EFFECTS OF THE ENVIRONMENT ON THE FLUORESCENCE OF AROMATIC COMPOUNDS IN SOLUTION

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I. INTRODUCTION

Solvents, pH, concentration, and temperature bring about significant changes in fluorescence spectra which lend insight into the influence of substituent groups in molecules, base–acid equilibria, hydrogen bonding, and the nature of the excited state.

An examination of these effects is also of importance because of the increased use of fluorescence spectroscopy in structural studies, in biochemical and medical research, and in analytical assay procedures. The advent of improved instrumentation including automatic recording spectrofluorimeters, high resolution instruments, and linear energy instruments have widened the scope of these fundamental studies and their application. Furthermore, the sensitivity of the fluorescence method, as compared to ultraviolet and infrared spectroscopy, has made it not only attractive, but in some instances a most valuable tool where only trace amounts of fluorescent materials are available for investigation.

The subject matter of this article was last reviewed by Förster (53) in his book "Fluoreszenz Organischer Verbindungen," published in 1951, and earlier by Pringsheim (125). The present review covers advances in the specified areas made from 1950 through 1961 and including some 1962 publications. It is the purpose of this review to discuss the most notable of the observed effects of solvent, pH, concentration, and temperature on fluorescence spectra of aromatic compounds in dilute solutions and to discuss and coördinate the interpretations of the observed effects. Specialized aspects such as fluorescence polarization and quenching are not discussed in detail but only insofar as they relate to environmental factors in fluorescence.

Analytical applications of fluorescence spectroscopy has recently received considerable attention and will not be discussed here. The use of fluorescence spectroscopy in biology and medicine, for example, is the subject of a recent book (156). General analytical applications and instrumentation, also, were reviewed recently (5, 174).

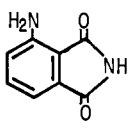
The fluorescence spectra referred to are of two types: (a) *fluorescence emission spectra*, obtained by scanning for fluorescence emission with a fixed excitation wave length, and (b) *fluorescence excitation spectra*, obtained by scanning for fluorescence excitation with a fixed emission wave length. The fluorescence excitation spectrum gives a band or series of bands which is theoretically at the same wave lengths as the ultraviolet absorption maxima since the wave lengths of maximum fluorescence excitation are also those wave lengths at which light is maximally absorbed.

II. Solvent Effects

Although the fluorescence of gases (18, 68, 117) and solids (129) have been measured the vast majority of fluorescence spectra have been measured in solution. As in the case of ultraviolet absorption spectra (64), solvent effects are observed in fluorescence measurements. These effects are manifested by wave length shifts and/or quenching of fluorescence. Fluorescence solvent effects are less well known than ultraviolet absorption solvent effects and the effects of pH on absorption and fluorescence spectra; nevertheless, several groups of compounds are known in which change of solvent brings about remarkable changes. These changes are in some instances accompanied by comparable changes in the fluorescence excitation spectra, and hence also in the ultraviolet absorption spectra; in other instances shifts in the position of the fluorescence emission maxima are not accompanied by comparable changes in the ultraviolet absorption spectra. Displacements in the ultraviolet and fluorescence emission spectra imply interaction with solvent in both the ground state and the excited state of the molecule. On the other hand, when the fluorescence emission spectra alone are effected by change of solvent, interaction between solvent and the excited state, but not with the ground state of the molecule, is indicated. An attempt will be made to discuss here the observed fluorescence solvent effects in the light of accompanying changes, or lack thereof, in the ultraviolet absorption spectra.

A. Wave Length Shifts

As early as 1904 Kauffmann and Beisswenger (78) observed changes in the fluorescence color of 3-aminophthalimide, I, with change in solvent from violet in hydrocarbons to blue-green in ethanol. These workers ascribed the fluorescence changes in 3-aminophthalimide and 9-dimethylaminobenzo(a)phenazine, II, to association in solution and to the effect of the dielectric constant of the solvent. The latter effect was stressed



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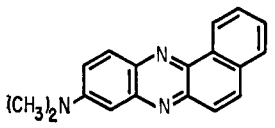


II

in early papers (132). Since then wave length shifts brought about by solvent have been observed in both absorption and fluorescence spectra of many compounds. The effect of the dielectric constant of the solvent was used in explaining some fluorescence solvent effects (38, 73, 160, 164) and hydrogen bonding has been invoked in other instances (29, 122, 123). However, the interpretation of solvent effects is often complicated because the observed changes may be the resultant of several quite different forces which may either augment or minimize each other and these effects have been discussed by various authors (6, 7, 75, 104, 122, 141).

Bayliss and McRae (6, 7) discussed the origin of solvent effects and point out that all spectra undergo a *polarization shift* to longer wave length. This shift results from the induced polarization of the solvent produced by the transition dipole of the solute. It is more clearly observed in nonpolar solvents and solutes, *i.e.*, a red shift occurs with increasing dielectric constant of the solvent. However, it is often obscured by associated effects, *e.g.*, in polar or hydrogen bonding compounds.

An important aspect in the examination of solvent effects in absorption and fluorescence spectra is the *Franck–Condon principle* (6). The relationship between solute and solvent in terms of orientation, dipole moment, and charge distribution is suited to the ground state of the solute. When the molecule changes to the excited state by the absorption of energy this relationship is upset. An excited solute molecule in this situation is in the *Franck–Condon* state. This state prevails for a very brief period of time but the solvent molecules cannot reorient themselves during the transition from the ground to the excited state. It follows then from the Franck–Condon principle that there may be a difference in solvation energy of the ground and excited states and this will be reflected in changes in absorption and emission spectra. The "blue shift," *i.e.*, hypsochromic shift observed in absorption spectra in solvents of high dielectric constant have been described (104, 122) in the light of the Franck–Condon principle. The blue shift is associated with $n \rightarrow \pi^*$ transitions which refers to the excitation of a nonbonding electron to an antibonding orbital (141). However, it was pointed out that $n \rightarrow \pi^*$ transitions are usually

TABLE I

SOLVENT EFFECTS IN THE FLUORESCENCE OF QUINOLINE AND BENZQUINOLINES[a]

	Quinoline			Acridine			5,6-Benzquinoline			3,4-Benzquinoline			7,8-Benzquinoline		
	Exc.[b]	Em.[b]	F.I.[c]	Exc.[b]	Em.[b]	R.F.I.[d]	Exc.[b]	Em.[b]	R.F.I.[d]	Exc.[b]	Em.[b]	R.F.I.[d]	Exc.[b]	Em.[b]	R.F.I.[d]
Cyclohexane	317	330	Very weak[e]	250, 365	400	Very weak[e]	273, 324, 337	344, 361, 379	1	290, 330, 345	350, 367, 385	...	270, 285, 313, 330, 345	343, 362, 380	1.6
Benzene			0.001	...	...	...	278, 320, 333	345, 362, 380	1	300, 337	345, 365, 385	0.35	280, 310, 325, 335, 350	345, 365, 380	1.5
Ethyl alcohol		305–370	0.03	250, 340, 355, 380	415, 440	1	275, 286, 315, 329, 342	348, 365, 380	3.2	298, 330, 347	350, 367, 385	1.0	265, 310, 325, 342	350, 367, 385	2.7
Water			1.0	250, 355, 382	430, 455, 475	8	272, 285, 315, 329, 342	350, 365	4.0	272, 298, 331, 347	352, 367, 385	1.7	265, 275, 310, 325, 342	350, 365	4.0

[a] (162, 163). [b] mμ. [c] Calculated relative fluorescence yield (99). [d] Relative fluorescence intensities (microammeter readings at same sensitivity setting of instrument and same slit width; concentration: 1.0 γ/ml). [e] Very weak fluorescence observed only with higher source intensities.

not observed in fluorescence spectra and when present are weak (76).

Pimentel (122), Brealey and Kasha (29), and Mataga and Tsuno (102) have stressed the significance of hydrogen bonding in solvent effects. In another study (100) it was assumed that solvation energy is mainly contributed by dipolar interaction between solute and solvent, *i.e.*, the observed solvent shifts in absorption and fluorescence spectra of the compounds studied are due to dipolar interaction only. In this work the fluorescence and absorption spectra of 2-methylnaphthalene, 2-napthylmethyl ether, and 1- and 2-naphthol were measured in a series of solvents and the differences in solvation energy of the ground and excited states were used to calculate differences in dipole moment of the two states.

In order to gain a clearer insight into the significance of the various solvent effects, the observed effects in several groups of related compounds are described below.

1. Quinoline, Benzquinolines, and Derivatives

Quinoline and acridine show pronounced shifts in fluorescence emission maxima and increased fluorescence intensities with increasing dielectric constant of the solvent (99, 163). Both quinoline and acridine fluoresce very weakly in cyclohexane and benzene (163) and in some instruments fluorescence could not be detected for acridine in hexane solution (99). On the other hand, both compounds show intense fluorescence in butyl alcohol, ethyl alcohol, and water (99, 163).

