty on given numbers) and how these errors were propagated to ultimately yield the error bars used on the final quenching rate constants reported.

(3) Answers to the 3 questions posed throughout this manual (in a separate section).

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