RESONANCE ENERGY TRANSFER STUDIES: DEPENDENCE OF TRANSFER PROPERTIES ON THE STRUCTURE OF THE DONOR MOLECULE

BARTON STEPHEN SOLOMON

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BY

BARTON STEPHEN SOLOMON

B. S., Union College, 1962

A THESIS
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This thesis is dedicated to the author's wife, Doris, and to his parents.

[Signature]

[Name]
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INTRODUCTION

The transfer of electronic excitation energy from an excited donor atom or molecule to a ground-state acceptor atom or molecule, was first observed by Carlo and Franck in their classical experiments on the sensitized fluorescence of thallium atoms by mercury atoms in the vapor phase. The first observations of energy transfer in solution were made by J. Perrin and Mlle. Choucroun. Since these early experiments, energy transfer has been studied extensively in organic scintillators, biological systems and photochemical systems.

Singlet-singlet resonance energy transfer by a dipole-dipole mechanism is illustrated by the energy level diagram of Figure 1. First the donor molecule is excited to a vibrational-rotational level of an excited electronic state, generally the first excited state (A). From there the molecule is converted to lower vibrational-rotational levels of the first excited state by obtaining thermal equilibrium (B) with its surroundings. This process takes place in $10^{-13}$ to $10^{-12}$ seconds and the excited molecule remains in one of its lowest vibrational-rotational levels for the remainder of its $\sim 10^{-8}$ second lifetime. After this time-interval, the molecule returns to the ground-state by either a radiative (fluorescence) (C) or a non-radiative (internal conversion) (D) process. The excited donor molecule
Figure 1  ENERGY LEVEL DIAGRAM

A - Absorption  E - Intersystem Crossing
B - Thermal Relaxation  F - Phosphorescence
C - Fluorescence  G - Energy Transfer by Coupled Transitions
D - Internal Conversion
may also undergo intersystem crossing (E) to a triplet state and from there return to the ground-state by phosphorescence (F) or intersystem crossing. In the presence of an acceptor molecule another deactivation path is available to the excited donor molecule; it can transfer its energy to a ground-state acceptor molecule (G). In order for this transfer to take place, the energy difference for one of the possible deactivation transitions in the donor molecule must correspond exactly to the energy of an excitation transition in the acceptor molecule. If there is sufficient energetic coupling between these molecules, both processes, the deactivation of the excited donor and the activation of the acceptor, occur simultaneously. This is referred to as a coupled transition and results in a transfer of excitation energy from donor to acceptor. The energetic coupling is directly dependent upon the overlap of the donor's fluorescence spectrum with the acceptor's absorption spectrum.

The excited acceptor molecule resulting from the energy transfer process can then either fluoresce, undergo internal conversion, or intersystem crossing. The fluorescence of the acceptor may then provide a method of observing the energy transfer process. It may appear that one is observing the absorption of the donor fluorescence by the acceptor and subsequent emission by the acceptor; this is known as the trivial process. The mechanism of resonance energy transfer is entirely different occurring before the emission of donor fluorescence with the result that there
is a decrease in the lifetime of the excited donor.

Energy transfer was first treated theoretically by J. Perrin\textsuperscript{3,4} using classical physics. He considered the molecule to be represented as one electron bound to a molecular framework by a quasi-elastic force so that the electron can vibrate with a certain frequency, $v$. The system was then considered to be a collection of oscillators which can interact with one another. The interaction energy decreases like that of two dipoles which is proportional to the inverse third power of the intermolecular distance. If a second molecule is nearby the excited one and has available the same frequency of oscillation, then the excitation energy can be transferred before the excited molecule can emit radiation. Transfer distances of $\frac{\lambda}{2\pi}$, where $\lambda_0$ is the wavelength of the oscillators, are predicted by this theory to be on the order of 1000Å. This theory qualitatively describes energy transfer but fails quantitatively since the largest transfer distances observed experimentally are about 80Å.\textsuperscript{5}

The quantum mechanical description of resonance energy transfer is much more successful in its quantitative treatment but it is more difficult to visualize physically. The classical theory was first revised by F. Perrin\textsuperscript{6} using quantum mechanics. Perrin's refinement of the classical treatment of resonance energy transfer was used to describe energy transfer between like molecules in solution. According to Förster\textsuperscript{5}, Perrin's theoretical approach to energy transfer does not give a good quantitative description of
the process. The quantitatively successful quantum mechanical treatment of resonance energy transfer has been given by Förster\textsuperscript{7,8}. The interaction between molecules is governed by their quantum mechanical transition moments which also determine the absorption and emission characteristics of the molecules. The interaction energy resulting from the interaction of the transition moments is of a dipole-dipole nature and has an inverse proportionality to the third power of the intermolecular distance. The probability of energy transfer, which is proportional to the square of this interaction energy, therefore, decreases with the sixth power of the intermolecular distance.

Förster's theory\textsuperscript{7,8} of resonance energy transfer is an extension of earlier ideas by J. Perrin\textsuperscript{3,4}, F. Perrin\textsuperscript{6}, Kallman and London\textsuperscript{9}, and Vavilov.\textsuperscript{10} This theory leads to the following equation for the critical transfer distance \((R_0)\), the distance at which there is a 50\% probability of the energy being transferred.

\[
R_0^6 = \frac{9000 \ln(10) k^2 \eta_D^0}{128 \pi n^4 N} \int_0^\infty f_D(\nu) \epsilon_A(\nu) \frac{d\nu}{\nu^4}
\]  

(1)

Here, \(\nu\) is the wavenumber, \(\epsilon_A(\nu)\) is the molar absorptivity of the acceptor, \(f_D(\nu)\) is the spectral distribution of fluorescence of the donor (measured in quanta and normalized to unity on a wavenumber scale), \(N\) is Avogadro's number, \(n\) is the refractive index of the solvent, \(\eta_D^0\) is the fluorescence quantum yield of the donor and \(k\) is a numerical factor which
depends on the mutual orientation of both molecules.

Equation (1) is sometimes inconvenient to use for two reasons. Fluorescence spectra measured with most spectrofluorometers must be corrected for the wavelength dependence of the detector system (monochromator and photodetector); this involves a time-consuming and difficult procedure. The precise measurement of fluorescence quantum efficiencies is also experimentally difficult. In this work, corrected fluorescence spectra and fluorescence quantum efficiencies have been measured; therefore, equation (1) can be used quite easily. The use of equation (1) to calculate \( R_0 \) will be referred to as method A.

Equation (1) has been simplified using the approximate mirror-image symmetry of the fluorescence and absorption spectra of the donor. Equation (2) is the result of this treatment.

\[
R_0^6 = \frac{9 \cdot 10^6 (\ln 10)^2 k^2 c t_D}{16 \pi^4 n^2 (2\nu_o - \nu)^2} \int_0^\infty \varepsilon_A(\nu) \varepsilon_D(2\nu_o - \nu) d\nu
\]  

(2)

For a random directional distribution \( k^2 \) equals \( 2/3 \) and the substitution of this into equation (2) leads to equation (3).

\[
R_0^6 = \frac{3 \cdot 10^6 (\ln 10)^2 c t_D}{8 \pi^4 n^2 (\nu_o)^2} \int_0^\infty \varepsilon_A(\nu) \varepsilon_D(2\nu_o - \nu) d\nu
\]  

(3)

Here, \( c \) is the velocity of light, \( \nu_o \) is the arithmetic mean of the absorption and fluorescence maxima of the donor in wavenumbers, \( \varepsilon_D(2\nu_o - \nu) \) is the molar absorptivity of the
donor and $t_D$ is the actual mean lifetime of the excited donor.

The integral in equations (1), (2) and (3) is called the overlap integral and reflects the region of coincidence between the donor's fluorescence spectrum and the acceptor's absorption spectrum; this is shown graphically in Figure 2. The second term of the integral in equations (2) and (3) gives an approximation to the donor's fluorescence spectrum by reflecting its absorption spectrum about $v_0$ to give the fluorescence spectrum of the donor in absolute units. This reflection procedure involves the assumption of the mirror-image symmetry of the absorption and fluorescence spectra of the donor. Since energy transfer takes place from a molecule's first excited state, it is necessary to separate the portions of the donor's absorption spectrum which are responsible for transitions to the first and second excited states. Only the portion of the spectrum which represents transitions to the first excited state is used. If the donor lifetime is not known, it can be calculated using equation (4) given by Karreman and Steele.  

$$\frac{1}{t_D} = \frac{8\pi n^2 (\ln 10) c}{\eta_D^0 N'} \int_0^\infty \frac{(2v_0 - \nu)^3}{\nu} \epsilon_D(\nu) d\nu$$

Here, $\eta_D^0$ is the fluorescence quantum efficiency of the donor and $N'$ is the number of molecules per cubic centimeter in a 1.0 molar solution.

Equations (3) and (4) can be used, with the fluorescence
Figure 2

The Overlap of the Donor's Fluorescence Spectrum with the Acceptor's Absorption Spectrum
quantum efficiency of the donor, the absorption spectra of
the donor and the acceptor and the fluorescence spectrum
of the donor, to calculate theoretical values of the critical
transfer distance. The use of equations (3) and (4) will
be referred to as method B.

Theoretical critical transfer distances may be
calculated using either method A or method B. Method A
gives more accurate values than method B for two reasons.
First, method B is based upon the approximate mirror-image
symmetry of the fluorescence and absorption spectra of the
donor. This is only true for molecules which have nearly
the same geometry in both the ground-state and the first
excited-state. Second, method B involves integrals over
the donor absorption spectrum corresponding to transitions
to the first excited-state. The separation of that portion
of the donor's absorption spectrum corresponding to trans­
itions to the first excited-state may involve considerable
error. For these two reasons, method A is expected to
give more accurate values for the critical transfer distance.

Resonance energy transfer can be observed experi­
mentally by many different techniques. The first experi­
mental observations of energy transfer in solution were
made by J. Perrin and Mlle. Choucroun\textsuperscript{2} and were of a
qualitative nature. Förster\textsuperscript{12,13} quantitatively followed
the quenching of the donor fluorescence in his studies of
energy transfer with trypanflavine and rhodamine B. He was
able to establish that transfer occurred over distances of
70\AA \ and showed it to be the non-trivial kind by the decrease
in donor lifetime indicated in his quenching experiments. Förster was the first investigator to measure critical transfer distances. A large number of like systems have been studied with similar results by Galanin and Levshin by measuring the decrease in donor lifetime directly.

Some of the most important experiments in energy transfer were performed by Bowen, Brocklehurst and Livingston where any possible trivial mechanism was excluded. In these experiments a constant ratio of donor (1-chloroanthracene) to acceptor (perylene) was used so that donor and acceptor absorb constant fractions of excitation light. Therefore, the increase of acceptor fluorescence with increasing concentration must be due to energy transfer from the donor. The trivial re-absorption process was taken into account in the authors' kinetic treatment of energy transfer by assuming that all of the light emitted by the donor is in fact absorbed by the acceptor. Then the probability of the "trivial" process is just the fluorescence quantum efficiency of the donor. This method enabled the authors to show that energy was transferred by a mechanism different from the trivial mechanism. The authors also studied the effect of solvent viscosity upon energy transfer. Energy transfer was found to be independent of solvent viscosity which showed the process not to be diffusion controlled. The transfer was shown to occur over mean intermolecular distances between donor and acceptor of about 40Å, corresponding to concentrations of $10^{-3}$ to $10^{-2}$M. Actual critical
transfer distances were not measured in this study.