In the case of quinoline the ultraviolet absorption maxima do not show shifts to longer wave length with increasing solvent polarity but the extinction coefficients do undergo changes (99). In the fluorescence excitation and ultraviolet absorption spectra of acridine a broadening of the long wave length band is observed with increasing solvent polarity (99, 163) but the bands at shorter wave length (249, 325, 339, and 355 mμ) are not affected (163). These changes are very similar to the effect of pH on these compounds, which will be dealt with below.

In remarkable contrast the ultraviolet absorption and fluorescence maxima of 3,4-, 5,6-, and 7,8-benzquinoline are not affected by change of solvent. Moreover, the fluorescence intensities of the compounds are high even in nonpolar solvents such as cyclohexane and benzene and increase with increasing solvent polarity (162, 163).

The effects of solvents on the fluorescence of quinoline and the benzquinolines are summarized in Table I.

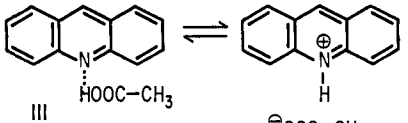
These solvent effects, and the associated pH effects discussed below, become more reasonable when the differences between the electronic structures of the nonfluorescent heterocyclics such as pyridine, pyrazine, and phenazine and the hydrocarbon analogs, benzene, naphthalene, etc., are examined. According to Kasha (75) an electron of the unshared electron pair on the nitrogen atom(s) in these heterocyclics undergoes a transition to an antibonding π molecular orbital. This excited state in turn undergoes a radiationless transition to the so-called *triplet state;* consequently such compounds do not exhibit fluorescence, *i.e.*, they undergo *inner quenching* but they do show phosphorescence at low temperature. When the unshared electrons on the nitrogen atom become involved in hydrogen bonding with proton-donating solvents, transitions to Kasha's "triplet state" become less likely or nonexistent and hence enhanced fluorescence is observed in proton-donating solvents. This interpretation, however, does not explain why the angular benzquinolines are not as remarkably affected by solvent as in the case of acridine.

Additional studies along these lines were made on the absorption and fluorescence spectra and intensities of quinoline and acridine in benzene in the presence of increasing quantities of chloroform, ethyl alcohol, mono-, di-, and trichloroacetic acid (101, 102).

The absorption spectrum of quinoline, in hexane solution showed small increases in ϵ values when ethyl alcohol was added but the bands were not shifted. When trichloroacetic acid was added, there was considerable enhancement of ϵ values of the long wave length bands. Acridine in benzene solution in the presence of ethyl alcohol showed only slight changes in ϵ values. The addition of trichloracetic acid caused the appearance of a broad long wave length band in the absorption spectrum between 380 and 440 mμ, *i.e.*, almost identical with the change in absorption spectrum that occurs on acidification of acridine in aqueous solution. With all the solvents used the fluorescence intensities increased markedly. The fluorescence wave length maxima were not given; however, the spectral range of fluorescence of acridine in benzene is changed by addition of trichloroacetic acid. It was also observed that the increase of fluorescence intensity in the quinoline–trichloroacetic acid system is small by comparison with the increase of fluorescence intensity of the acridine–trichloroacetic acid system.

The effects of trichloroacetic acid on the absorption and fluorescence of quinoline and acridine were ascribed to hydrogen bond formation (102). It should be noted that recent work (147) established the formation of charge transfer complexes between aliphatic amines and halomethanes, including chloroform which was used in the study described above (102). These charge transfer complexes result in a red shift in the absorption spectra (147), and such complexes may well account for some of the spectral shifts described above.

Kokubun (81) studied the effect of added acetic acid on the absorption and fluorescence of acridine in benzene and made the same observations as those described above (102), *i.e.*, minor changes in absorption and increased fluorescence intensity on addition of acetic acid. It was concluded that hydrogen bond formation, III, took place in the ground state but that in the excited state an ion-pair predominates, IV, *i.e.*, in the



III IV

excited state the equilibrium is shifted to the right as a result of the increased basicity. This difference in basicity between the ground and excited states of fluorescent organic compounds has been carefully examined and will be dealt with in section III.

The effect of solvents on the absorption and fluorescence spectra of acridone was studied by Kokubun (82); the spectra were measured in a series of solvents varying in polarity between benzene and aqueous ethyl alcohol. The absorption and fluorescence maxima showed the expected shift to longer wave length with increasing dielectric constant of the solvent. Thus, in benzene the absorption maxima occur at 361 and 387 mμ, whereas in aqueous ethyl alcohol bands are observed at 296, 364, 383, and 401 mμ. The fluorescence emission maxima in benzene occur at 397 and 414 mμ and at 414, 437, and 461 mμ in aqueous ethyl alcohol. In the other solvents the maxima fall between these two extremes. Since the acridone molecule can behave as both donor and acceptor in hydrogen bond formation these solvent effects are expected. In proton-donating solvents the fluorescence intensities are increased (23, 82). The quantum yield of fluorescence of acridone is close to unity in methyl-, ethyl-, isopropyl alcohol, and in water from $-70°$ to $+20°$ (23). In proton-accepting solvents (dioxane, ether, ethyl acetate, and acetone) the fluorescence intensity is decreased (23, 82).

Fluorescence was also reported for the N-oxides of pyridine, quinoline, isoquinoline, and related compounds (86, 87). In most, but not all, compounds examined a small shift to longer wave length occurs with increasing solvent polarity in the fluorescence spectra. These shifts are small compared to shifts observed in other compounds, *e.g.*, the indoles discussed below, and, in addition, shifts to longer wave length occurred also in the fluorescence spectra of some of the parent compounds. The ultraviolet absorption spectra of these compounds, all showed significant shifts to shorter wave length with increasing solvent polarity in contrast to the normal shift to longer wave length associated with $\pi \rightarrow \pi^*$ transitions (87). The solvents of higher solvent polarity were all hydrogen bonding solvents and hence it is reasonable to conclude that hydrogen bonding played an important role in the observed blue shifts in the absorption spectra.

2. Indole, Substituted Indoles, and Carbazole

Indole and substituted indoles show pronounced shifts to longer wave lengths in their fluorescence emission maxima with increasing dielectric constant of the solvent (164), but comparable shifts are not observed in the ultraviolet absorption and fluorescence excitation spectra. Fluorescence data on indole and some substituted indoles are presented in Table II.

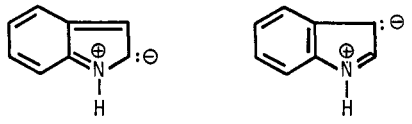
Polar mesomeric forms of indole, such as structures V and VI, may make a larger contribution in the excited state of indole than in the ground state and such structures are expected to be more sensitive to the dielectric constant of the surrounding solvents. This will explain why changes occur in the emission spectrum

TABLE II

SOLVENT EFFECTS IN THE FLUORESCENCE OF INDOLE AND SUBSTITUTED INDOLES[a]

	Cyclohexane			Benzene			Dioxane			Ethyl alcohol			Water		
	a[b]	b[b]	c[c]	a	b	c	a	b	c	a	b	c	a	b	c
Indole	285	297	6.0	285	305	0.6	285	310	5.8	285	330[e]	6.1	285	350[d]	4.0
													280	350	...
2-Methylindole	280	306	6.2	280	305	Very weak	280	325	8.9	280	335	6.5	280	355[d]	2.0
													280	355	...
3-Methylindole	280	315	6.6	280	317	Very weak	280	329	8.0	280	350	6.0	280	370	6.3
													290	370[d]	...
1,2-Dimethylindole	291	315	10.0	291	326	11.0	291	329	7.7	291	340	11.0	291	362	7.9
2,3-Dimethylindole	282	320	6.0	282	330	0.1	282	340	11.0	282	360	9.1	282	376	3.6
3-Hydroxymethylindole	285	305	4.8	285	325	0.85	285	327	7.6	285	338	8.4	285	360	7.4
Indole-3-acetic acid	No fluorescence			No fluorescence			290	325	8.5	290	340	6.3	290	360	5.3
													285	360[d]	...
1-Methyl-2-phenyl indole	310	360	64.0	310	370	63.0	310	370	72.0	310	370	61.0	305	380	17.0
Tryptophane	...	...	...	...	...	...	...	...	...	...	...	...	280	345[f]	...
														348[g]	...
														360[d]	...

[a] Except where indicated otherwise data were taken at room temperature and are from ref. 164. [b] a. Fluorescence excitation maximum, mμ; b. Fluorescence emission maximum, mμ. [c] c. Relative fluorescence intensities, uncorrected; microammeter readings at same sensitivity setting, slit width, and concentration, 4.7×10^{-5} mole/l. [d] Reference 142. [e] Indole shows maxima at 310 and 320 mμ (63) at −196° in ether–*n*-pentane–ethyl alcohol rigid glass. [f] −196°; in the presence of 0.5 to 10% glucose in water this maximum is shifted to 325 mμ and its intensity is doubled (63, 143). [g] Reference 153.



