Role of Heterogeneity of the Solvation Site in Electronic Spectra in Solution*

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Abstract. The emission spectra of polar aromatic molecules in rigid, polar solution are shown to depend on the exciting wavelength. Occurrence of the phenomenon depends on both the excited-state lifetime of the chromophore and the degree of rigidity of the medium. The results are interpreted in terms of a model which stresses the contribution of micro-environmental heterogeneity to electronic absorption and emission spectra.

Shifts in chromophore electronic absorption and emission spectra as a function of the polarity and polarizability of the solvent are well known and have been interpreted by Bayliss and McRae in terms of permanent and induced dipolar interactions between a molecule in its ground and excited states and the solvent.[1] The interactions have been qualitatively treated as occurring between the chromophore and a general molecular environment. For aromatic amino acid residues in proteins[2-4] and for aromatic hydrocarbons trapped in polycrystalline matrices[5] a considerable body of evidence exists that indicates that chromophores of the same species can have different electronic energies as a consequence of the existence of several distinct local environments. Even in a homogeneous phase, solute molecules can be expected to occupy a variety of solvation sites at any given time, generating an array of electronic transition energies whose summation comprises the absorption or emission spectrum of the sample as a whole. Such variation in solute–solvent local interactions is thought to be a significant source of the broadening of the absorption and emission spectra of a chromophore in a condensed phase.[6]

In the present article we provide evidence for the contribution of micro-environmental heterogeneity to solution spectra through our observation of an exciting-wavelength dependence for fluorescence and phosphorescence spectra of aromatic molecules in dilute rigid solution. In exploring the consequences of this environmental heterogeneity we show that it provides a rationalization for the failure of fluorescence concentration depolarization at the red edge of the solute absorption band[7-9]—the so-called "Weber red edge effect."[10]

The Model. No attempt is made in this paper to present a quantitative model for the contribution of environmental heterogeneity to electronic spectra. We only emphasize three qualitative assumptions: (1) that electronic energies of chromophores in solution are a function of geometry-dependent solute–solvent interactions, (2) that at any instant in time there is an ensemble of interac-

tions that gives rise to a spectrum of electronic energies in the sample, and (3) that the interactions of a solute molecule with its solvent sphere, which rapidly fluctuate with time in fluid solution, become static in a rigid solution†.

The first assumption is well founded on the basis of theoretical treatments of intermolecular interactions. These interactions can be described in terms of electrostatic forces which can be written as a sum of terms (dipole–dipole, dipole–induced dipole, etc.) that are dependent on the relative distance and orientation of the interacting molecules.[11] The electronic energy of a chromophore at any instant in time in solution can be expected, therefore, to be a function of the geometry of its solvent shell at that time. A distribution of solvation sites within the sample will generate a spectrum of electronic energies for the chromophore. Solvent–solute interactions will be the largest and the distribution of electronic energies the widest for polar molecules in polar solvents, where the forces are largely dipole–dipole in nature.

If the solution is a rigid glass, there will still be a distribution of solvation sites for a chromophore because of the random nature of the glass structure. In view of the absence of rapid molecular motion in the glassy state[12], however, solvent sphere geometries will persist for relatively long periods of time so that a static array of chromophore electronic energies throughout the sample will result.

Since a molecule possesses a different electronic distribution in its ground and excited states, ground- and excited-state interactions with the local environment of the chromophore will, in general, be quite different.[1] As a result, a distribution of ground-state electronic energies arising from environmental heterogeneity is accompanied by a spectrum of transition energies in absorption and emission. If in a polar medium the chromophore dipole moment is larger in the excited than in the ground state, solvent interactions will be greater in the excited state[13], so that this distribution of transition energies in the sample may be much larger than kT.