Many other workers have measured critical transfer
distances by different methods and have obtained good
quantitative results. Resonance energy transfer between
aromatic amino acids in proteins has been studied by Weber\textsuperscript{18,19},
who used fluorescence polarization spectra to measure critical
transfer distances. Melhuish\textsuperscript{20} has studied the effect of
solvent viscosity upon energy transfer and measured critical
transfer distances by relating the rate constant of energy
transfer, which was measured using the procedure of Bowen
and Livingston\textsuperscript{16}, to \( R_0 \). Critical transfer distances for
energy transfer between aromatic hydrocarbons have been
measured by Ware\textsuperscript{21} using the variation of the donor life-
time with acceptor concentration. The time dependence of
the decay of excited donor molecules was used to measure
critical transfer distances by Bennett and co-workers\textsuperscript{22,23}
in their studies of singlet-singlet and triplet-singlet
energy transfer. Basu and Greist\textsuperscript{24} have studied energy
transfer by measuring energy transfer efficiencies. The
transfer efficiency, \( T_{12} \), was given as

\[
T_{12} = \frac{(I_{21}/I_{22}) - \alpha_{21}}{\alpha_{11}},
\]

where \( I_{21} \) and \( I_{22} \) are the acceptor fluorescence intensities
under indirect (by transfer) and direct excitation with equal
fluxes of exciting photons, \( \alpha_{11} \) is the fraction of indirect
excitation absorbed by the donor and \( \alpha_{21} \) is the fraction of
indirect excitation absorbed by the acceptor. Cohen and
Weinreb$^{25,26}$ have also measured energy transfer efficiencies and from their variation with acceptor concentration have been able to measure critical transfer distances for organic scintillator systems. Birks and co-workers$^{27-29}$ have used a similar approach to study energy transfer in organic scintillators and wavelength shifters. A wavelength shifter is a secondary solute added to a scintillator solution to obtain more efficient matching between the wavelength of maximum emission and the wavelength of maximum sensitivity of the detector. Birks has given a complete theoretical justification and experimental description of his method. This method, which has been used in this investigation, is described below.

In Birks' method, the critical transfer distance is evaluated from the dependence of the transfer efficiency upon the acceptor concentration; the donor concentration is high and held constant. The energy transfer efficiency is determined from the acceptor fluorescence intensity measured under "direct" and "indirect" excitation of solutions which contain both the donor and acceptor. Light, which is absorbed almost exclusively by the acceptor and results in acceptor fluorescence, is used for "direct" excitation. "Indirect" excitation is provided by light of a different frequency which is absorbed primarily by the donor; the majority of the acceptor's fluorescence is the result of energy transfer from excited donor molecules.
Birks derived the following equation:

\[ f = \frac{I_A}{I_{oA}} - pc \left(1 - \frac{I_A}{I_{oA}}\right) \]  

(6)

which relates the transfer efficiency \( f \) to the experimentally measured \( I_A \), the acceptor fluorescence intensity excited indirectly (by transfer), \( I_{oA} \), the maximum acceptor fluorescence intensity excited directly at high c (acceptor concentration), and \( p \), the ratio of the molar absorptivities of acceptor to donor at the wavelength used for the indirect excitation.

\[ p = \frac{\varepsilon_A}{\varepsilon_D} \]  

(7)

A requirement for the valid application of equation (6) is that the donor concentration must be high so that most of the energy at the wavelength used for "indirect" excitation is absorbed by the donor. The last term in equation (6) is a correction factor for the portion of the excitation absorbed by the acceptor at the wavelength used for "indirect" excitation. This term is negligible except at high acceptor concentrations.

Birks also derived, by a kinetic treatment of resonance energy transfer, an equation which describes the dependence of the transfer efficiency upon the concentration of the acceptor.

\[ f = \eta^o_D + \sigma c (1-f) \]  

(8)

In equation (8) \( \eta^o_D \) is the molecular fluorescence quantum
efficiency of the donor and $\sigma$ is the ratio of the rate constant for energy transfer to the sum of the rate constants for fluorescence and internal conversion. Equation (8) is valid at high acceptor concentrations ($c > 10^{-4}$ M). Equation (8) can be rearranged to equation (9) which is just the Stern-Volmer equation for energy transfer given by Förster\textsuperscript{5}

$$f = \frac{\eta^0_D}{1 + \sigma c}$$

(9)

where

$$\sigma = \frac{2.05}{c_0}$$

(10)

The critical concentration ($c_0$) is related to the critical transfer distance by equation (11) which has been given by Förster\textsuperscript{5,8}.

$$c_0 = \frac{3000}{4\pi NR_0^3}$$

(11)

Using equations (10) and (11), one can relate the critical transfer distance to the experimentally measurable variable $\sigma$ by

$$R_0^3 = \frac{3000 \sigma}{4\pi N(2.05)}$$

(12)

The derivation of the Stern-Volmer equation for energy transfer by Förster and Birks' kinetic derivation of equation (12) are given in Appendix I.

The transfer efficiency ($f$) is experimentally determined using equation (6) as a function of acceptor concentration. This data is then plotted as $f$ versus $c(1 - f)$ and the slope
(σ) of the straight line obtained at high acceptor concentrations is used in equation (12) to calculate the experimentally measured values of $R_0$. These are then compared with the theoretical values obtained by methods (A) and (B). This approach has been used by Birks and co-workers\textsuperscript{27-29} and by Basile\textsuperscript{30} in their studies with organic scintillators.

Although resonance energy transfer has been studied in many chemical and biological systems, effects due to the systematic variation of the molecular structure of either the donor or the acceptor have received little attention. In this investigation two series of model compounds have been used as donors to study the effects of variation in molecular structure on the energy transfer properties of donor molecules. Since olefinic bonds may have some importance in photochemical systems\textsuperscript{31-33}, it was planned to study naphthalene derivatives with increasing numbers of olefinic bonds in a single side chain. However, we were unable to synthesize the higher members of the series with the result that the change in the compounds studied represents only small variations in the molecular structure. Naphthalene, 1-allylnaphthalene, 1-methylnaphthalene and 1-vinyl naphthalene were used as donors and acriflavine was used as the acceptor. These systems contain a weak donor and a strong acceptor. Normally with weak donors, small critical transfer distances would be expected, but reasonably large distances are obtained due to the long lifetimes of the naphthalene derivatives. The variation of molecular structure within this series of compounds is small, therefore
small differences in $R_0$ are expected.

The second series of compounds was chosen to study the effect of heteroatoms and of heteroatom containing groups placed between two pi-electron systems. The compounds have the general formula

![Chemical structure](image)

where $X$ is $-\text{CH}_2-$ for fluorene, $-\text{NH}-$ for carbazole, $-\text{CHOH}$ for 9-fluorenol, $-\text{O}-$ for dibenzofuran, and $-\text{S}-$ for dibenzothiophene. These compounds were used as donors and 9-phenylanthracene was used as an acceptor in this study of resonance energy transfer. In this series of compounds the variation of $X$ is a more drastic change of the molecular structure, therefore larger $R_0$ differences are expected.
EXPERIMENTAL

Absorption Spectra. Absorption spectra of naphthalene, the naphthalene derivatives and acriflavine were measured with a Beckman Model DU spectrophotometer; the spectra of carbazole, fluorene, 9-fluorenol, dibenzofuran, dibenzothiophene and 9-phenylanthracene were measured with a Bausch and Lomb 505 spectrophotometer. Quartz cells of 1.0 cm. path length were used in measuring all absorption spectra.

Fluorescence Spectra. A spectrofluorometer was designed and built for measuring fluorescence spectra. Excitation was provided by a 1000 watt mercury arc lamp (General Electric AH-6) in conjunction with a Bausch and Lomb High Intensity Grating Monochromator. The fluorescence was detected at a right angle to the excitation beam with a Jarrell-Ash Model 82-000 half-meter scanning monochromator equipped with a 1P28 photomultiplier tube. The output of the photomultiplier was amplified by a Leeds and Northrup Co. Microvolt Indicating Amplifier (Cat 9835-A) and could be displayed on a meter or recorded on a Houston Omnigraphic Corporation Model HR-96T x-y recorder. A block diagram of this apparatus is shown in Figure 3. Dilute solutions with absorptions of less than five percent were used in measuring fluorescence spectra in order to avoid inner filter effects.
Figure 3
The Spectrofluorometer
The emission monochromator-detector combination was calibrated so that "corrected emission spectra" could be obtained from the measured spectra. This calibration is necessary to correct for the wavelength-sensitive response of the monochromator-detector combination. Several methods have been described for correcting fluorescence spectra; the method of Parker, in which a precisely measured quantity of light, having a known frequency distribution, is directed into the monochromator-photomultiplier combination, was followed. The intensity of the light is recorded as a function of frequency, and a correction curve for the instrument is obtained by comparing the measured curve with the true curve for the source. This procedure is illustrated graphically in Figure 4. Curve A is the output of a calibrated tungsten lamp calculated directly from Wien's law using the known color temperature of the lamp under specified operating conditions. A General Electric type #1C.P. miniature photometric standard lamp (#431-2655), when operated at 5.39 volts and 0.2394 amperes producing a filament temperature of 2738°C, was used in the calibrations. The output curve of the calibrated lamp was calculated using the computer program in Appendix II. Curve B of Figure 4 is the photomultiplier output. The correction curve for the instrument is curve C, which was obtained by dividing curve B by curve A using the computer program in Appendix III. The correction factors, from which curve C is drawn, are given in Appendix V.
Figure 4 - Calculation of correction factors for a grating emission monochromator and a 1P28 photomultiplier tube. Curve A: output of a standard tungsten lamp in relative quanta per unit frequency interval. Curve B: photomultiplier output using fixed slit widths. Curve C: sensitivity curve (correction factors as a function of wavenumber).
Corrected Fluorescence spectra were calculated using the computer program in Appendix IV which is similar to one published by Drushel.\textsuperscript{41}

**Quantum Efficiencies.** The quantum efficiencies of the donor molecules were measured by the method described by Parker and Rees.\textsuperscript{38} In this method, the quantum efficiency ($\phi$) is determined by measuring the fluorescence yields of the unknown and of a reference compound of known quantum efficiency. This method requires that the fluorescence yields of the unknown and reference be measured on the same spectrofluorometer with the same intensity and wavelength of excitation light and at the same temperature. The fluorescence yields must also be corrected for the variation of the detector system's response with wavelength. The fluorescence yields, along with absorption data, make possible the calculation of the unknown quantum efficiency using equation (13).

$$\frac{F_2}{F_1} = \frac{\phi_2A_2}{\phi_1A_1}$$  \hspace{1cm} (13)

$F$ is the fluorescence yield and $A$ is the absorption of the solution at the wavelength of excitation. The reference compound used was 1.0 p.p.m. quinine sulfate in 0.10 N sulfuric acid; its quantum efficiency of 0.51 was measured by Melhuish.\textsuperscript{42,43} The fluorescence yields were corrected for the variation of detector system's response with wavelength using the computer program given in Appendix IV.
Initial Experimental Set-up for Measurement of $R_0$'s.

The first attempts at observing energy transfer experimentally were made using the apparatus pictured in Figure 3 with the excitation monochromator and the mercury arc rotated $90^\circ$ to give a straight-thru arrangement. The straight-thru configuration was used to obtain a longer path length of solution. The long path length is needed to ensure complete absorption of the excitation light. This is necessary because the experimental method of separating the radiative (trivial) and radiationless processes, which is described below, requires that all of the excitation be absorbed in an initial thin layer of the solution.