V VI

only. On the other hand, indole can behave as proton donor or acceptor with hydrogen bonding solvents and this factor also was taken into consideration. Thus, the two nitrogen-substituted indoles included in Table II cannot undergo hydrogen bonding with dioxane and yet their fluorescence emission maxima follow the same shift to longer wave length with increasing solvent polarity exhibited by the other indoles, not substituted on the nitrogen atom, so that it would appear that solvent polarity is an overriding factor in this series (164).

The fluorescence intensities of the indoles are very similar in cyclohexane, dioxane, and ethyl alcohol, but are generally lower in water and very weak in benzene except in the cases of 1-methyl-2-phenylindole and 1,2-dimethylindole. This suggests that the free $\rangle$NH is involved in interactions in benzene solutions.

As in the case of indole, the unshared electrons of the nitrogen atom in carbazole contribute to the π-electronic state of the whole molecule. Solvent effects similar to those observed for indole are therefore to be expected for carbazole. Shifts in fluorescence emission maxima to longer wave length were observed for carbazole with solvents of increasing dielectric constants. These shifts are smaller than in the indoles, *e.g.*, in cyclohexane solution carbazole shows emission maxima at 332 and 348 mμ; in ethyl alcohol and in water the bands are shifted to 342 and 355 mμ, respectively. In addition comparable shifts are observed in the ultraviolet absorption spectra with change of solvent (162, 164).

3. Naphthols and Naphthylamines

In an examination of the effect of hydrogen bond formation on fluorescence, the absorption and fluorescence spectra of 2-naphthol and 1- and 2-naphthylamine were measured in hexane and benzene alone and in these solvents in the presence of several proton acceptors; dioxane, methyl, ethyl, and butyl acetates (96, 98) were used as acceptors. All these solvent mixtures caused increases in fluorescence intensity compared to the fluorescence emission in the hydrocarbon solvent alone. The fluorescence of 2-methoxynaphthalene in hexane was not affected by these solvents. Carbon tetrachloride and ethylchloroacetate caused a quenching of the fluorescence of 2-naphthol (98). The ultraviolet absorption spectra showed small increases in ε values and small shifts to longer wave lengths with both types of proton acceptors enumerated above. There is also a slight shift of fluorescence emission maxima to longer wave length in going from hexane to methyl alcohol. Thus, 1- and 2-naphthol in hexane solution show fluorescence maxima at 325.6 and 335.6 mμ, respectively; in methyl alcohol these bands are shifted to 329.1 and 343.5 mμ (100). In the case of 2-methoxynaphthalene fluorescence is shifted from 344 mμ in hexane solution to 346.7 mμ in methanol solution (100). Pyridine caused quenching of the fluorescence of 2-naphthol and 1- and 2-naphthylamine (96, 97, 103), but quenching was not observed in the presence of phenol or aniline (97).

Mataga and Tsuno (97, 101, 103) also studied the effect of hydrogen bonding solvents on the fluorescence intensity of acridine, 3,6-diaminoacridine, and 3,6-

dimethylaminoacridine in benzene or hexane solution. When phenol, aniline, or pyrrole were used as hydrogen bonding solvents, considerable quenching of fluorescence of 3,6-dimethylaminoacridine and 3,6-diaminoacridine was observed (101, 103). These two compounds, in contrast to acridine, normally fluoresce in nonpolar solvents. This quenching effect was independent of solvent viscosity, *i.e.*, it is not a diffusion controlled process and was ascribed to interaction between the π electron systems of the fluorescer and quencher molecules *via* hydrogen bonds between the proton donor and acceptor. This will result in delocalization of the π electrons of the excited state and hence loss of fluorescence. When proton donors such as benzyl alcohol or 2-phenylethyl alcohol were used enhancement of fluorescence was observed (102). In these donors the hydrogen atom is separated from the π electron system by one or more aliphatic carbon atoms and hydrogen bonding then leads to enhancement of fluorescence as was explained above. In contrast to the naphthols and naphthylamines, the fluorescence of the acridine derivatives is not quenched by pyridine.

4. *Chlorophyll*

Chlorophyll shows, in nonpolar solvents such as benzene, very little fluorescence when water is rigorously excluded (95). Addition of water of a polar solvent causes a remarkable enhancement of fluorescence. This phenomenon is probably due to hydrogen bonding between the polar solvent (methyl or ethyl alcohol or water) and chlorophyll and has been studied in relation to its effect on the absorption spectra of chlorophyll and related compounds (61).

5. *Anthracene and Derivatives*

Kortüm and Finckh (83) and Sambursky and Wolfson (128) have measured the wave length shift of anthracene in various solvents. These earlier results were summarized by Förster (53, p. 135). Not only is there a shift to longer wave length in this series with increasing polarizability but the distance between the last absorption band and the first fluorescence band increases in going from vapor to hexane to chlorobenzene. It was concluded that increased interaction between solute and solvent gave rise to increased broadening of the bands, and that this is not due to electrostatic interaction between solute and solvent, but rather to dispersion forces.

The fluorescence red shift in different solvents was examined more recently (165) for anthracene and several substituted anthracenes in 19 different solvents. The shifts were ascribed, as concluded earlier by Förster (53, p. 135), to dispersion forces. In the case of 9-cyanoanthracene the wave length shift is in the opposite direction from anthracene and its other derivatives, *i.e.*, the red shift is inversely proportional to the polarizability of the solvent. This was ascribed (165) to a polar–polar interaction between solvent and 9-cyanoanthracene; such interaction would cancel out dispersion force interactions and hence the observed effect. On the other hand, 9-oximinoanthracene which contains the polar oximino group does not show the same fluorescence solvent effect observed for the cyano compound and it is possible that in this case hydrogen bonding takes place between solute and solvent (165).

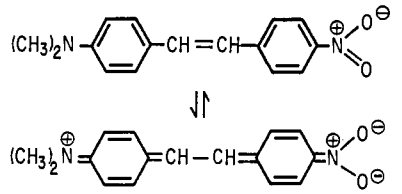
The fluorescence of 1- and 2-acetylanthracenes was examined in a series of solvents (38). In nonpolar solvents the shift of fluorescence maximum increases with increasing refractive index of the solvent. In polar solvents the displacement is larger but is independent of the dielectric constant. Significant shifts occur with hydroxylic solvents and hydrogen bond formation was invoked to account for the effect of hydroxylic solvents on the positions of the fluorescence emission maxima.

6. *Stilbene Derivatives*

Lippert and co-workers (93, 94) examined the fluorescence and absorption characteristics of polar compounds of the general type

D—R—A

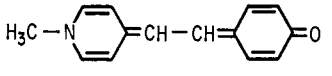
where D is an electron-donating group, A an electron-accepting group, and R a system of conjugated double bonds. This group of compounds belong to two types: (i) exemplified by 4-nitro-4′-dimethylaminostilbene, VII, which can exist in a zwitterionic mesomeric form, VIII. Other compounds of this type include those in



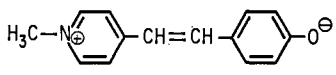
VII

VIII

which D = $(CH_3)_2N$—, —NH_2, —OH, —OCH_3, and —CH_3; A = —NO_2, —CN, —CF_3, —CCl_3; and R = 4,4′-disubstituted stilbene or diphenyl; and (ii) exemplified by IX which can exist in the ionic form X.



IX



X

The polar mesomeric form, VIII, probably makes a larger contribution in the excited state of the molecule and the polar mesomeric form X, of the pyridine derivative, IX, makes a larger contribution in the ground state of the molecule. In type (i) compounds the position of the fluorescence emission maxima is strongly

TABLE III

QUANTUM YIELD OF FLUORESCENCE OF AROMATIC HYDROCARBONS AND DERIVATIVES IN VARIOUS SOLVENTS[a]

	Hexane	Acetic acid	Benzene	Toluene	p-Xylene	Pyridine	Chlorobenzene	Thiophene	Chloroform
Anthracene	0.32	0.31	0.24 0.241[b]	0.23	0.17	0.16	0.15	0.12	0.10
1-Chloroanthracene	...	...	0.09	...	...	...	...	...	.05
1,5-Dichloroanthracene	...	...	0.065	...	...	...	...	...	.04
9,10-Dichloroanthracene	...	...	0.65	...	...	...	...	...	.50
9-Phenylanthracene	...	...	0.74	...	...	...	...	...	.40
9,10-Diphenylanthracene	...	...	0.80 0.840[b]	...	...	...	...	...	.65
Perylene	...	...	0.96 0.800[b]	...	...	...	...	...	.88

[a] At 20° and 365 mμ excitation, except for perylene, excitation 313.5 mμ (18, 25, 26). These values were obtained by direct conversion of measurements into absolute quantum yields by using a value of 0.24 for the quantum yield (F_0) of anthracene in a deoxygenated benzene solution. (F_0 = quanta emitted/quanta absorbed.) [b] Reference 108, 25°.

solvent dependent and shifts to longer wave length (red shift) with increasing solvent polarity. The absorption spectra are not much affected by solvent, although small shifts are observed. 4-Dimethylamino-4′-nitrodiphenyl, for example, fluoresces very weakly in cyclohexane. Addition of increasing quantities of *o*-dichlorobenzene to a cyclohexane solution of this compound caused an increase in fluorescence intensity and a progressive shift of the fluorescence emission maxima to longer wave length. A smaller shift to longer wave length occurs in the absorption spectrum (93). In type (ii) compounds, exemplified in this work (94) only by IX, the absorption maxima are strongly solvent dependent (94); the blue shift becomes more pronounced with increasing solvent polarity. The fluorescence of IX is weak and green in color, and the maxima could not be carefully determined.