Results and Discussion. Exciting-wavelength dependence for emission spectra of molecules in rigid solution: Fig. 1 displays the fluorescence and 0–0 region phosphorescence spectra of indole in a 1:1 ethylene glycol–water glass excited near the center (280 nm) and the red edge (295 nm) of the absorption band. Clearly the emission spectrum is *not* independent of the exciting wavelength as is generally assumed, for the spectra generated at the longer exciting wavelength are red-shifted by about 900 cm^{-1} in fluorescence and 90 cm^{-1} in phosphorescence. The failure to observe a decrease in the emission shift for indole over a 100-fold dilution (10^{-2} M to 10^{-4} M) argues against the possibility that this effect arises from solute–solute interactions, such as dimer or excimer formation. With concentrations greater than 10^{-1} M, the emission shift in fact decreases with *increasing* concentration; at 0.5 M the shift in the fluorescence of indole is 600 cm^{-1} for 280 and 295 nm excitation.

We have observed this emission dependence on exciting wavelength in such polar aromatic molecules as indole, tryptophan, β-naphthol, 9-aminoacridine cation, and proflavin. Notably, small or undetectable shifts were observed for nonpolar aromatic hydrocarbons, such as anthracene, perylene, and naphthalene in polar solvents.
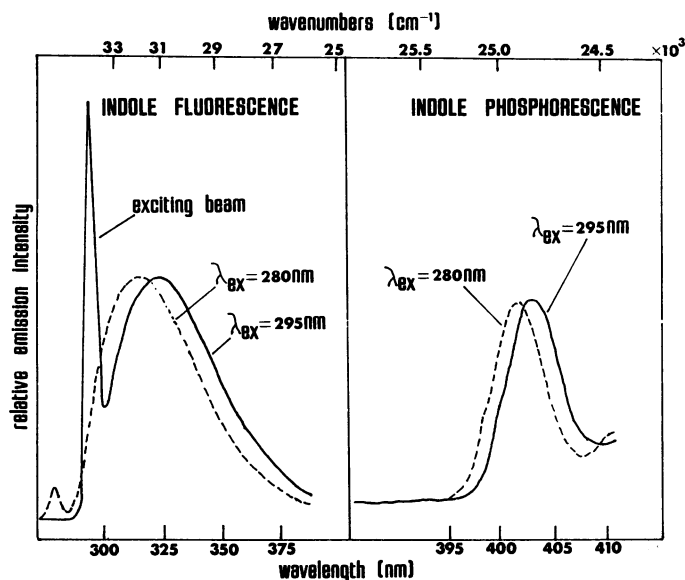
FIG. 1.   Fluorescence and 0–0 region phosphorescence spectra of
10^{-3} M indole in 1:1 ethylene glycol–water media at 202 and 77° K,
respectively, demonstrating the shift between the emission excited
at 280 and 295 nm.

The dependence on exciting wavelength is also a function of the solvent, decreasing as one passes from polar to nonpolar media.   For β-naphthol in a 4:1 methylcyclohexane–cyclohexane glass at 77°K the fluorescence spectrum shift with variation in the exciting wavelength is essentially zero (<1 nm) compared with a shift observed in 1:1 ethylene glycol–water of about 5 nm.

The observation of an exciting-wavelength dependence for emission spectra of chromophores in rigid solutions is consistent with our model, which emphasizes the role of environmental heterogeneity on emission spectra.   Excitation into the red edge of the chromophore absorption band selects a population of molecules which, because of their particular local environments, have low 0–0 transition energies.   In a rigid medium the solvent shell about each chromophore molecule remains fixed over the excited state lifetime of the chromophore and those molecules subsequently emit at long wavelengths.   As the exciting wavelength becomes shorter, new subclasses of molecules containing members with 0–0 transitions of higher energy are excited, yielding a corresponding blue shift in the emission spectrum.   The decrease in the emission dependence upon exciting wavelength at higher concentrations (about 10^{-1} M or greater) is anticipated as a consequence of singlet–singlet nonradiative transfer from molecular subclasses of higher transition energies to those of lower energy emissions.   As a result, emission obtained by excitation into the middle of the chromophore absorption band becomes red-shifted at higher concentrations, which decreases the observed exciting-wavelength dependence.   In the high concentration limit it is expected that essentially the long wavelength emission would be observed at all exciting wavelengths.