A number of difficulties were encountered using this experimental arrangement. First, it was impossible to eliminate all of the scattered radiation, which made it difficult to interpret the results. Second, the complete absorption of the excitation in a thin layer was not assured because of the use of the high intensity mercury arc for excitation. Third, the use of a monochromator to monitor the fluorescence was inconvenient because of the necessity to either record a complete fluorescence spectrum or to set the monochromator at a specific wavelength and measure the intensity only at that wavelength. For these reasons, the method of observing energy transfer was changed.

Final Experimental Set-up for Measurement of $R_0$'s.

An experimental set-up for the energy transfer studies was then designed and built. This apparatus is shown in Figure 5. The output of a Beckman hydrogen lamp is chopped mechanically
Figure 5

The Apparatus used for Energy Transfer Measurements
and, with a Bausch and Lomb High Intensity Grating Monochromator provides the excitation light. This light is reflected downward with a front-surface mirror and impinges upon the solution contained in a glass cell. The emission from the sample is detected with a 1P28 photomultiplier tube placed underneath the cell. The light filters between the sample and the photomultiplier tube provide for the separation of acceptor and donor emissions. The slits and the shutter between the filters and the photomultiplier allow for the regulation of the amount of light hitting the photomultiplier. The output of the photomultiplier is amplified by an Electronic Missiles and Communications Lock-In Amplifier Model RJB (Serial No. 188) and displayed on a meter. The light from a tungsten lamp, which is mechanically chopped by the same chopper used in the excitation light path, shines on a RCA 918 phototube producing the reference signal for the lock-in-amplifier. The high voltage for the 1P28 photomultiplier tube is provided by a John Fluke Manufacturing Company High Voltage Power Supply Model 412B. This experimental set-up has the following advantages: (1) the use of a low intensity source eliminates the problem of scattered radiation and assures complete absorption of the excitation in a thin layer; (2) the cell configuration allows for the variation of solution depth; and (3) fluorescence intensities are easily obtained.

Energy Transfer Studies. The donor concentration in these studies was $1.0 \times 10^{-1} \text{M}$ for naphthalene and naphthalene derivatives as donors and was $2.0 \times 10^{-2} \text{M}$ for carbazole,
fluorene, 9-fluorenol, dibenzofuran and dibenzothiophene; the acceptor concentration was varied from $1.0 \times 10^{-6}$ to $4.0 \times 10^{-3}$ M, with particular emphasis being placed on the region from $1.0 \times 10^{-4}$ to $4.0 \times 10^{-3}$ M. The high donor concentration is necessary to insure that all incident light is absorbed in a thin layer at the top of the solution. A path length of 1.15 cm. was used and could be varied by changing the volume of the solution.

Quenching curves were obtained for all the systems studied by monitoring the donor fluorescence as a function of the acceptor concentration. The energy transfer studies were accomplished by monitoring the acceptor fluorescence as a function of acceptor concentration for both "direct" excitation of the acceptor and "indirect" excitation of the acceptor. "Indirect" excitation was provided by light which is absorbed by the donor. Acceptor fluorescence resulted exclusively by energy transfer from the donor molecules. "Direct" excitation was provided by light which is absorbed primarily by the acceptor and results in acceptor fluorescence directly. Table 1 lists the various donors and acceptors, the excitation wavelength used for these compounds, and the filters used to monitor the fluorescence of the compounds.

Duplicate determinations of these measurements were made in all cases. All solutions were prepared in 95% ethanol and were deaerated by flushing with nitrogen for 15 minutes prior to use. The fluorescence of the standard solution of fluorescein in methanol was measured each time measurements were made to provide a correction for light intensity changes.
Table 1

Excitation Wavelengths and Emission Filters for Various Donors and Acceptors

<table>
<thead>
<tr>
<th>Compound</th>
<th>Donor or Acceptor</th>
<th>Excitation Wavelength</th>
<th>Emission Filter Color Spec.#</th>
<th>Turner* #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>Donor</td>
<td>310\textmu m</td>
<td>7-60</td>
<td>110-811</td>
</tr>
<tr>
<td>1-Methyl-naphthalene</td>
<td>Donor</td>
<td>310\textmu m</td>
<td>7-60</td>
<td>110-811</td>
</tr>
<tr>
<td>1-Allyl-naphthalene</td>
<td>Donor</td>
<td>310\textmu m</td>
<td>7-60</td>
<td>110-811</td>
</tr>
<tr>
<td>1-Vinyl-naphthalene</td>
<td>Donor</td>
<td>310\textmu m</td>
<td>7-60</td>
<td>110-811</td>
</tr>
<tr>
<td>Fluorene</td>
<td>Donor</td>
<td>300\textmu m</td>
<td>7-60</td>
<td>110-811</td>
</tr>
<tr>
<td>Carbazole</td>
<td>Donor</td>
<td>300\textmu m</td>
<td>7-60</td>
<td>110-811</td>
</tr>
<tr>
<td>Dibenzofuran</td>
<td>Donor</td>
<td>300\textmu m</td>
<td>7-60</td>
<td>110-811</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>Donor</td>
<td>300\textmu m</td>
<td>7-60</td>
<td>110-811</td>
</tr>
<tr>
<td>9-Fluorenol</td>
<td>Donor</td>
<td>300\textmu m</td>
<td>7-60</td>
<td>110-811</td>
</tr>
<tr>
<td>Acriflavine</td>
<td>Acceptor</td>
<td>400\textmu m</td>
<td>2A-12</td>
<td>110-818</td>
</tr>
<tr>
<td>9-Phenylanthracene</td>
<td>Acceptor</td>
<td>365\textmu m</td>
<td>2A</td>
<td>110-816</td>
</tr>
</tbody>
</table>

*G. K. Turner Assoc., Palo Alto, California
The energy transfer efficiency was then determined as a function of the acceptor concentration using the following equation.

\[ f = \frac{I_A}{I_{0A}} - pc \left( 1 - \frac{I_A}{I_{0A}} \right) \]  

(6)

Here, \( I_A \) is the acceptor fluorescence intensity excited indirectly, \( c \) is the acceptor concentration, \( I_{0A} \) is the acceptor fluorescence intensity excited directly at high acceptor concentration, and \( p \) is the ratio of the molar absorptivities of acceptor to donor at wavelength used for indirect excitation. The transfer efficiency \( (f) \) was then plotted versus \( c(1-f) \), and the slope \( (\sigma) \) of the straight line, which is obtained at high acceptor concentrations, was determined using a least squares procedure. The slope \( (\sigma) \) was then used in the following equation to determine the experimental value of the critical transfer distance.

\[ \frac{3}{R_0^3} = \frac{3000 \sigma}{4\pi n (2.05)} \]  

(12)

Calculation of Theoretical \( R_0 's \). Theoretical critical transfer distances have been calculated by methods A and B described previously. Bennett\(^{22}\) used method A for these calculations and substituted values for the physical constants into equation (1) to arrive at

\[ R_0 = \frac{9.0 \times 10^{-25} (k^2) \gamma_D}{4 n} \int_0^\infty f_D(\nu)\epsilon_A(\nu)\frac{d\nu}{\nu^4} \]  

(14)

where \( k^2 = 2/3 \), \( n \) is the refractive index of the solvent.
(1.34)^{45}, \eta_D^0 is the fluorescence quantum efficiency of the donor, and \textit{f}_D(\nu) is the corrected fluorescence spectrum of the donor. The integrals were evaluated graphically.

Method B was also used to calculate critical transfer distances using equations (3) and (4). All integrals were evaluated graphically.

**Chemical Preparations.** Acriflavine (Trypaflavine), obtained from the Bios Laboratories, was purified using the method of Gailliot\(^{46}\) by treating with excess freshly precipitated silver oxide and left standing at 0°C overnight. The solution was filtered, neutralized to pH 7 with hydrochloric acid, and evaporated to dryness. The resulting solid was recrystallized four times from methanol, at which point a constant fluorescence intensity was reached.

Naphthalene (Fisher Reagent Purified Crystals) was recrystallized twice from methanol. (m.p. 80.2°C, lit. 80.2°C.)

1-Methylnaphthalene (Eastman Kodak white Label) was fractionally distilled through a 38 cm. Vigreaux column and then redistilled twice through a 15 mm. Vigreaux column equipped with greaseless O-ring joints. A middle fraction was taken each time. (b.p. 241-242°C, lit. 244.8°C; n_D^{20} 1.6150, lit. 1.6149.)

1-Vinylphenanthrene was prepared by reacting the Grignard reagent of 1-bromonaphthalene with acetaldehyde and dehydration of the resultant alcohol using the procedure of Klemm, Sprague and Ziffer\(^{49}\). It was purified by fractional distillation under reduced pressure twice through a 38 cm.
Vigreaux column and twice through a 15mm. Vigreaux column equipped with greaseless O-ring joints. The middle fraction was taken each time. (b.p. 4\textsubscript{mm} 98-99°C, lit.\textsuperscript{49} b.p. 3.7\textsubscript{mm} 100-101°C; n\textsubscript{D}\textsuperscript{20} 1.6412, lit.\textsuperscript{50} 1.6404).

1-Allylnaphthalene (Aldrich Chemical Co.) was fractionally distilled at reduced pressure once through a 38 cm. Vigreaux column and twice through a 15mm. Vigreaux column, the middle fraction being taken each time. (b.p. 8\textsubscript{mm} 127-128°C, lit.\textsuperscript{51} b.p. 8\textsubscript{mm} 127.5-128.5°C; n\textsubscript{D}\textsuperscript{25} 1.6085, lit.\textsuperscript{51} 1.6089).

1-(1-Naphthyl)-1,3 butadiene. The preparation of this compound was attempted by three different methods. Isaqulyants and Esayan\textsuperscript{52,53} report the preparation of 1-(1-Naphthyl)-1,3 butadiene by the dehydrohalogenation of 1-naphthyl-3-chlorobut-2-ene. This method was attempted, but no product identifiable as 1-(1-Naphthyl)-1,3 butadiene could be isolated from the reaction mixture. The second method which was attempted was the dehydration of 1-naphthyl allyl carbinol. Our results confirm those of Arnold and Coyner\textsuperscript{54}, in that the diene appears to polymerize too rapidly to allow isolation. Wittig and Schollkopf\textsuperscript{55} have prepared 1-phenyl-butadiene by reacting triphenylphosphinenvinylmethylene with benzaldehyde. A similar procedure was used with 1-naphthaldehyde for the third attempt at preparing 1-(1-naphthyl)-1,3 butadiene. No product could be isolated from the reaction mixture.

Carbazole (Eastman Kodak White Label) was recrystallized three times from ethyl acetate and then vacuum sublimed.
Dibenzofuran (Eastman Kodak technical grade) was sublimed under vacuum, recrystallized three times from ethyl acetate and finally sublimed under vacuum. (m.p. 244.5-245°C., lit. 244.8°C.).

Dibenzothiophene (Eastman Kodak White Label) was recrystallized three times from ethyl acetate and sublimed under vacuum. (m.p. 82.5-83.0°C., lit. 82.0-83.9°C.).

Fluorene (Eastman Kodak White Label) was recrystallized three times from ethyl acetate and sublimed under vacuum. (m.p. 98.5-99.0°C., lit. 98.8-101.2°C.).