B. SOLVENT QUENCHING

In studying the quenching effect of solvents on the fluorescence of aromatic compounds it is of importance to determine the *quantum yields of fluorescence*, also referred to as fluorescence efficiency, in different solvents, rather than uncorrected relative intensities.

The fluorescence efficiency (F_0) is the ratio of the number of light quanta absorbed to the number of light quanta emitted and there are formidable instrumental and experimental difficulties which have to be overcome in its measurement (18, 25, 108). Many measurements have been made of the quantum yield of fluorescence (18, 24, 25, 26, 37, 43, 65, 108, 183) and these measurements are of significance in the study of fluorescence quenching either by internal conversion or intersystem crossing (*i.e.*, singlet–triplet transitions) and in the examination of transfer of electronic excitation energy. The fluorescence efficiency which is an inherent property of a compound is influenced by solvent, temperature, and concentration. The last two factors are discussed in other sections of this review.

Bowen points out (18) that in the vapor phase at 300° anthracene shows a quantum yield near unity. In solution the quantum yield is much lower and varies with the solvent. The quantum yield of anthracene, anthracene derivatives, and other aromatic hydrocarbons have been examined by several workers (18, 24, 25, 26, 108) and some of these results are tabulated in Table III in order to show the magnitude of the effect of solvent on fluorescence efficiency. Of incidental interest here is the remarkable difference in fluorescence efficiency, under the same conditions of solvent and temperature, between 1,5-dichloroanthracene and 9,10-dichloroanthracene. In general the fluorescence efficiency of the *meso*-substituted anthracenes are higher than those anthracene derivatives with substituents in other positions. Melhuish (108) also reports a high quantum yield of fluorescence for 9-cyanoanthracene.

The question arises then as to the mechanism of degradation of excitation energy in solution. According to Bowen and West (26) solvent quenching is a complex effect depending not only on the orientation of the excited molecule in the solvent cage but also on van der Waals interactions between different parts of the solute and solvent.

Aromatic hydrocarbons have been found to exhibit a lower fluorescence intensity in solvents such as acetone or chloroform compared to nonpolar solvents such as hexane. This phenomenon has been ascribed to extensive van der Waals interaction in acetone or chloroform. Rubrene is more fluorescent in a mixture of acetone and chloroform than in either solvent alone at low temperature. This was ascribed to strong van der Waals interaction between solvent and rubrene (27).

Fluorescence quenching in solution has also been linked to singlet–triplet changes (26, 124). Some observations on the fluorescence and phosphorescence of indole and its derivative are noteworthy in this respect. The fluorescence of indole in cyclohexane solution at room temperature is quenched by the addition of 1% acetone (164). In ether–pentane–alcohol rigid glass at −196° the fluorescence of indole occurs at 310 and 320 mμ (excitation 280 mμ); addition of 2% acetone caused a decrease in intensity to one-half of

its value in the absence of acetone. The longer wave length blue phosphorescence (430, 450 mμ) of indole undergoes a fourfold increase in intensity under these conditions (63). The fluorescence and phosphorescence maxima are not shifted by the addition of acetone. Similarly, tryptophane shows, at −196° in water, a peak at 345 mμ which is completely quenched in an acetone–water (8:2) rigid glass. The longer wave length phosphorescence peak of tryptophane is increased in intensity by the addition of acetone.

McRae and Kasha (107) have commented on the enhancement of quantum yield of phosphorescence at the expense of fluorescence emission in the aggregation of dyes and it is possible that acetone facilitates internal conversion from the singlet to the triplet state. In another report on the fluorescence and phosphorescence of indole and related compounds (60) it was pointed out that the phosphorescence of indole in rigid glass at −196° was enhanced by the addition of almost any solute, *e.g.*, electrolytes. Also, the addition of glucose enhances the phosphorescence of tyrosine and tryptophane at −196° (143). Other instances have also been observed in which phosphorescence increases at the expense of fluorescence, *e.g.*, mesoporphyrin exhibits an intense fluorescence emission spectrum and weak phosphorescence. When magnesium is complexed to mesoporphyrin there is a sharp decrease of fluorescence and an increase in phosphorescence (1).

Specific examples of solvent quenching in which wave length shifts also occur have already been referred to in Section A above. Several cases of solvent quenching were observed and described in which interpretations were either not provided or are not clear. The influence of hydrogen bonding in solvent quenching varies from increased fluorescence intensities, *e.g.*, acridine (102) to decreased fluorescence intensities, *e.g.*, acridine N-oxide (84). The fluorescence of acridine oxide is quenched in phenol, ethyl alcohol, or acetic acid but is not quenched by anisole.

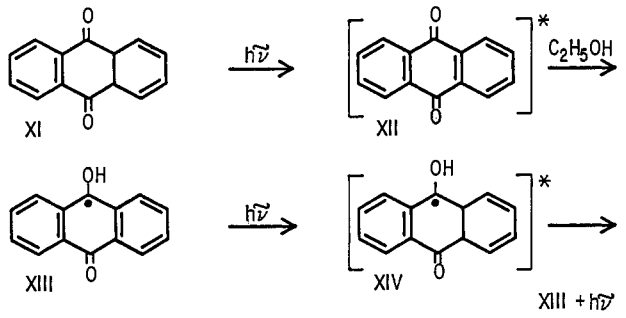
C. SOLVENT VISCOSITY EFFECTS

In a discussion on concentration quenching, Förster (53, p. 230) classified compounds which undergo such quenching into various classes. One of the criteria used in this classification was solvent viscosity. Aromatic hydrocarbons, for example, undergo increased quenching with decreasing viscosity of the solvent. The quenching of dyes in nonaqueous solution, on the other hand, are not affected by solvent viscosity. These effects of solvent viscosity have been used extensively in studies of quenching of fluorescence (80, 101, 103, 109, 152) and other fluorescence phenomena such as the dimerization of the excited state of pyrene (58, 77), electron interaction between acridine derivatives and phenol, aniline, or pyrrole (101) and transfer of excitation energy (169). These studies are discussed in appropriate sections of this review.

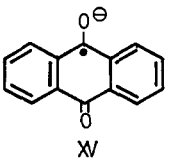
Polarization of fluorescence is intimately associated with the examination of viscous solutions. The theory of this specialized aspect of fluorescence spectroscopy was discussed in detail by Förster (53, p. 160) and by Bowen (27, p. 15) and has been reviewed recently particularly with respect to its use in the examination of materials of biological origin (89, 145, 156) and need not be discussed further.

D. FLUORESCENCE OF RADICALS

It is generally accepted that quinones do not exhibit fluorescence (53, p. 105, 171). Recent work (150) has shown that 1,4-naphthoquinone, 9,10-anthraquinone, and aromatic ketones can be converted to fluorescent free radicals under certain conditions. The absence of oxygen is required and also the use of a solvent such as ethyl alcohol from which an hydrogen atom can be abstracted. Thus anthraquinone shows fluorescence in absolute ethyl alcohol solution at 412 and 481 mμ. This fluorescence is ascribed to the formation of a fluorescent semiquinone radical. In the presence of oxygen such radicals will be trapped and no fluorescence will be observed. The radical formation probably takes place as shown in structures XI through XIV.



In this scheme the abstraction of a hydrogen atom from ethyl alcohol is accomplished by the excited anthraquinone molecule. Supporting evidence for the formation of a semiquinone radical is supplied by the examination of the fluorescence of anthraquinone in alkaline absolute ethyl alcohol. The fluorescence emission maxima are shifted to longer wave lengths (597 (sh), 559 (sh), 538 mμ) probably due to the formation of a radical ion, XV. The formation of intermediates of this kind was postulated earlier (30) in a study of the flash photolysis of quinones. The fluorescence spectra of anthraquinone and its derivatives, 2-chloro-, 2-methyl-, and 2-aminoanthraquinone have also been studied in dilute solutions (10^{-4} to 10^{-5} mole/l.) at −196° in hexane, heptane, and octane (135) and fine structure was observed in the fluorescence spectra. Caution should be exercised in the interpretation of fluorescence data obtained on compounds where prolonged irradiation produces more profound changes,



XV

e.g., the formation of the hydroquinone in the flash photolysis of anthraquinone (30) and the dimerization of 1,4-naphthoquinone (54).