The exciting-wavelength dependence we observe for emission spectra can be correlated with the breadth of the spectrum, since this is also a function of the heterogeneity in solvent–solute interactions.   Fig. 2 displays the fluorescence spectra of β-naphthol and naphthalene. β-Naphthol has a poorly resolved fluorescence spectrum and exhibits an emission shift of about 5 nm, whereas the naphthalene spectrum, which is essentially independent of exciting wavelength, displays much more highly resolved vibronic bands.   The greater polarity of the β-naphthol as opposed to the parent hydrocarbon permits a wider spectrum of interactions with its polar environment.
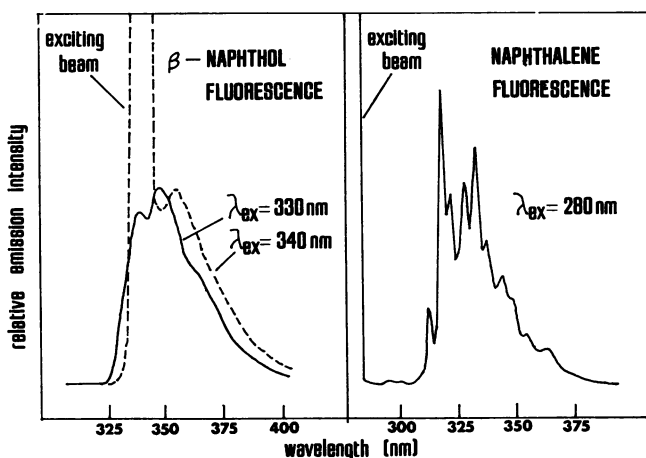

FIG. 2.   Fluorescence spectra of 10^{-3} M β-naphthol, demonstrating the emission shift between exciting wavelengths of 330 and 340 nm, and 10^{-3} M naphthalene in 1:1 ethylene glycol–water at 150°K.   Note the sharply defined structure in the naphthalene fluorescence spectrum.   The naphthalene spectrum position was only slightly, if at all, affected by changes in the exciting wavelength.

While the exciting-wavelength dependence is most clearly observed near the red edge of the absorption spectrum, in favorable cases (e.g. anthracene and 9-aminoacridine), where the absorption vibronic bands are moderately well resolved, the effect recurs at the red edge of each vibronic band.

Since the entire emission spectrum—fluorescence and phosphorescence—is produced by the same subclass of chromophore molecules for any given exciting wavelength, any difference in exciting-wavelength dependence between fluorescence and phosphorescence from the same chromophore must arise from a difference in the electronic distribution in the excited singlet and triplet states. That the fluorescence shift was greater than the phosphorescence shift for indole in Fig. 1 requires that the triplet-state electronic distribution in indole be closer to the electronic distribution in the ground singlet state than is that of the first excited singlet state.

Dependence of the exciting-wavelength effect on the rigidity of the solution: On the basis of the model we have proposed, emission spectra should become in-

dependent of the excitation wavelength when randomization of the chromophore local environments occurs in times that are short compared to the excited-state lifetime of the chromophore. A distribution of electronic energies would still exist at the instant of absorption and emission, but there would be no correlation between the two events.

An exciting-wavelength dependence is not observed for the fluorescence spectra of any of the molecules we have examined at room temperature. This dependence on solution "rigidity" for the exciting wavelength effect is depicted in Fig. 3. The differences in emission wavelength for both the phosphorescence
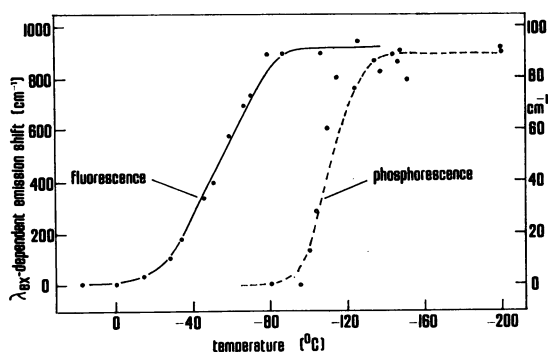