9-Fluorenol (Aldrich Chemical Co.) was recrystallized three times from benzene and sublimed under vacuum. (m.p. 154.6-155.1°C., lit. 154-155°C.).

9-Fluorenone (Eastman Kodak White Label) was recrystallized three times using benzene and petroleum ether according to the procedure of Huntress, Hershberg and Cliff and then sublimed under vacuum. (m.p. 83.2-83.5°C., lit. 83.0-83.5°C.).

9-Phenylanthracene (Aldrich Chemical Co.) was recrystallized once from ethanol followed by two vacuum sublimations. (m.p. 153-153.5°C., lit. 153°C.).

95% Ethanol was distilled using a 38 cm. Vigreaux column.

All melting points were obtained using a Hoover melting point apparatus.
DISCUSSION

I. Naphthalene Derivatives as Donors in Resonance Energy Transfer

Absorption and Fluorescence Spectra. The absorption spectra of the four donor molecules (naphthalene, 1-allylnaphthalene, 1-methylnaphthalene and 1-vinylnaphthalene) and of the acceptor (acriflavine) were measured and were found to agree with those published in the literature. These spectra were used in calculating the theoretical values of the critical transfer distances.

The fluorescence spectra of naphthalene, 1-methylnaphthalene, 1-allylnaphthalene and 1-vinylnaphthalene, which were corrected using the procedure outlined earlier, are shown in Figure 6. These spectra have also been corrected for differences in absorption at 313\(\mu\)m, the excitation wavelength. The added conjugation found in 1-vinylnaphthalene produces the expected bathochromic shift of the fluorescence spectrum.

The fluorescence quantum efficiencies of naphthalene, 1-methylnaphthalene, 1-allylnaphthalene and 1-vinylnaphthalene were measured using the comparison method of Parker and Rees, which has been described earlier. Quinine Sulfate was used as the fluorescent reference compound; its quantum efficiency has been found by Heuwhish to be 0.51. The measured fluorescence quantum efficiencies of the four donor
Fluorescence Intensity

Wavenumber ($\nu^{-1}$)

Figure 6 - The corrected fluorescence spectra of 1-vinyl naphthalene (--), 1-methylnaphthalene (---), 1-allylnaphthalene (----), and naphthalene (-----). (excitation is at 313nm $\nu$).
molecules are listed in Table 2. The differences among the fluorescence quantum efficiencies are small and, therefore, are expected to contribute little to any differences in the critical transfer distances.

**Calculation of theoretical \( R_0 \)'s.** Theoretical critical transfer distances were calculated using both methods A and B. The overlap integrals and theoretical critical transfer distances calculated using method A are shown in Table 3. Method B was used to calculate the overlap integrals, lifetimes and theoretical critical transfer distances presented in Table 4. The differences between methods A and B are discussed in the introduction. The rather large differences between the two calculated critical transfer distances for naphthalene is probably caused by the difficulty in separating the portion of the absorption spectrum causing the transition to the first as opposed to the second excited state. The separation of these two portions of the absorption spectrum is necessary when using method B for the calculation of critical transfer distances. The small differences between the critical transfer distances for the other three systems, as calculated by the two different methods, are likely caused by the approximations used in deriving equations (3) and (4) which are used in method B.

The change of critical transfer distance with the molecular structure of the donor agrees with that which would be predicted from the structural changes. The ultraviolet absorption spectrum of 1-vinyl naphthalene should show a bathochromic shift with respect to the spectrum of naphthalene.
<table>
<thead>
<tr>
<th>Donor</th>
<th>Fluorescence Quantum Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>.19</td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>.21</td>
</tr>
<tr>
<td>1-Allylnaphthalene</td>
<td>.19</td>
</tr>
<tr>
<td>1-Vinylnaphthalene</td>
<td>.22</td>
</tr>
</tbody>
</table>
Table 3

Calculated Overlap Integrals and Critical Transfer Distances
(calculated using method A)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Overlap Integral</th>
<th>Theoretical $R_0$ in Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>$8.24 \times 10^{-15}$</td>
<td>26.</td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>$7.74 \times 10^{-15}$</td>
<td>26.</td>
</tr>
<tr>
<td>1-Allylnaphthalene</td>
<td>$9.94 \times 10^{-15}$</td>
<td>27.</td>
</tr>
<tr>
<td>1-Vinylnaphthalene</td>
<td>$11.73 \times 10^{-15}$</td>
<td>28.</td>
</tr>
</tbody>
</table>
Table 4

Calculated Overlap Integrals, Lifetimes and Critical Transfer Distances
(calculated using method B)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Overlap Integral</th>
<th>Lifetime (sec.)</th>
<th>Theoretical $R_0 (Å)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>$12.00 \times 10^{10}$</td>
<td>$4.38 \times 10^{-9}$</td>
<td>28.</td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>$7.06 \times 10^{10}$</td>
<td>$4.96 \times 10^{-9}$</td>
<td>26.</td>
</tr>
<tr>
<td>1-Allylnaphthalene</td>
<td>$12.45 \times 10^{10}$</td>
<td>$4.59 \times 10^{-9}$</td>
<td>28.</td>
</tr>
<tr>
<td>1-Vinylnaphthalene</td>
<td>$12.92 \times 10^{10}$</td>
<td>$4.62 \times 10^{-9}$</td>
<td>29.</td>
</tr>
</tbody>
</table>
This can be rationalized by consideration of the free-electron model of pi-electron systems.\(^{64}\) 1-Vinyl-1-naphthalene will have a longer potential well, than naphthalene because of the ethylene side chain. The energy of excitation \(\Delta E\), is given in the free-electron model by

\[
\Delta E = \frac{(2k + 1) \hbar^2}{8ma^2},
\]

where \(k\) is a quantum number, \(\hbar\) is Planck's constant, \(m\) is the mass of the electron and \(a\) is the length of a one-dimensional box.\(^{64}\) Thus a molecule with a larger pi-electron system will have a smaller excitation energy or an absorption spectrum at longer wavelengths. This should shift the fluorescence spectrum of 1-vinyl-1-naphthalene to longer wavelengths and should result in a greater overlap integral and critical transfer distance for 1-vinyl-1-naphthalene than for naphthalene (see Tables 3 and 4). Alkyl substituents on aromatic compounds have little or no effect upon the fluorescence spectrum,\(^{65}\) therefore, naphthalene and 1-methylnaphthalene should have similar critical transfer distances. If there is any effect due to the methyl group, it will be a very small bathochromic shift caused by participation of electron pairs in the methyl group with an adjacent double bond, called hyperconjugation. Therefore, naphthalene and 1-methylnaphthalene are expected to have similar overlap integrals and critical transfer distances as shown in Tables 3 and 4. The high value of \(R_0\) for naphthalene calculated by method B has been discussed above. The bathochromic shift
of the absorption and fluorescence spectra of 1-allylnaphthalene
is expected to be greater than that for 1-methylnaphthalene
because of hyperconjugation between the methylene group
and both the aromatic system and the side chain double bond.
Qualitatively naphthalene and 1-methylnaphthalene are ex­
pected to have the smallest \( R_0 \), 1-allylnaphthalene should
have a larger \( R_0 \) and 1-vinyl naphthalene is expected to have
the largest \( R_0 \). The differences among the four \( R_0 \)'s are
expected to be small since the structural differences among
the four donors are small. These qualitative considerations
are in good agreement with the theoretical critical transfer
distances given in Tables 3 and 4.

Initial Measurements of \( R_0 \)'s. Critical transfer
distances were measured experimentally using the procedure
of Birks\(^2^9\) which is discussed in the experimental section.
The energy transfer efficiency \( (f) \) versus acriflavine
(acceptor) concentration curves for the four donor molecules
are shown in Figures 7 and 8. These curves were obtained
using the experimental set-up consisting of the mercury
arc, excitation monochromator, sample, emission monochromator
and detector, which is described earlier. "Indirect" ex­
citation was at 310 m\( \mu \), which is absorbed primarily by the
donor. "Direct" excitation was at 400 m\( \mu \), which is absorbed
principally by the acceptor.

In each curve, three distinct concentration regions
are observed. In the first region, from \( 10^{-5} \) to \( 10^{-4} \) M.,
radiational energy transfer (the trivial process) is dominant.
Figure 7 - Dependence of energy transfer efficiency on acriflavine concentration with naphthalene (○) and 1-methylnaphthalene (△) as donors. All donor concentrations are .1M. Direct excitation is at 400mμ and indirect excitation is at 310mμ.
Figure 8 - Dependence of energy transfer efficiency on acriflavine concentration with 1-vinylanthalene (□) and 1-allylnaphthalene (○) as donors. All donor concentrations are .1M. Direct excitation is at 400 m√ and indirect excitation is at 310 m√.
The transfer efficiency in this region is high and decreases with increasing concentration of the acceptor, a phenomenon resulting from inner filter effects, particularly self-absorption. In the second region, from $10^{-4}$ to $10^{-3}M$, a much more slowly decreasing and then slightly increasing transfer efficiency indicates that radiationless energy transfer is operative and is building to a maximum value. However, the mechanisms of excitation found in the first region are also found here. The third region, greater than $10^{-3}M$, is distinguished by a very gradual decrease in transfer efficiency; this is probably due to inner filter effects operant on the acceptor molecules which have been excited by radiationless energy transfer.

In the second region, where several paths of acceptor excitation are available, the data was treated in the following manner. Because the major peak reflects fluorescence from direct excitation and from radiational energy transfer, it is assumed that the shape of this curve will be similar to that found for the fluorescence from direct excitation of the acceptor at high concentrations. With this general profile, the major peak, found principally in region one and partially in region two, was extrapolated to $f = 0$. The higher concentration curves which are attributed to radiationless energy transfer were also extrapolated. Both these extrapolations are shown in Figures 9 and 10; the sum of these two components equals or nearly equals the measured transfer efficiency (indicated by the points in Figures 9 and 10).

The initial slopes of the radiationless energy transfer
Figure 9 - Graphical Extrapolation of transfer efficiency curves for naphthalene (●) and 1-methyl-naphthalene (△) as donors.
Figure 10 - Graphical Extrapolation of transfer efficiency curves for 1-vinylnaphthalene (□) and 1-allyl-naphthalene (●) as donors.
curves were measured, and the experimentally determined $R_0$ for each donor-acceptor system was calculated using equation (12). The experimental $R_0$'s, obtained are in good agreement with the theoretical values as shown in Table 5.

Even though there is very good agreement between the experimental and theoretical $R_0$'s, the experimental values of the critical transfer distance are questionable for four reasons. First, scattered radiation from the high intensity mercury arc may be complicating the interpretation of the transfer efficiency versus concentration curves. Second, this method of studying energy transfer requires that all of the exciting radiation be absorbed in the initial thin layer of the solution. This is not assured with the experimental set-up used because of the intense excitation source and relatively short path length. Third, the graphical extrapolation procedure is open to question and may involve considerable error. Fourth, the transfer efficiency versus concentration curves obtained in this work are not similar to those obtained by Birks. For these four reasons, the experimental set-up was redesigned as described in the experimental section to more nearly conform to the apparatus used by Birks.