1,4-Naphthoquinone fluoresces at 420 mμ in absolute ethyl alcohol in the absence of air. The fluorescence maxima for some aromatic ketones and radicals are given in Table IV (150).

TABLE IV

FLUORESCENCE OF AROMATIC KETONES AND SOME RADICALS[a]

	Emission maxima, mμ
Acetophenone	400
4-Bromoacetophenone	370
4-Nitroacetophenone	382
Benzaldehyde	368
Benzoin	367
Benzophenone	373
4-Bromobenzophenone	373
Benzoyl peroxide (C_6H_5COO)·	344, 370 (sh)
Diphenylamine ($C_6H_5)_2N$·	333, 360
Phenazine radical	469

[a] In absolute ethyl alcohol under nitrogen.

Phenazine on irradiation shows fluorescence at 420 mμ in the presence of air and at 469 mμ in the absence of air. The latter fluorescence is ascribed to that of the phenazine radical and is of interest since phenazine is well known for its lack of fluorescence (27, p. 26; 171, p. 731).

III. INFLUENCE OF pH

The basicity or acidity of a molecule is determined by its electronic structure and this may undergo detailed changes during excitation from the ground state by the absorption of light, *i.e.*, the basicity or acidity of a compound may change during excitation. A difference in the basicity or acidity of the ground and excited states will be reflected in differences between the absorption and fluorescence spectra with change in pH. The nature of the changes will depend on whether the basicity is increased or decreased during ionization. Also, in order for the expected changes in pK to be observed, it is essential that the ionization equilibrium be established during the lifetime of the excited state. Weller (170) discussed the absorption and fluorescence spectral shifts in terms of the energy changes involved. A simplified energy diagram is presented in Fig. 1. This diagram represents the energy terms involved in the dissociation of an acid (HA) and the corresponding base (B). ΔH represents the enthalpy of the dissociation reaction in the ground state and ΔH^* the same

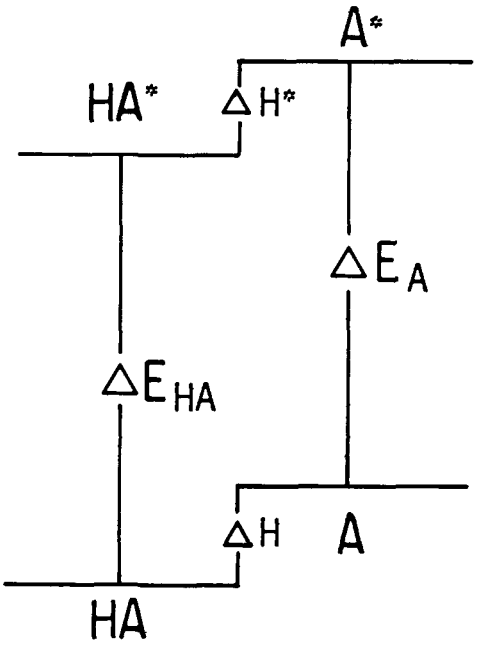


Fig. 1.—Energy diagram for dissociation of an acid (HA) in the ground and excited states.

for the excited state. When $\Delta H > \Delta H^*$ a red shift occurs in absorption and fluorescence spectra on acid dissociation; when $\Delta H < \Delta H^*$ a blue shift occurs on acid dissociation, and when $\Delta H = \Delta H^*$ there is no shift in either absorption or fluorescence spectra.

Since,

$$\Delta H - \Delta H^* = \Delta E_{HA} - \Delta E_A \qquad (1)$$

where

ΔE_{HA} = excitation energy for the 0–0 transition of the acid, and

ΔE_A = the excitation energy for the 0–0 transition of the base, and

assuming that the entropy of the reaction of acid dissociation is the same for the ground and the excited states it is possible to calculate the difference in pK between the two states from equation 2.

$$pK - pK^* = \frac{\Delta E_{HA} - \Delta E_A}{2.3\,RT} \qquad (2)$$

When $\Delta E_{HA} - \Delta E_A > 0$ the excited state is more acidic than the ground state, *e.g.*, oxy- and amino aromatic compounds; when $\Delta E_{HA} - \Delta E_A < 0$ the excited state is less acidic, *e.g.*, acridine and acridone, and, of course, when $\Delta E_{HA} - \Delta E_A = 0$ there is no difference in pK between the ground and excited states, *e.g.*, pyridine. From the position of the 0–0 transition for acridine and the acridinium cation, Weller (170), using the method described above, calculated the pK for the excited state of acridine to be 10.35 and that for the ground state, 5.45. On this basis then it is expected that in aqueous solution acridine will show a fluorescence change at pH 10 which is independent of the ab-

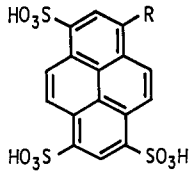
sorption change at pH 5.45. The green fluorescence of the cation should change to the blue fluorescence of the free base between pH 9 and 11. Neutral aqueous acridine solutions, however, fluoresce blue so that it has to be concluded that the required ionization equilibrium is not established during the lifetime of the excited state.

In recent years a great deal of work has been accomplished in the study of the effects of pH on fluorescence and accompanying changes in absorption spectra. A discussion of the notable studies follows.

A. HYDROXY- AND AMINOPYRENESULFONIC ACIDS

Early studies (44) on pH effects were concerned with the fluorescence of 1-naphthol-4-sulfonic acid, 2-naphthol-3,6-disulfonic acid, and 1-naphthol-2-sulfonic acid. In this work the visible fluorescence of these compounds was examined at various pH values and found to show in certain pH ranges such sharp changes in fluorescence intensities (wave length shifts were not examined) that these changes could be used for acid–alkali titrations. Since the time of these early observations a number of other compounds have become useful as fluorescent indicators because of the pronounced changes in visible fluorescence which they exhibit with change in pH (171, p. 714; 156, p. 470).

Weber (167) in his studies on the fluorescence of 1-aminonaphthalene-4-sulfonic acid found a shift to longer wave length in going from a neutral to an alkaline solution. Förster (50) observed that this shift from a blue-violet to a green fluorescence was not a continuous displacement but instead involved the disappearance of one maximum and the appearance of a new maximum at a longer wave length. This fluorescent change was not accompanied by a change in the ultraviolet absorption spectrum. In further explorations of these phenomena Förster (51) used the more intensely fluorescent 3-hydroxy- and 3-aminopyrene-1,6,8-trisulfonic acid, XVI and XVII (121) and measured their ultraviolet absorption and fluorescence emission spectra at various pH values.



XVI: R = -OH
XVII: R = -NH2

3-Hydroxypyrene-1,6,8-trisulfonic acid, XVI, showed in its ultraviolet absorption spectrum a shift to longer wave length above pH 7 due to dissociation of the phenolic hydroxyl. The fluorescence emission spectrum, λ_{max} 510 mμ, shows little change in the pH range in which the ultraviolet absorption is effected. As the solution becomes more acid between pH 2 and 0 the 510 mμ band decreases in intensity, and a new band appears at 455 mμ. In stronger acids the 510 mμ band disappears completely and is replaced by the 455 mμ band.

At pH 0 the ultraviolet absorption spectrum of the 3-aminopyrene-1,6,8-trisulfonic acid, XVII, is very similar to that of pyrene. Above pH 2 the spectrum changes to that of the free amine and remains unchanged to pH 14. Between pH 0 and 12 the fluorescence emission maximum remains unchanged at 500 mμ. Between pH 12 and 14 this maximum is shifted to 580 mμ. This shift is ascribed by Förster to the abstraction of a proton from the amine

$$[RNH_2]^* + OH^- \rightarrow [RNH^-]^* + H_2O \qquad (3)$$

In both these and other compounds examined by Förster (51) changes in the ultraviolet absorption spectra occur at pH values which are different from those at which the fluorescence spectra change, *i.e.*, the acidity of these compounds differ in the ground and excited states. Förster concluded that in the compounds in question the ionization equilibrium is established during the lifetime of the excited state. For the hydroxypyrenesulfonic acid the dissociated form occurs at a lower pH value in the excited state than in the ground state, *i.e.*, dissociation is favored in the excited state.