FIG. 3. Plot of the exciting-wavelength dependence for the indole (10^{-3} M) fluorescence and phosphorescence spectra versus the temperature of the 4:1 glycerol–water medium. Fluorescence and phosphorescence shifts were measured as the average of the red edge and blue edge differences between the spectra at 280 and 295 nm excitation.

and fluorescence spectra of indole excited at 280 and 295 nm in 4:1 glycerol–water are plotted as a function of temperature. The exciting-wavelength dependence is lost in either case, but the temperature at which the transition occurs for phosphorescence is much lower than that for fluorescence. The results indicate that at $-110°C$ the mobility of the glassy solution is sufficient to randomize the chromophore solvation sites within the triplet-state lifetime of indole (about 7 sec), whereas a temperature of $-53°C$ is necessary to render the solution fluid enough to average the chromophore local environments within the nanosecond excited singlet state lifetime. The temperatures at which these transitions occur are distinctly different when indole is dissolved in another solvent system. It should be possible to utilize this effect to measure a rather wide range of molecular reorganization times.

That different temperature dependencies for phosphorescence and fluorescence shifts were observed is persuasive evidence that the emission shift with variation in exciting wavelength does not arise through any type of solute–solute interactions but stems from a heterogeneity in solute–solvent interactions for isolated molecules in the solution.

The Weber "red edge" effect: In 1960, G. Weber reported[7] that fluorescence concentration depolarization in rigid solutions, which results from singlet–singlet intermolecular energy transfer, failed to occur upon excitation into the red edge of the chromophore absorption band. More recent studies[8,9] have indicated that this is a general phenomenon in aromatic molecules.

Our model, in its emphasis upon the composite nature of both absorption and emission spectra in rigid solutions, provides a simple rationalization for the red

edge failure of concentration depolarization.   Excitation into the red edge of the chromophore absorption band selects a certain subpopulation of the solute molecules of small absolute concentration, which by virtue of their particular local environments are characterized by relatively low 0–0 singlet electronic transition energies.   Because the bulk of the chromophore molecules in the solution possess 0–0 transitions of higher energy than the subclass of molecules at the red edge, members of the latter subclass have, in general, only neighboring molecules with higher transition energies.   Singlet-singlet energy transfer and, as a result, fluorescence depolarization, thus cannot occur for those molecules selectively excited at the red edge of the chromophore absorption band.   On the other hand, at shorter exciting wavelengths, molecules of generally higher transition energies are excited and readily undergo energy transfer and corresponding fluorescence depolarization.

Interestingly, while this manuscript was in preparation, Weber and Shinitzky[9] reported a small emission dependence on exciting wavelength in many of the compounds in which they studied the "red edge effect."   They suggest that the failure to observe transfer at the red edge indicates the presence of two nearly isoenergetic excited electronic states in any single chromophore molecule.   However, this interpretation requires the assumptions that intramolecular energy transfer does not readily occur from the higher to the lower electronic state, and that intermolecular energy transfer is forbidden when a molecule is selectively excited in the lower state.   As pointed out by the authors themselves, both of these assumptions are difficult to justify.

Experimental Details.   Sample concentrations varied from 0.5 to 10^{-4} M, with most spectra recorded at 10^{-3} M.   Several optical path lengths (3×10^{-3} to 0.4 cm) and solvents (1:1 and 3:2 ethylene glycol–water, 4:1 glycerol–water, glycerol, absolute ethanol, 4:1 methylcyclohexane–cyclohexane) were used.   All materials were of reagent grade.   Spectra were recorded on an emission spectrometer described elsewhere.[4] The exciting band width was 6.3 nm, while the emission monochromator band width was 3.3 nm.

† For our purposes a "rigid" medium is one in which the geometry of the average solvation sphere persists for a time much greater than the excited-state lifetime of the chromophore.

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