Final Measurements of $R_0$'s. The energy transfer efficiency ($f$) versus acriflavine (acceptor) concentration curves for the four donor molecules determined using the new experimental set-up are shown in Figure 11. These curves are very similar to those obtained by Birks. Birks and Kuchela
Table 5

Observed and Theoretical Critical Transfer Distances*  
(the acceptor is acriflavine)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Observed $R_0$ (Å) (method A)</th>
<th>Theoretical $R_0$ (Å) (method A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Allynaphthalene</td>
<td>28.</td>
<td>27.</td>
</tr>
<tr>
<td>1-Vinynaphthalene</td>
<td>29.</td>
<td>28.</td>
</tr>
</tbody>
</table>

* Using initial method of measurement
Figure 11 - Dependence of the transfer efficiency on the acriflavine concentration. All donor concentrations are 0.1 M. Direct excitation is at 400 nm and indirect excitation is at 310 nm.
have discussed this type of curve thoroughly and have shown that for acceptor concentrations of less than $10^{-4}$ M, the primary mechanism of energy transfer is the trivial process, the emission of donor fluorescence followed by acceptor absorption and emission. By analogy with the work of Birks and Kuchela, the low values of $f$ observed in the concentration region below $2 \times 10^{-4}$ M. indicate that little energy is transferred by the trivial process in the four donor-acceptor systems studied.

In the concentration region around $2 \times 10^{-4}$ M. a sharp break in the transfer efficiency versus concentration curve is evident. This is caused by the increased importance of resonance energy transfer as the concentration of the acceptor is increased. The concentration region from $2 \times 10^{-4}$ M. to $2 \times 10^{-3}$ M. is of interest in the determination of critical transfer distances, since in this concentration region, resonance energy transfer predominates. The critical transfer distances were determined by plotting $f$ versus $c(1-f)$ for $c > 10^{-4}$ M. These plots are shown in Figure 12. The straight lines obtained for all four donor-acceptor systems indicate the validity of equation (8) at high acceptor concentrations ($c > 10^{-4}$ M.). The slopes of the straight lines were measured using a least squares procedure and substituted into equation (12). The critical transfer distances determined from these data are given in Table 6 and are compared with theoretical values calculated by methods A and B. The uncertainties in the theoretical and experimental critical transfer distances are $\pm 1.2 \, \AA$ and $\pm 0.8 \, \AA$ respectively. These un-
Figure 12 - Transfer efficiency versus c(l-f) plots for naphthalene (○), 1-methylnaphthalene (▲), 1-allylnaphthalene (●) and 1-vinyl naphthalene (□) as donors.
Table 6

Observed and Theoretical Critical Transfer Distances*  
(the acceptor is acriflavine)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Observed ( R_0 ) (( \AA ))</th>
<th>Theoretical ( R_0 ) (( \AA ))</th>
<th>(method A)</th>
<th>(method B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Allynaphthalene</td>
<td>27.</td>
<td></td>
<td>27.</td>
<td>28.</td>
</tr>
<tr>
<td>1-Vinylnaphthalene</td>
<td>12.</td>
<td></td>
<td>28.</td>
<td>29.</td>
</tr>
</tbody>
</table>

* Using final method of measurement
certainties were obtained from the precision of the quantities used to calculate the transfer distances and in no way reflect the approximations used to derive the expressions for the theoretical and experimental critical transfer distances.

The quenching curves of the donor emission with increasing acriflavine concentration shown in Figure 13 provide additional evidence for the occurrence of resonance energy transfer in these systems. The quenching in these systems occurs at higher acceptor concentrations than Birks and Kuchela\textsuperscript{29} observed for different systems in their work. This is consistent with the smaller critical transfer distances observed and predicted for the systems studied in this work.

The theoretical and measured critical transfer distances shown in Table 6 agree very well for naphthalene, 1-methylnaphthalene and 1-allylnaphthalene as donors. The measured $R_0$ for 1-vinylnaphthalene as donor is much lower than the theoretically calculated value. The anomalous behavior of 1-vinylnaphthalene is clearly seen in Table 6, in the energy transfer efficiency ($f$) versus acriflavine concentration curve of Figure 11 and in the quenching curve of Figure 13. The quenching curves provide a clue to one possible explanation for this behavior.

The fluorescence intensity of 1-vinylnaphthalene in the absence of any acriflavine is shown by these quenching curves to be much less than the fluorescence intensity of any of the other donors. From the fluorescence quantum
Figure 13 - Dependence of donor fluorescence intensity on the acriflavine concentration. All donor concentrations are 0.1 M. and excitation is at 310 nm.
efficiencies of Table 2, one would expect 1-vinylnaphthalene to have the most intense fluorescence, but the quantum efficiencies were measured at very low donor concentrations while the quenching curves were measured at very high donor concentrations. If in addition to self-quenching of fluorescence, the self-quenching process also competes successfully with energy transfer, then the energy transfer would be decreased as observed. Therefore, one possible explanation for the anomalous behavior of 1-vinylnaphthalene could be self-quenching of 1-vinylnaphthalene at high concentrations, i.e., a decrease in the quantum efficiency of the donor.

The self-quenching also explains the disagreement between the fluorescence quantum efficiency and the quenching curves. The self-quenching by 1-vinylnaphthalene has been investigated by studying the variation of 1-vinylnaphthalene fluorescence with concentration. The self-quenching of 1-vinylnaphthalene is compared with similar data for naphthalene in Figure 14. Naphthalene was chosen for comparison since its behavior is typical of that observed for the three other donor molecules. It is evident from Figure 14 that more self-quenching is observed with 1-vinylnaphthalene, than with naphthalene, especially at high concentrations. Since the energy transfer studies are conducted using high donor concentrations, the self-quenching present in the 1-vinylnaphthalene system may account in part for the very low $R_0$ observed experimentally.

Three other brief studies concerning the anomalous behavior of 1-vinylnaphthalene were carried out. These were
Figure 14 - Dependence of the fluorescence intensity of naphthalene (△) on its concentration and 1-vinylnaphthalene (■) on its concentration. Excitation is at 310nm.

Donor Concentration

Donor Fluorescence Intensity (Arbitrary Units)

10^{-5} M.  10^{-4} M.  10^{-3} M.  10^{-2} M.  10^{-1} M.
quenching of the acriflavine fluorescence by 1-vinylnaphthalene, and formation of ground-state and excited-state complexes between 1-vinylnaphthalene and acriflavine.

The effects of naphthalene and 1-vinylnaphthalene upon the acriflavine fluorescence were studied, and the results are shown in Figure 15. Naphthalene was again chosen for comparison with 1-vinylnaphthalene in these studies since its behavior is typical of that observed for the three donors other than 1-vinylnaphthalene. Figure 15 shows clearly that naphthalene has no effect upon the acriflavine fluorescence, but 1-vinylnaphthalene significantly quenches the acriflavine fluorescence, especially at high 1-vinylnaphthalene concentrations. Two possible explanations for this behavior are (a) quenching of the acriflavine fluorescence by 1-vinylnaphthalene and (b) complex formation between acriflavine and 1-vinylnaphthalene, which would result in quenching of the acriflavine fluorescence.

Ground-state complex formation between acriflavine and 1-vinylnaphthalene was investigated using absorption spectra of mixed solutions, but no evidence for a ground-state complex could be found. Evidence for the formation, to a small extent, of excited-state complexes between acriflavine and both naphthalene and 1-vinylnaphthalene was found in the fluorescence spectra of mixed solutions. The evidence for the formation of the excited-state complexes was the appearance of a fluorescence peak in mixed solutions, which was not present in the spectra of either of the pure substances.
Figure 15 - Dependence of the acriflavine fluorescence ($10^{-4}$ M.) on the naphthalene ($\triangle$) concentration and 1-vinylnaphthalene ($\Box$) concentration. Excitation is at 400 nm.
The new fluorescence peak was at 394 µm for solutions of naphthalene and acriflavine and at 402 µm for solutions of 1-vinyl naphthalene and acriflavine (see Figure 6 for the fluorescence spectra of naphthalene and 1-vinyl naphthalene). The excited-state complexes were detected only when the naphthalene or 1-vinyl naphthalene and acriflavine concentrations were greater than 10^{-3} M. Since complexes are formed in both systems, only to a slight extent and only at concentrations greater than 10^{-3} M., the observation, that 1-vinyl naphthalene quenches acriflavine fluorescence while naphthalene does not, is not explained by complex formation. The difference illustrated in Figure 15 is attributed to the specific quenching of acriflavine fluorescence by 1-vinyl naphthalene. Since the donor concentration is held constant for the measurement of R_0, it is difficult to see how the quenching of acriflavine fluorescence by 1-vinyl naphthalene can affect the experimental R_0.

The good agreement between the measured and theoretical values of the critical transfer distances for the systems of naphthalene, 1-methylnaphthalene, and 1-allylnaphthalene as donors and acriflavine as the acceptor indicates the applicability of Förster's dipole-dipole mechanism of energy transfer to these systems. The straight lines obtained for the plots of f versus c(1-f) for all four donor-acceptor systems demonstrates the validity of Birks' method of measuring these critical transfer distances. 1-Vinyl naphthalene, which was expected to give the largest measured R_0, gave a much smaller experimental critical transfer distance than predicted theor-
etically. The best explanation of this result is self-quenching of the 1-vinylnaphthalene fluorescence at high concentrations, which decreases the population of excited 1-vinylnaphthalene molecules. Since the experimental and theoretical critical transfer distances for the 1-vinylnaphthalene-acriflavine system do not agree, two conclusions can be reached. Either the experimental \( R_0 \) is the true value and, therefore, for this system the theoretical description of energy transfer is not correct, or the theoretical \( R_0 \) is the actual value and, therefore, the experimental method of measuring \( R_0 \) is lacking in this system. It is believed that in this case the self-quenching is interfering with the measurement of the critical transfer distance and invalidates the experimental method.

II. The Effect of Heteroatoms in Donor Molecules on Resonance Energy Transfer

Absorption and Fluorescence Spectra. The absorption spectra of the donors (carbazole, fluorene, 9-fluorenone, dibenzofuran, dibenzothiophene) and the acceptor, 9-phenylanthracene were measured and were found to agree with those published in the literature. These spectra were used to calculate the theoretical values of the critical transfer distances.

The corrected fluorescence spectra of the five donor molecules, carbazole, fluorene, 9-fluorenone, dibenzofuran and dibenzothiophene, are shown in Figures 16 & 17. These spectra have also been corrected for differences in absorption at 300\( \mu \text{m} \).
Figure 16 - The corrected fluorescence spectra of carbazole (---), fluorene(---), and dibenzofuran (---). (excitation is at 300mμ).
Figure 17 - The corrected fluorescence spectra of dibenzothiophene (----) and 9-fluorenol (-----). (excitation is at 300m\(\mu\)).
The corrected fluorescence spectrum of 9-phenylanthracene is shown in Figure 18.

The comparison method of Parker and Kees, which has been described in the experimental section, was used to measure the fluorescence quantum efficiencies of carbazole, fluorene, 9-fluorenol, dibenzofuran, dibenzothiophene and 9-phenylanthracene. Quinidine sulfate was used as the fluorescent reference compound. The measured fluorescence quantum efficiencies of the five donors are listed in Table 7. The fluorescence quantum efficiency of 9-phenylanthracene is 0.66. The fluorescence quantum efficiency of fluorene (0.50) obtained from this study is in reasonable agreement with the value of 0.53 determined by Weber and Teale. The decrease of the fluorescence quantum efficiency in the series fluorene > carbazole > dibenzofuran > dibenzothiophene > 9-fluorenol is in qualitative agreement with results obtained by Nur'mukhametov and Gobov. They found in the two series of compounds

\[
\text{[Diagram of compounds]} 
\]

where \( X = -\text{CH}_2-, -\text{O}-, -\text{S}-, \) and \(-\text{NH}-\), that the presence of a heteroatom \((-\text{O}-, -\text{S}-, -\text{NH}-\) facilitated excited singlet to triplet \((S\rightarrow T)\) intersystem crossing. This was observed as an increase of the phosphorescence yield when \( X \) was a heteroatom, especially sulfur.