In the case of the aminopyrenesulfonic acid, XVII, the amino group is more acidic in the excited state than in the ground state. In order to abstract a proton from the amino group in the ground state stronger basic conditions are therefore required. This was accomplished (16) by the use of sodium amide in liquid ammonia. The long wave length absorption maxima of XVII is shifted from 415 mμ in water solution and 433 mμ in liquid ammonia to 575 mμ in liquid ammonia in the presence of sodium amide. This latter absorption is ascribed to the anion, RNH^-. The fluorescence emission spectrum in water between pH 0 and 12 occurs at 500 mμ and in liquid ammonia at 490 mμ with a shoulder at 578 mμ. Between pH 12 and 14 in aqueous solution the 500 mμ maximum is shifted to 580 mμ which is close to the observed fluorescence emission maxima in liquid ammonia in the presence of sodium amide; in the latter medium the maximum occurs at 592 mμ, and this maximum is ascribed to the excited anion $[RNH^-]^*$. However, in the presence of an excess sodium amide the fluorescence emission maximum is shifted to *ca.* 700 mμ while the absorption spectrum remains unchanged; this band is ascribed to the anion $[R-N^{--}]^*$. 3-Methylaminopyrene-1,6,8-trisulfonic acid undergoes similar absorption and fluorescence changes with increasing basicity. However, no further changes occur in the presence of an excess of sodium amide in liquid ammonia, since loss of a second proton cannot occur.

3-Dimethylaminopyrene-1,6,8-trisulfonic acid shows the same absorption spectrum in water, liquid ammonia,

and liquid ammonia in the presence of sodium amide, with maxima at 380 and 403 mμ. The fluorescence emission spectrum, as expected, also remains unchanged in all these media with a maximum at 483 mμ.

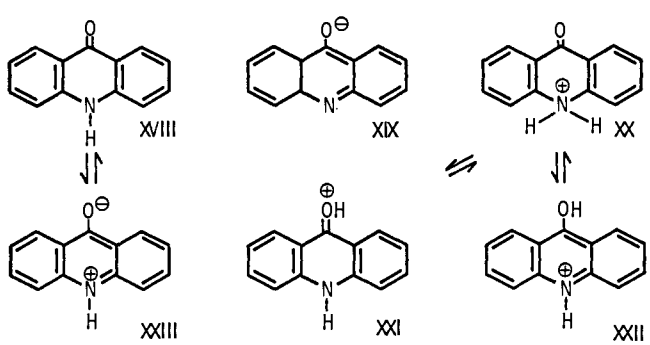
B. QUINOLINE, BENZQUINOLINES, AND DERIVATIVES

Both quinoline and acridine show shifts in their fluorescence emission spectra with change of pH (99, 163, 170, 181).

On changing the pH of aqueous acridine solutions from 3.7 to 8.0 the positions of the long wave length absorption maxima remain unchanged, but ϵ values increase and a new broad band appears in the region 380 to 440 mμ (99). From these studies of the effect of pH on the absorption spectrum it was concluded that the pK of acridine is ±5. The same workers (99) measured the fluorescence of acridine at various pH values and concluded that the shift in fluorescence emission to shorter wave length with increasing pH occurs at a much higher pH (between 9.5 and 10.0) than the corresponding change in absorption spectrum on protonation. In water the fluorescence emission maxima of acridine occur at 430, 455, and 475 mμ, whereas in acid the maxima are at 457, 484, 505, and 515 mμ (163). An apparent change in fluorescence color at pH 5 is ascribed (99) to reabsorption of fluorescence by the 380–440 mμ broad absorption band which becomes prominent below pH 5. Quinoline and 2,8-diaminoacridine show behavior very similar to that exhibited by acridine in their changes in absorption and fluorescence spectra with pH. It follows then that the N-heterocyclics are stronger bases in the excited state than in the ground state (99, 170). In order to explain increased basicity in the excited state, it was suggested that polar structures in which electrons are localized on the nitrogen atom make a large contribution in the excited state of the molecule (99).

The effect of pH on the absorption and fluorescence spectra of acridine, phenazine mono, and phenazine di N-oxides was also studied and it was concluded that the basicity of acridine-N-oxide was the same in the ground and excited states while the phenazine-N-oxides are stronger bases in the excited state (85).

Acridone XVIII is amphoteric and gives in alkali the anion XIX and in acid the cation XX, which can exist



in the various mesomeric forms XXI and XXII. The neutral molecule may exist partly as the zwitterion, XXIII (82, 185, 187), in which case it can be considered to be a derivative of the acridine cation (82). In both acid and in alkali the fluorescence and absorption maxima of acridone, and 9-aminoacridine are shifted to longer wave length, Table V (82). Zanker and Wittwer

TABLE V

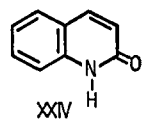
FLUORESCENCE EMISSION MAXIMA OF ACRIDONE AND 9-AMINOACRIDINE

Medium	Fluorescence emission maxima, mμ
Acridone	
Aqueous ethyl alcohol	414, 437, 461
Concentrated sulfuric acid	437, 460, 485
Aqueous ethyl alcohol, 0.2 mole/l. sodium hydroxide	444, 471, 496
9-Aminoacridine	
Aqueous ethyl alcohol, 0.1 mole/l. perchloric acid	429, 454, 481
Aqueous ethyl alcohol, 0.1 mole/l. sodium hydroxide	444, 471, 494

(187) point out that the cation of 9-aminoacridine and acridone in neutral form have very similar electronic distributions and presented their absorption spectra which are, as expected, closely similar. Comparison of the fluorescence data of these two compounds given in Table V also shows close similarities.

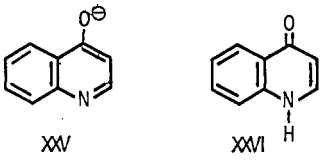
A related series of amphoteric compounds were examined by Williams (175). These materials, 2-, 3-, 4-, 6-, and 7-hydroxyquinoline showed pronounced differences in the fluorescence changes which they undergo with changes in pH. By contrast 5- and 8-hydroxyquinoline do not fluoresce and 6-hydroxyquinoline fluoresces only very weakly. However, metal complexes of 8-hydroxyquinoline are well known for their intense fluorescence (171, p. 736) and this is not unusual since a number of other nonfluorescent organic compounds chelate with metal ions in solution to give fluorescent products (62).

The absorption maxima of 2- and 4-hydroxyquinoline (47) are not affected by change in pH. The fluorescence emission maxima of 2-hydroxyquinoline also remains unchanged (excitation 280 and 325 mμ; emission 380 mμ) with change in pH from 1 to 14 and this compound probably is in the keto (lactam) form XXIV.



In the case of 4-hydroxyquinoline, however, the fluorescence emission shifts from 365 mμ at pH 1 to 390 mμ at pH 14 and the fluorescence intensity increases with increase in pH. The long wave length emission at

pH 14 must be ascribed to the anion XXV, whereas at lower pH values the quinolone form, XXVI, is predominant. This compound differs in its behavior from acridone in that in the latter the absorption also changes with change in pH; but both exhibit a shift to longer wave length in their emission spectra.



XXV XXVI

3-Hydroxyquinoline shows a fluorescence maximum at 450 mμ from pH 1 to 7 and at 420–430 mμ at pH 10 to 14. 7-Hydroxyquinoline also shows a shift of the fluorescence emission maximum to shorter wave length with increasing pH; at pH 14 this maximum is at 490 mμ and at pH 1 to 10 it occurs at 510 mμ. Changes occur also in the absorption spectra of these two compounds with change in pH. 7-Hydroxyquinoline is probably in the cation, XXVII, form at pH 1 to 10 and the anion form, XXVIII, at pH 14.

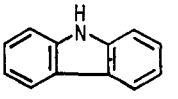
XXVII XXVIII

C. INDOLES AND CARBAZOLE

Indole in water fluoresces at 350 mμ; in the presence of 2 *N* sulfuric acid this band remains at 350 mμ but its intensity is reduced to one-third (164). This is ascribed to the loss of aromaticity in the indole cation and hence decrease of fluorescence. In cyclohexane in the presence of trichloroacetic acid the fluorescence emission maximum of indole remains at 297 mμ but the fluorescence intensity is reduced to one-tenth its value in cyclohexane (164). Similarly tryptophane, tyramine, and related compounds, all of which fluoresce in neutral pH range, lose fluorescence in acid solution (63, 157, 173). The fluorescence of these compounds is also quenched at pH values above 9.5 (173). This may be due to abstraction of a proton to give the anion of the indole nucleus as was described above for 3-aminopyrene-1,6,8-trisulfonic acid. This suggestion is supported by the observation that indole and its derivatives exhibit no detectable fluorescence in 1 *N* sodium hydroxide; however, the fluorescence of N-methylindole remains unchanged even in 5 *N* sodium hydroxide (173).

The ultraviolet absorption and fluorescence emission maxima of carbazole are not affected by acidification with 2 *N* sulfuric acid (164). However, in alkaline N,N-dimethylformamide (prepared from N,N-dimethylformamide containing aqueous tetraethylammonium hydroxide) the ultraviolet and fluorescence spectra are shifted to longer wave length, due to the formation of the carbazole anion (131). The fluorescence excitation and emission maxima of carbazole in various media are given in Table VI.

TABLE VI
FLUORESCENCE OF CARBAZOLE IN VARIOUS MEDIA



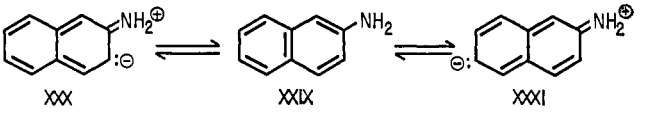
Medium	Fluorescence excitation maxima, mμ	Fluorescence emission maxima, mμ	Reference
Cyclohexane	290, 318, 330	332, 348	164
Ethyl alcohol	290, 323, 335	342, 355	164
Water or 2 *N* sulfuric acid	290, 323, 335	342, 355	164
Dimethylformamide	298, 330, 342	347, 360	131
Alkaline dimethylformamide	289, 312, 383, 400	425, 450, 475	131

D. 1- AND 2-NAPHTHYLAMINES

Förster (55) examined the fluorescence of 1- and 2-naphthylamine over a range of pH values. 2-Naphthylamine, which has a p*K* of 4.07, shows an unchanged fluorescence emission spectrum from pH 2 to 9 with a maximum at 420 mμ. The position of this maximum is the same as that of 2-naphthylamine in hexane solution, *i.e.*, the fluorescence in aqueous solution between pH 2 and 9 must also be due to the neutral molecule. The existence of fluorescence of the neutral molecule in acid solution is explained by the loss of a proton from the excited naphthylammonium ion.

$$RNH_3^+ \xrightarrow{h\bar{\nu}} [RNH_3^+]^* \rightarrow [RNH_2]^* + H^+ \qquad (4)$$
$$\downarrow$$
$$RNH_2 + \text{visible fluorescence}$$

In highly acid solutions only ultraviolet fluorescence is observed and this is ascribed to the fluorescence of the naphthylammonium ion, which cannot exist in mesomeric forms, such as are possible for the free amine (structures XXIX–XXXI), and therefore exhibits in



XXX XXIX XXXI

ultraviolet absorption and fluorescence a similarity to naphthalene. At intermediate acidity (pH −1.5) both free amine and naphthylammonium ions are present. The same will apply for aniline also, which fluoresces in aqueous solution between pH 7 and 10 with a maximum at 340 mμ but the aniline cation does not fluoresce (175).

In strong base (pH 14) the absorption spectrum of

2-naphthylamine remains unchanged but a new peak appears in the fluorescence emission at 530 mμ while the fluorescence peak at 420 mμ disappeared. The fluorescence change is reversible and therefore does not involve a deep-seated change in the molecule. This has been ascribed by Förster to abstraction of a proton from the excited naphthylamine molecule

$$[RNH_2]^* \rightarrow [RNH^-]^* + H^+ \qquad (5)$$

as was concluded in his studies on the aminopyrenesulfonic acids (51) described above. An identical interpretation was given independently by Boaz and Rollefson (12). The change of fluorescence with pH and the structures to which they are ascribed are shown in Table VII. N-Methyl-2-naphthylamine shows the same

TABLE VII

EFFECT OF pH ON THE FLUORESCENCE OF 2-NAPHTHYLAMINE (55)[a]

pH	Fluorescence emission, mμ	Structure[c]
14	420, 530	$RNH^- + RNH_2$
9	420	RNH_2
2	420	RNH_2
−1.5[d]	Ultraviolet fluorescence,[b] and 420	$RNH_2^+ + RNH_2$
−5[d]	Ultraviolet fluorescence[b]	RNH_2^+

[a] Excitation at 313 mμ. [b] Wave length approximately 350 mμ from diagram (52). [c] R = 2-naphthyl. [d] As defined by Hammett and Deyrup (66).

change in the fluorescence exhibited by the primary amine but N-dimethyl-2-naphthylamine does not, in accordance with this proton abstraction theory.

The fluorescence in strong alkaline solution is partly quenched and, in fact, in 1-naphthylamine the fluorescence is completely quenched in strong alkali (12, 52).

Similar observations were made for 1-aminonaphthalene-4-sulfonic acid (12) and it was concluded that these amines are weaker bases in the excited state than in the ground state.

E. PHENOLS AND NAPHTHOLS

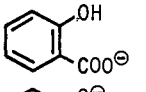
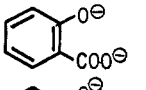
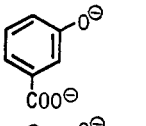
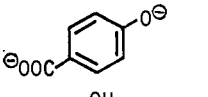
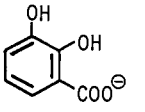
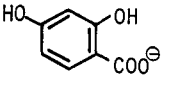
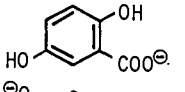
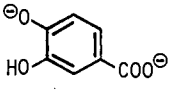
Phenol, tyrosine, tyramine, tyrosine methyl ester, and related compounds fluoresce in neutral solution but undergo partial to complete quenching of fluorescence in aqueous acid and alkaline solution (153, 173, 175). The decrease of fluorescence in alkaline solution follows closely the titration curve of the phenolic hydroxyl (173) suggesting that the ionized species is nonfluorescent. The fluorescence emission maximum of phenol for example, occurs at 310 mμ (excitation at 270 mμ) and decreases to zero at pH 13. Methyl-substituted phenols and diphenols of benzene also do not fluoresce in alkaline pH (175). When the phenolic hydrogen is replaced by methyl, *e.g.*, in anisole quenching of fluorescence does not occur even up to pH 14 (173, 175); the fluorescence intensity of anisole (fluorescence emission maximum: 300 mμ) remains the same from pH 1 to 14, and is about the same as that of phenol at pH 1 (175).

Williams (175) suggests that in dihydroxybenzenes, which fluoresce in the undissociated forms, ionization of the first hydroxyl causes almost complete loss of fluorescence. This result is in contrast with that obtained with mono- and dihydroxynaphthalenes described below. For the latter compounds quinoid mesomeric forms of the anions are held responsible for shifts to longer wave length in alkaline pH and the same is expected for phenol and substituted phenols. It is possible that phenol and substituted phenols do fluoresce weakly in alkaline solution and this aspect deserves further investigation.

Benzoic acid does not fluoresce at any pH but salicylic acid and the corresponding *m*- and *p*-hydroxybenzoic acids fluoresce in alkaline pH. In the case of salicylic acid the mono- and diionic forms fluoresce, but only the diionic forms of the other two isomers fluoresce. This may be associated with hydrogen bond formation in the monoionic form of salicylic acid. The fluorescence maxima of salicylic acid and other hydroxycarboxylic acids of benzene are given in Table VIII (175).

TABLE VIII

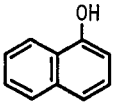
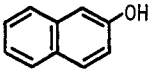
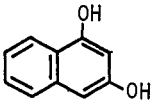
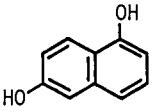
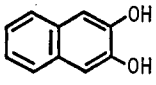
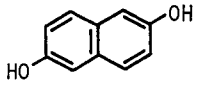
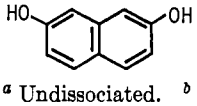
FLUORESCENCE OF DISSOCIATED AROMATIC HYDROXYCARBOXYLIC ACIDS (175)

Structure	Fluorescence excitation maxima, mμ	Fluorescence emission maxima, mμ
	230, 295	420
	310	435
	250, 315	425
	295	350
	240, 305	440
	250, 295	400
	235, 325	455
	300	370

The ultraviolet fluorescence of the diionic form of *p*-hydroxybenzoic acid is noticeable since the other isomers exhibit visible fluorescence. Change of pH affects also the ultraviolet absorption spectra of these compounds. In the undissociated dihydroxybenzoic acids

TABLE IX

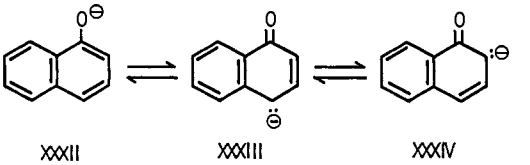
EFFECT OF pH ON GROUND AND EXCITED STATE DISSOCIATION OF NAPHTHOLS

Compound	State of dissociation	Medium	Ultraviolet absorption maxima, mμ	Medium[e]	Fluorescence emission maxima, mμ[g]	Intensity[h]
	a[a]	Ethyl alcohol	292, 307, 322	Methyl alcohol	360	2.75
	b[b]	0.2 *N* NaOH	327 (sh), 332	0.2 *N* NaOH	500, 480 (exc. 330 mμ)[f]	2.60
	a	Ethyl alcohol	255, 264, 273, 285, 316, 328	0.2 *N* H_2SO_4	358	3.20
	b	0.2 *N* NaOH	272 (sh), 282, 292, 346	0.2 *N* NaOH	429, 460 (exc. 350 mμ)[f]	5.90
	a	Ethyl alcohol	277 (sh), 288, 298, 327, 337	0.2 *N* H_2SO_4	385	0.75
	c[c]	pH 10.4	255 (sh), 321	pH 6.1	453	3.30
	d[d]	2 *N* NaOH	318, 355	0.2 *N* NaOH	487	4.85
	a	Ethyl alcohol	276, 287, 300, 323, 336	Ethyl alcohol	365	4.00
	b	0.1 *N* NaOH	303, 315 (sh), 350	pH 9.7	488	2.50
				0.1 *N* NaOH	459	2.95
	a	Ethyl alcohol	263 (sh), 273, 282, 292, 306, 312, 320, 327	0.2 *N* H_2SO_4	345	6.50
	b	0.2 *N* NaOH	286 (sh), 324 (sh), 338, 347	0.1 *N* NaOH	401	2.45
	a	Ethyl alcohol	272, 282, 293 (sh), 320, 339	0.2 *N* H_2SO_4	363	1.35
	b	0.2 *N* NaOH	268, 301 (sh), 348	0.1 *N* NaOH	440	8.45
	a	Ethyl alcohol	287, 306 (sh), 315, 321, 329 (sh)	0.2 *N* H_2SO_4	347	5.80
	b	0.2 *N* NaOH	329, 343	pH 8.4	418	1.75