The increased phosphorescence yield results from increased competition between intersystem crossing and fluorescence. This can be explained by considering spin-orbital coupling and
Figure 18 - The corrected fluorescence spectrum of 9-phenylanthracene (excitation is at 365 μm).
<table>
<thead>
<tr>
<th>Donor</th>
<th>Fluorescence Quantum Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorene</td>
<td>.50</td>
</tr>
<tr>
<td>Carbazole</td>
<td>.35</td>
</tr>
<tr>
<td>Dibenzofuran</td>
<td>.29</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>.03</td>
</tr>
<tr>
<td>9-Fluorenol</td>
<td>.01</td>
</tr>
</tbody>
</table>
Transitions between pure singlet and pure triplet states are forbidden because of the orthogonality of the spin wave functions; however, singlet-triplet transitions are observed. These transitions must take place between "impure" singlet and "impure" triplet states. These "impure" states are obtained by perturbing the pure states or mixing the pure states to obtain "impure" states. The perturbation causing this mixing of pure states is provided by the spin-orbital operator which arises from magnetic interactions between the orbital motion of an electron and the electron's spin magnetic moment. The quantum mechanical treatment of this leads to the following results: the degree of mixing is directly dependent upon $\mathcal{S}$ and inversely proportional to $(E_T - E_S)$. The term $\mathcal{S}$ is the radial part of the matrix element of the spin-orbit operator between the triplet and the perturbing singlet. $\mathcal{S}$ generally increases with increasing atomic number.

The term $(E_T - E_S)$ is the energy difference between unperturbed triplet and singlet states. The important result here is that the presence of a heteroatom increases the mixing of the singlet and triplet states and consequently increases the probability of singlet-triplet transitions; also, the probability of singlet-triplet transitions will generally increase with increasing atomic number of the heteroatom.

The differences among the fluorescence quantum efficiencies are large for this series of compounds. The differences reflect the increased intersystem crossing in the molecules with the heavier heteroatoms and are expected to
contribute to differences in the critical transfer distances. From the fluorescence quantum efficiencies one would expect 9-fluorenol and dibenzothiophene to have smaller critical transfer distances than fluorene, carbazole and dibenzofuran.

**Calculation of Theoretical $R_0$'s.** Method B has been used to calculate overlap integrals, lifetimes and theoretical critical transfer distances for the five donor-acceptor systems. These overlap integrals, lifetimes and theoretical critical transfer distances are shown in Table 8. The carbazole--9-phenylanthracene system has the largest $R_0$ since carbazole has the largest lifetime of the five donors and this system has the second largest overlap integral. The fluorene--9-phenylanthracene system has the largest overlap integral, but the short fluorene lifetime lowers the theoretical $R_0$ to 23 Å. The intermediate values of the overlap integral and lifetime for the dibenzofuran--9-phenylanthracene result in a theoretical $R_0$ of 21 Å. The short lifetimes of 9-fluorenol and dibenzothiophene result in the smallest theoretical critical transfer distances for these two donor-acceptor systems.

Theoretical critical transfer distances were also calculated by method A using the corrected fluorescence spectra of the donors, the fluorescence quantum efficiencies of the donors and the absorption spectrum of the acceptor. These critical transfer distances are shown in Table 9 along with the overlap integrals. The low fluorescence quantum efficiencies of dibenzothiophene and 9-fluorenol clearly explain the low critical transfer distances since both of these systems have large overlap integrals. Fluorene and dibenzofuran both have
Table 8
Calculated Overlap Integrals, Lifetimes and Critical Transfer Distances
(calculated using method B)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Overlap Integral</th>
<th>Lifetime in sec.</th>
<th>Theoretical $R_0$ in Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorene</td>
<td>$0.441 \times 10^{12}$</td>
<td>$0.37 \times 10^{-9}$</td>
<td>23.</td>
</tr>
<tr>
<td>Carbazole</td>
<td>$0.205 \times 10^{12}$</td>
<td>$2.03 \times 10^{-9}$</td>
<td>27.</td>
</tr>
<tr>
<td>Dibenzofuran</td>
<td>$0.094 \times 10^{12}$</td>
<td>$1.01 \times 10^{-9}$</td>
<td>21.</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>$0.085 \times 10^{12}$</td>
<td>$0.25 \times 10^{-9}$</td>
<td>16.</td>
</tr>
<tr>
<td>9-Fluorenol</td>
<td>$0.168 \times 10^{12}$</td>
<td>$0.03 \times 10^{-9}$</td>
<td>13.</td>
</tr>
</tbody>
</table>
Table 9
Calculated Overlap Integrals and Critical Transfer Distances
(calculated using method A)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Overlap Integral</th>
<th>Theoretical $R_0$ in Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorene</td>
<td>$1.52 \times 10^{-15}$</td>
<td>23.</td>
</tr>
<tr>
<td>Carbazole</td>
<td>$6.60 \times 10^{-15}$</td>
<td>27.</td>
</tr>
<tr>
<td>Dibenzofuran</td>
<td>$1.89 \times 10^{-15}$</td>
<td>21.</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>$4.09 \times 10^{-15}$</td>
<td>17.</td>
</tr>
<tr>
<td>9-Fluorenol</td>
<td>$3.31 \times 10^{-15}$</td>
<td>13.</td>
</tr>
</tbody>
</table>
small overlap integrals, but their relatively large fluorescence quantum efficiencies result in longer transfer distances. Carbazole has the largest overlap integral and a moderately large fluorescence quantum efficiency resulting in the largest $R_0$.

The two methods (A and B) of calculating theoretical critical transfer distances give nearly identical transfer distances for these five donor-acceptor systems, indicating that the approximations inherent in method B must be valid for these donor-acceptor systems.

**Measurement of $R_0$'s.** The procedure of Birks and co-workers$^{27-29}$, which is discussed in the experimental section, was used to measure the critical transfer distances for the five donor-acceptor systems. The energy transfer efficiency ($f$) versus 9-phenylanthracene (acceptor) concentration curves for the five donor-acceptor systems are shown in Figure 19. Light with a wavelength of 300 nm, which is absorbed by the donor, was used for "indirect" excitation. "Direct" excitation was provided by light with a wavelength of 365 nm, which is absorbed by the acceptor and gives acceptor fluorescence directly. These $f$ versus $c$ curves are similar to those obtained for the naphthalene compounds as donors.

These curves, as found by Birks, show two concentration-dependent regions. Below acceptor concentrations of about $10^{-4}$ M, energy is transferred from donor to acceptor by the trivial process, the emission of donor fluorescence followed by acceptor absorption and re-emission. In the acceptor con-
Figure 19 - Dependence of energy transfer efficiency on 9-phenylanthracene concentration for carbazole (△), fluorene (○), dibenzofuran (□), dibenzothiophene (●) and 9-fluorenol (▲) as donors. All donor concentrations are 2.0 x 10^{-5} M. Direct excitation is at 365 nm and indirect excitation is at 300 nm.
centration region around $10^{-4}$ M. The sharp increase in the transfer efficiency versus concentration is indicative of the increased importance of resonance energy transfer at high acceptor concentrations. The transfer efficiency curve above an acceptor concentration of $2 \times 10^{-4}$ M. was used to determine the experimental critical transfer distances.

The experimental critical transfer distances were determined by plotting $f$ versus $c(l-f)$ for $c > 10^{-4}$ M. These plots are shown in Figure 20. The validity of equation (8) at high acceptor concentration is indicated by the straight lines obtained in Figure 20. The slopes of the straight lines were measured using a least squares procedure and substituted into equation (12). The critical transfer distances obtained from this treatment are compared in Table 10 with the theoretical values calculated above. The experimental and theoretical critical transfer distances agree very well for all five donor-acceptor systems. The uncertainties in the theoretical and experimental critical transfer distances are $\pm 1.2 \, \text{Å}$ and $\pm 0.8 \, \text{Å}$ respectively. These uncertainties were obtained from the precision of the quantities used to calculate the transfer distances and in no way reflect the approximations used to derive the expressions for the theoretical and experimental critical transfer distances.

The quenching of donor fluorescence with increasing 9-phenylanthracene concentration is shown in Figure 21 for the five donor-acceptor systems. These quenching curves are similar to those obtained by Birks and Kuchela\(^{29}\) and to
Figure 20 - Transfer efficiency versus c(1-f) plots for carbazole (△), fluorene (○), dibenzofuran (□), dibenzothiophene (●) and 9-fluorenol (▲) as donors.
### Table 10

Observed and Theoretical Critical Transfer Distances

(the acceptor is 9-phenylanthracene)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Observed $R_0$ in Å (method A)</th>
<th>Theoretical $R_0$ in Å (method B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorene</td>
<td>24.</td>
<td>23.</td>
</tr>
<tr>
<td>Carbazole</td>
<td>26.</td>
<td>27.</td>
</tr>
<tr>
<td>Dibenzofuran</td>
<td>22.</td>
<td>21.</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>16.</td>
<td>17.</td>
</tr>
<tr>
<td>9-Fluorenol</td>
<td>12.</td>
<td>13.</td>
</tr>
</tbody>
</table>
Figure 21 - Dependence of donor fluorescence intensity on the 9-phenylanthracene concentration for carbazole (△), fluorene (○), dibenzo[ f ]uran (■), dibenzothiophene (●), and 9-fluorenol (▲) as donors. All donor concentrations are $2.0 \times 10^{-2}$ M and excitation is at 300 nm.
those obtained above for the systems using naphthalene derivatives as energy transfer donors. The quenching in these systems occurs at higher acceptor concentrations (as before) than Birks and Kuchela\textsuperscript{29} report for their systems. This is consistent with the smaller critical transfer distances predicted and observed for these systems.

The applicability of Förster's dipole-dipole mechanism of resonance energy transfer to the systems with carbazole, fluorene and dibenzofuran as donors is indicated by the close agreement between the measured and theoretical values of the critical transfer distances. The straight lines obtained for the plots of $f$ versus $c(l-f)$ for all five donor-acceptor systems demonstrates the validity of Birks' method of measuring these critical transfer distances. The experimental and theoretical $R_0$'s for the systems with dibenzothiophene and 9-fluorenol as donors agree very well. However, the small critical transfer distances for these two systems are of the order of magnitude of contact distances. This makes the applicability of Förster's dipole-dipole mechanism of resonance energy transfer questionable in both of these cases.

The importance of the competition between intersystem crossing and energy transfer is indicated by the decrease of the experimental and the theoretical critical transfer distances with the decrease in the fluorescence quantum efficiency for the four donor-acceptor systems with fluorene, carbazole, dibenzofuran and dibenzothiophene as donors. The 9-fluorenol--9-phenylanthracene system is somewhat different from the other
four, since the heteroatom is not directly attached to the two benzene rings. As seen in Table 9, the overlap integral for this system is not responsible for the low theoretical $R_0$; the low $R_0$ must be attributed to the low fluorescence quantum efficiency. These same conclusions can be reached from the data in Table 8 where the short lifetime of 9-fluorenol, which is the result of the small fluorescence quantum efficiency, results in the low $R_0$. The phosphorescence quantum efficiency of 9-fluorenol is not available, therefore, it is impossible to tell whether the low fluorescence quantum efficiency is due to intersystem crossing or to interaction with the solvent.
SUMMARY

The fluorescence quantum efficiencies and corrected fluorescence spectra have been measured for naphthalene, 1-methylnaphthalene, 1-allylnaphthalene, 1-vinylnaphthalene, fluorene, carbazole, dibenzofuran, dibenzothiophene, 9-fluorenol, and 9-phenylanthracene. Theoretical critical transfer distances have been calculated by two methods for naphthalene and its derivatives as donors and acriflavine as the acceptor and for fluorene and its analogs as donors and 9-phenylanthracene as the acceptor. The critical transfer distances were experimentally measured using the method of Birks and Kuchela.

Effects of the variation of the molecular structure of the donor on resonance energy transfer have been studied using the two different series of donor molecules. Naphthalene, 1-methylnaphthalene, 1-allylnaphthalene and 1-vinylnaphthalene were used as donors and acriflavine as an acceptor in an initial investigation of the roles of small hydrocarbon side chains, particularly ones containing unsaturation, upon resonance energy transfer. The experimental values of \( R_0 \) for the systems using naphthalene, 1-methylnaphthalene, and 1-allylnaphthalene as donors were between 25\( \AA \) and 28\( \AA \) and were in good agreement with theoretical values calculated from the absorption and fluorescence spectra by two methods. The 1-vinylnaphthalene-acriflavine system had an experimental \( R_0 \) of 12\( \AA \) and a theoretical value of 28\( \AA \).
observed self-quenching of 1-vinylpyridine fluorescence is probably responsible for this discrepancy.

The effect of placing heteroatoms and heteroatom containing groups between pi-electron systems on their resonance energy transfer properties was studied using compounds with the general formula,

\[
\begin{array}{c}
\text{H} \\
\text{X} \\
\text{H}
\end{array}
\]

where \(X\) is -NH for carbazole, -CH\(_2\) for fluorene, -O- for dibenzofuran, -S- for dibenzothiophene and -CH\(_2\)OH for 9-fluorenol, as donors and 9-phenylanthracene as an acceptor. The experimental and theoretical values of the critical transfer distances are in close agreement for all five donor-acceptor systems. The effect of the heteroatom for the compounds where \(X = \text{-NH, -O-}, \text{ and -S-}\), is a decrease in the fluorescence quantum efficiency, in the order indicated, caused by increasing intersystem crossing. The intersystem crossing competes not only with fluorescence but also with the energy transfer process and is responsible for the smaller observed and theoretical critical transfer distances.
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Appendix I

DERIVATIONS
1. FORSTER'S DERIVATION OF THE EXPRESSION FOR THE CRITICAL TRANSFER DISTANCE.

Forster's quantum mechanical treatment of resonance energy transfer considers a long-range, weak intermolecular interaction between an excited donor molecule and a ground-state acceptor molecule. The probability of transfer, which is defined as the total number of transfers per unit time per excited molecule, has been given by Dirac as

\[
\frac{2\pi}{\hbar} \int |U_{k1}(w'_k, w_1; w_k, w'_1)|^2 D \cdot dW,
\]

where

\[
U_{k1}(w'_k, w_1; w_k, w'_1) = \int \int \phi_{k}^{*}(w'_k, r_k) \phi_{1}^{*}(w_1, r_1) U(r_k, r_1) \phi_{k}(w_k, r_k) \phi_{1}(w'_1, r_1) dr_k dr_1
\]

are the matrix elements of the interaction operator

\(U(r_k, r_1)\) for two dipoles, \(\phi_{k}^{*}\) and \(\phi_{k}\) are the wave functions of the excited and ground-state donor molecule, \(\phi_{1}^{*}\) and \(\phi_{1}\) are the wave functions of the excited and ground-state acceptor molecule, \(w'_k\) and \(w_k\) are the energies of the excited and ground-state donor molecules, \(w'_1\) and \(w_1\) are the energies of the excited and ground-state acceptor molecules, \(r_k\) and \(r_1\) are coordinate vectors describing the electronic configuration of the donor and acceptor molecules, and \(D\) is
an operator to transform the expression, which is in terms of quantum numbers $v^i_k$ and $v^1_1$, to one in terms of $w^i_k$, $w^1_1$ and $\check{W}$ the average electronic excitation energy of the donor and the acceptor.

After normalizing the wavefunctions, transforming the coordinates and accounting for the mutual orientation of the donor and acceptor molecules, one arrives at the following equation for $F_{k1}$, the number of transfers $k-1$ per unit time.

$$
F_{k1} = \frac{2\pi}{h} \int_{w^i_k}^{\infty} \int_{w^1_1}^{\infty} \int_{\check{W}}^{\infty} g^1(w^i_k)g(w^1_1) | \mathcal{U}_{k1}(w^i_k,w^1_1; \check{W}_0-\check{W}+w^i_1, \check{W}_0+w^1_1) |^2 \, dw^i_k dw^1_1 d\check{W}
$$

The frequency distributions normalized to an energy scale of 1 are given by the distribution functions $g(w^i_1)$ and $g^1(w^i_k)$ for the ground- and excited-states. $\check{W}_0$ is the same for both molecules and is the pure electronic excitation energy. This leads to

$$
F_{k1} = \frac{4\pi}{3n^4 \hbar^6} R^6_{kl} \int_{\check{W}=0}^{\infty} \left[ \int_{w^1_1=0}^{\infty} g^1(w^i_k)M^2(\check{W}_0-\check{W}+w^i_k, w^i_k) \, dw^i_k \right] \, d\check{W}
$$

which takes into account the relative orientations of donor and acceptor molecules. In equation (4)

$$
M(\check{W}_0-\check{W}+w^i_k, w^i_k) = | \mathcal{M} |^2 = | - e \int \delta^i_k(\check{r}_k, \check{r}'_k) \check{r}_k \check{r}'_k(\check{w}_k, \check{w}'_k) \, d\check{r}_k |^2
$$

and similarly for $M(w^1_1, \check{W}_0+w^1_1)$.

The integrals within the brackets in equation (4) are related to the absorption and fluorescence spectra of
the molecules. The first integral is related to the fluorescence spectrum through the Einstein spontaneous emission coefficient. This is given by

$$A(W) = \frac{4\pi n^2}{3h^2 C^3} \int_{w_1=0}^{\infty} g'(w') \left| M(w_0-W+w', w') \right|^2 dw'$$

where \(A(W)\) is the number of quanta emitted with energy between \(W\) and \(W + dW\) per unit time per excited molecule. If one assumes the mirror image symmetry of the absorption and fluorescence spectra, i.e. \(g(W) \equiv g'(W)\), then \(A(W)\) may be related to the molar absorptivity by

$$B(W) = \frac{\pi^2 h^3 C^3}{n^3 (2W_\omega-W)^3} A(2W_\omega-W)$$

and \(n\) is the refractive index of the solvent. The second integral of equation (4) is related to the molar absorptivity by

$$B(w) = \frac{\pi^2 h^3 C^3}{n^3 N' W}$$

where \(N' = 6.02 \times 10^{20}\)

The combination of equations (4), (6), (7), (8) and (9) leads to the following

$$F_{k'l} = \frac{3hC^2(ln10)^2 J(W)}{4\pi n^2(N')^2 W_0 R_kl}$$

(10)
where
\[ J(W) = \int_{W = 0}^{\infty} \epsilon(W) \epsilon(2W_0 - W) dW. \] (11)

Equation (10) can also be written in the form
\[ F_{kl(t)} = \frac{1}{t_0} \left( \frac{R_o}{R_{kl}} \right)^6 \] (12)

with
\[ R_0 = \frac{3 \hbar t_0 c^2 (ln 10)^2 J(W)}{4 \pi^3 n^2 (N')^2 \overline{\nu}_o^2} \] (13)

Here \( R_o \) is the theoretical transfer distance, the distance between a donor and acceptor molecule at which there is a 50% probability of energy being transferred. The energy units of equation (13) can be switched to wavenumbers resulting in equation (14).
\[ R_0^6 = \frac{3 \ h \ t_0 \ c^2 \ (ln 10)^2 \ \ J(\overline{\nu})}{8 \ \pi^4 n^2 \ (N')^2 \ \frac{\overline{\nu}_o}{\overline{\nu}}^2} \] (14)

In equation (14) \( J(\overline{\nu}) \) is the overlap integral, the overlap between the donor's emission spectrum and the acceptor's absorption spectrum given by
\[ J(\overline{\nu}) = \int \epsilon_A(\overline{\nu}) \epsilon_D(2\overline{\nu}_o - \overline{\nu}) d\overline{\nu} \] (15)

where \( \overline{\nu}_o \) is the mean of the donor's absorption and emission maxima, \( \epsilon_A \) is the acceptor's molar absorptivity and \( \epsilon_D \) is the donor's molar absorptivity.
2. FORSTER'S $^5,^8$ DERIVATION OF THE EQUATION USED TO DETERMINE $R_o$ EXPERIMENTALLY.

The fluorescence quantum yield $\eta_A$ of the acceptor fluorescence excited by transfer from a donor molecule is given by

$$\eta_A = C \int_0^\infty \overline{Q(t)} \, dt$$  \hspace{1cm} (16)

where $C$ is a constant and $\overline{Q(t)}$ is the probability of finding at time $t$ an excited molecule which was excited at $t = 0$. The maximum quantum yield of acceptor fluorescence obtained either by direct excitation or by complete transfer is given by

$$\eta_A^{\text{max}} = C \int_0^\infty e^{-t/t_0} \, dt = C t_0$$  \hspace{1cm} (17)

Then the relative fluorescence output is

$$\frac{\eta_A}{\eta_A^{\text{max}}} = \frac{1}{t_0} \int_0^\infty \frac{\overline{Q(t)}}{Q(t)} \, dt.$$  \hspace{1cm} (18)

Forster $^7,^8$ has derived using the dipole-dipole mechanism of resonance energy transfer the following expression for $\overline{Q(t)}$,

$$\overline{Q(t)} = e^{-t/t_0} - \frac{2C}{C_0} \sqrt{\frac{t}{t_0}}$$  \hspace{1cm} (19)

where $C$ is the acceptor concentration and $C_0$ is the critical concentration corresponding to the critical transfer distance. This leads to the following equation for the relative fluor-
Forster\textsuperscript{5,8} has shown further that equation (21) is a good approximation to equation (20) when \( \beta = 2.05 \). Equation (21) may also be derived by a formal kinetic treatment of resonance energy transfer\textsuperscript{5,29}.

3. KINETIC TREATMENT OF RESONANCE ENERGY TRANSFER\textsuperscript{29}.

Resonance energy transfer can be considered from a kinetic standpoint by using the following mechanism.

\begin{align*}
D + hv & \rightarrow D^* & \text{(excitation)} \quad (22) \\
D^* & \underset{k_{FD}}{\rightarrow} D + hv' & \text{(fluorescence)} \quad (23) \\
D^* & \underset{k_{ID}}{\rightarrow} D + \text{heat} & \text{(internal quenching)} \quad (24) \\
D^* + A & \underset{k_t}{\rightarrow} D + A^* & \text{(energy transfer)} \quad (25)
\end{align*}

In equations (22, 23, 24, and 25) the rate parameters (sec\textsuperscript{-1}) \( k_{FD}, k_{ID} \) and \( k_t \) describe the fluorescence, internal quenching, and energy transfer processes respectively. When \( c \), the acceptor concentration, equals zero, the molecular fluorescence quantum efficiency of the donor is

\[
(q_D^o)_c = \frac{k_{FD}}{k_{FD} + k_{ID}}
\]
When \( c = 0 \), and non-radiative transfer occurs, \((q_{OD})_0\) is reduced to

\[
(q_{OD}) = \frac{kt}{kfD + k_{1D} + kt c}
\]

The radiative transfer (trivial process) efficiency is

\[
f_R = a(q_{OD})
\]

where \( a \) is the fraction of \( q_{OD} \) absorbed by the acceptor.

The non-radiative transfer efficiency is

\[
f_{NR} = \frac{kt c}{kfD + k_{1D} + kt c}
\]

The sum of the radiative and non-radiative transfer efficiencies is

\[
f = f_R + f_{NR} = a(q_{OD}) + \frac{kt c}{kfD + k_{1D} + kt c}
\]

\[
f = a \frac{kfD}{kfD + k_{1D} + kt c} + \frac{kt c}{kfD + k_{1D} + kt c}
\]

if

\[
\sigma = \frac{kt}{kfD + k_{1D}}
\]

then

\[
f = a(q_{OD})_0 + \sigma c
\]

At low acceptor concentrations where \( \sigma c \) is small, equation
(34) may be approximated by
\[ f = a (q_{oD})_0 \quad \text{(at low \( c \)).} \]  

At high acceptor concentrations, the acceptor absorbs all of the donor emission, therefore
\[ a \approx 1 \]  
and equation (34) may be approximated as
\[ f = \frac{(q_{oD})_0 + \sigma c}{1 + \sigma c} \quad \text{(at high \( c \))} \]  

(For the systems studied in this work (see Figures (11) and (19) in the preceding section) two concentration regions are observed. For low acceptor concentrations \( c < 10^{-6}\text{M.} \) equation (35) applies. For high acceptor concentrations \( c > 10^{-6}\text{M.} \) equation (37) applies.)

Equation (37) may be rewritten in the form
\[ f = (q_{oD})_0 + \sigma c \cdot (1 - f). \]  
If the experimental data are then plotted as \( f \) versus \( c(1 - f) \), the slope of the straight line obtained gives an experimental value for \( \sigma \).

Equation (37) is of the same form as the Stern-Volmer type equation (21) with
\[ \sigma = \frac{2.05}{c_o} \]  

Substitution of equation (39) into Förster's relationship between the critical concentration and the critical transfer distance
\[ c_o = \frac{3000}{4\pi NR_o^3} \]
yields the following equation for the experimental determination of the critical transfer distance.

\[ R^3 = \frac{3000 \sigma}{4\pi N (2.05)} \]  

(41)
Appendix II

A FORTRAN II PROGRAM TO CALCULATE THE ENERGY DISTRIBUTION OF A TUNGSTEN LAMP
FORTRAN II ENERGY DISTRIBUTION PROGRAM

TO CALCULATE THE AMOUNT OF LIGHT THROUGH THE SLIT OF THE MONOCHROMATOR FROM A TUNGSTEN LAMP AT A GIVEN DISTANCE AND OF KNOWN CANDLE POWER AND TEMPERATURE

THE UNITS OF THE RESULTS ARE WATTS FOR A 1 MILLIMICRON CONE = 37.403
CTWO = 14384.

1 READ 583,TEMP,WAVEF WAVE, DELTA, CANDLE, DIST, AREA
2 CFACT = (CANDLE*1.656)/((0.567E-11)*(TEMP**4))
3 STERAD = AREA/(DIST*DIST)
4 WAVE = WAVEF
5 GO TO 2
1 WAVE = WAVE + DELTA
2 XNUMER = CONE/(WAVE**5)
3 DENOM = -1. + EXPP (CTWO/(WAVE*TEMP))
4 ENER = CFACT * (XNUMER/DENOM)
5 NOW MULTIPLY BY THE NUMBER OF STERADIANS (SLIT AREA/
6 DIST. SQUARED)
7 ACTUAL = ENER*STERAD
8 NWAVE = WAVE *1000.
9 NTEMP = TEMP
10 PUNCH 584,NTEMP, NWAVE, ACTUAL, DIST, AREA
3 TYPE 585
11 PAUSE
12 GO TO 4
13 583 FORMAT (F5.0,2X,F5.3,2X,F5.3,2X,F5.3,2X,F5.3,2X,F4.2,2X,
14 E10.5,2X,E10.5)
584 FORMAT (15X,5X,15X,E14.8,5X,E11.5,5X,E11.5)

585 FORMAT (14HEND OF PROBLEM)

END
Appendix III

A FORTRAN II PROGRAM TO CALCULATE
THE CORRECTION FACTORS
C PROGRAM TO CALCULATE CORRECTION FACTORS
C ALL HEIGHTS NORMALIZED TO ATTENUATION OF 1K
C AND RECORDER SENSITIVITY OF 1MV.

DIMENSION CFACT(70),XHGT(70),RESPN(70),WAVE(70),FREQ(70)

DO 5 J = 1, 69
   5 READ 581,XHGT(J)
   READ 582,AREA

DO 10 K = 1, 69
   10 RESPN(K) = XHGT(K)*40.

C CALCULATION OF ENERGY FROM CALIB. LAMP

J = 0

DO 80 J = 1, 69
   XJ = J
   WAVE(J) = ((XJ*5.) + 305.)/1000.
   XNUM = (37.403)/(WAVE(J)**5)
   DENOM = -1. + EXPF(5.25346/WAVE(J))
   ENER = (AREA)*(2.987E-5)*(XNUM/DENOM)
   CFACT(J) = WAVE(J) + (ENER/RESPN(J))*WAVE(J)*WAVE(J)

30 FREQ(J) = 1./WAVE(J)

J = 0

DO 90 J = 1, 69
   PUNCH 583, FREQ(J), CFACT(J)

90 PRINT 584, WAVE(J), CFACT(J), FREQ(J)

581 FORMAT (25X,F11.5)
582 FORMAT (F7.5)
583 FORMAT (5X,F9.5,5X,E12.5)
584 FORMAT (1H5X,F6.3,5X,E12.5,5X,F9.5)

END
Appendix IV

A FORTRAN II PROGRAM FOR THE CORRECTION OF FLUORESCENCE SPECTRA
C FORTRAN II PROGRAM TO CORRECT FLUORESCENCE SPECTRA
C CFACi is the correction factor for the particular slits used
C XHGT is the fluorescence intensity of the substance versus wave length
C RESP is the normalized intensity
C Y is the corrected intensity
C WAVE is the wave length in microns
C X is the wave number in reciprocal microns
C W is the interpolated intensity on a scale linear in wave number
C V is the interpolated wave number
C ATT is the lock-in-amplifier attenuation
C SPAN is the span in millivolts of the recorder

DIMENSION CFAC(100), XHGT(100), RESP(100), Y(100), WAVE(100), X(100)
DIMENSION FREQ(100), ENER(100), W(100), V(100)
READ 579, ATT, SPAN, QS, Q51
C READ IN THE CORRECTION FACTORS
J=0
DO 10 J=1,69
10 READ 580, CFAC(J)
C READ IN THE SPECTRUM TO BE CORRECTED
K=0
DO 20 K=1,69
20 READ 581, XHGT(K)
C NORMALIZE SPECTRUM TO AN ATTENUATION OF 2K, A RECORDER
SENSITIVITY OF 1Mv/100 DIVISIONS, AND NORMALIZE TO
A CONSTANT EXCITATION LIGHT INTENSITY WITH QUININE SULFATE. QS IS PEAK HEIGHT OF 1PPM QUININE SULFATE UNDER STANDARD CONDITIONS.

DO 30 L=1,69
RESP(L)=XHGT(L)*ATT*SPAN*(QS/QS1)
ENER(L)=CFACT(L)*RESP(L)
XL=L
WAVE(L)=( (XL*5.)+305. )/1000.
30 FREQ(L)=1./WAVE(L)

LAGRANGIAN INTERPOLATION PROGRAM

DO 80 J=1,5
X(J)=FREQ(J)
80 Y(J)=ENER(J)

M=1
V(K)=3.20
N=0
GO TO 101

100 M=M+1
101 XN1=(V(M)-X(1))*(V(M)-X(2))*(V(M)-X(3))*(V(M)-X(4))*(V(M)-X(5))
XN2=(V(M)-X(1))*(V(M)-X(2))*(V(M)-X(3))*(V(M)-X(4))*(V(M)-X(5))
XN3=(V(M)-X(1))*(V(M)-X(2))*(V(M)-X(3))*(V(M)-X(4))*(V(M)-X(5))
XN4=(V(M)-X(1))*(V(M)-X(2))*(V(M)-X(3))*(V(M)-X(4))*(V(M)-X(5))
XN5=(V(M)-X(1))*(V(M)-X(2))*(V(M)-X(3))*(V(M)-X(4))*(V(M)-X(5))
XD1=(X(1)-X(2))*(X(1)-X(3))*(X(1)-X(4))*(X(1)-X(5))
XD2=(X(2)-X(1))*(X(2)-X(3))*(X(2)-X(4))*(X(2)-X(5))
XD3=(X(3)-X(1))*(X(3)-X(2))*(X(3)-X(4))*(X(3)-X(5))
$XD_4 = (X(4) - X(1)) \cdot (X(4) - X(2)) \cdot (X(4) - X(3)) \cdot (X(4) - X(5))$

$XD_5 = (X(5) - X(1)) \cdot (X(5) - X(2)) \cdot (X(5) - X(3)) \cdot (X(5) - X(4))$

$XNUM_1 = XN_1 \cdot Y(1)$

$XNUM_2 = XN_2 \cdot Y(2)$

$XNUM_3 = XN_3 \cdot Y(3)$

$XNUM_4 = XN_4 \cdot Y(4)$

$XNUM_5 = XN_5 \cdot Y(5)$

$W(M) = \frac{XNUM_1}{XD_1} + \frac{XNUM_2}{XD_2} + \frac{XNUM_3}{XD_3} + \frac{XNUM_4}{XD_4} + \frac{XNUM_5}{XD_5}$

$L = M + 1$

$V(L) = V(M) - 0.02$

IF ($V(K) - 1.56 < 120, 105, 105$

$105 \ IF \ (V(K) - X(3) < 108, 108, 100$

$108 \ IF \ (N - 64 < 110, 100, 100$

$110 \ N = N + 1$

DO 115 I = 1, 5

K = N + I

$X(I) = FREQ(K)$

$Y(I) = ENBR(K)$

GO TO 100

120 DO 130 M = 1, 84

130 PRINT 562, V(K), W(M)

562 FORMAT (1H, 5X, F6.3, 5X, E12.5)

579 FORMAT (F5.1, 2X, F4.1, 2X, F5.1, 2X, F5.1)

580 Format (19X, E12.5)

581 FORMAT (4X, F6.1)

END
Appendix V

SPECTRA CORRECTION FACTORS FOR THE JARRELL-ASH MONOCHROMATOR AND 1P28 PHOTOMULTIPLIER TUBE COMBINATION (0.1MM SLITS).
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<th>Correction Factor</th>
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<td>5.35267E⁻¹⁰</td>
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