[a] Undissociated. [b] Dissociated. [c] Monoionic. [d] Diionic. [e] Concentration of solute: 5×10^{-5} mole/l. [f] Reference 175; all other data from reference 70. [g] Excitation at 313 mμ. [h] Uncorrected relative fluorescence intensities.

little or no fluorescence was observed; however, ionization of the carboxyl group results in fluorescence. In the case of the 2,3-, 2,4-, and 2,5-dihydroxybenzoic acids no fluorescence was observed when the phenolic hydroxyl groups dissociate; however, 3,4-dihydroxybenzoic acid did fluoresce in the diionic form. The most intensely fluorescent compound in the series was 2,5-dihydroxybenzoic acid. 3,4,5-Trihydroxybenzoic acid did not show any fluorescence. Thus fluorescence measurements at various pH values can be valuable in structural analysis of these compounds. Caution should be exercised in the interpretation of some of these data since it is stated, *e.g.*, that coumarin is nonfluorescent (175). Coumarin does however fluoresce in the ultraviolet (171, p. 732) with a fluorescence emission maxima at 351 mμ (172).

Several studies were carried out on the effects of pH on the fluorescence of 1- and 2-naphthol (52, 55, p. 12; 70, 175) and several dihydroxynaphthalenes (70). The ultraviolet absorption and fluorescence emission data are summarized in Table IX. In both 1- and 2-naphthol there is a shift to longer wave length in the absorption and fluorescence emission maxima on dissociation of the phenol, and the shifts of fluorescence maxima are much more pronounced than those in the absorption maxima. These shifts to longer wave length can be ascribed to the contribution made to the napholate ion, XXXII, by anionic quinoid mesomeric forms such as XXXIII and XXXIV, which are not possible for the



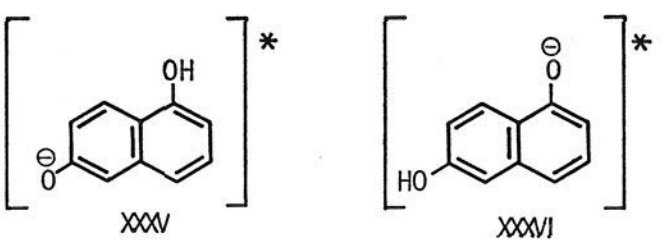
XXXII XXXIII XXXIV

undissociated phenol. The fluorescence emission maxima of the undissociated phenols would therefore be at shorter wave lengths similar to that of naphthalene. The fluorescence emission maximum of undissociated 1-naphthol was measured in methanol solution since fluorescence could not be observed in aqueous acid solution (52, 70) although 2-naphthol did fluoresce in aqueous solution (52, 55, p. 12; 70). Williams (175) reported absence of fluorescence of both 1- and 2-naphthol in aqueous solution at pH 1. Förster (55, p. 12) examined the fluorescence emission spectra of 2-naph-

thol from pH 0 to 13. At pH 0 (1 *N* hydrochloric acid) only the short wave length maximum corresponding to the undissociated phenol was present and at pH 13 (0.1 *N* sodium hydroxide) only the long wave length maximum corresponding to the phenolate anion was present. At intermediate pH values both peaks were present.

From the absorption and fluorescence emission spectra measured at various pH values the pH at which dissociation occurs for the ground and excited states of 1- and 2-naphthol were determined (70). It is clear from these data that in both compounds dissociation occurs at a much lower pH in the excited state than in the ground state, *i.e.*, these naphthols are stronger acids in the excited state than in the ground state. However, the fluorescence spectra show, over a wide pH range, the presence of both ionized and unionized phenols. This is illustrated in Fig. 2 for 1- and 2-naphthol; these data were taken from the paper of Hercules and Rogers (70) which is in agreement with that given by Förster for 2-naphthol (55, p. 12). According to Förster the wide pH range in which ionized and unionized species occur together is due to the incomplete establishment of the ionization equilibrium during the brief lifetime of the excited state.

Hercules and Rogers (70) studied also the absorption and fluorescence spectra of several dihydroxynaphthalenes at various pH values. The spectral shifts observed were comparable to those of 1- and 2-naphthol, *i.e.*, shifts occur to longer wave length in absorption and fluorescence emission maxima with increasing pH and increased acidity in the excited state (Table IX). In the case of 1,3-dihydroxynaphthalene three types of absorption and fluorescence emission spectra were observed corresponding to the undissociated, monoionic, and diionic species. For the 2,3-, 2,6-, and 2,7-diphenols only two types of absorption and fluorescence spectra were obtained, even in 0.1 *N* sodium hydroxide. The long wave length maxima in these cases were ascribed to the monoionic species but this interpretation is not clear. 1,6-Dihydroxynaphthalene showed two types of absorption spectra and three types of fluorescence emission spectra. The two fluorescent species in alkaline pH were ascribed to the existence of two monoionic forms, XXXV and XXXVI, in the excited state.



XXXV XXXVI

F. PURINES AND PYRIMIDINES

Recent studies (158) on the fluorescence characteristics of purines and pyrimidines and their derivatives

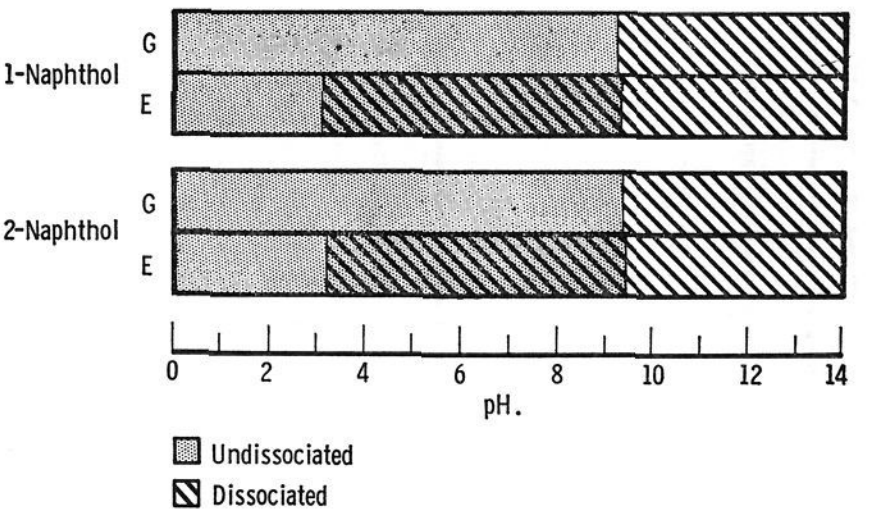
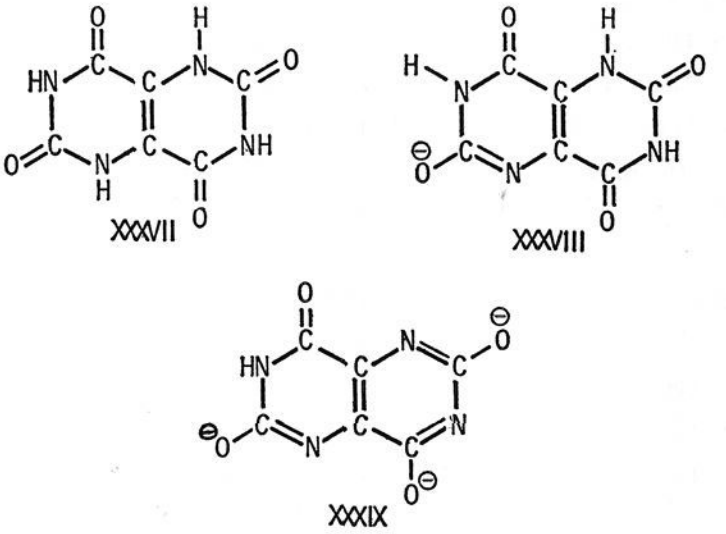


Fig. 2.—Ground (G) and excited state (E) dissociation of 1- and 2-naphthol.

indicated that at certain pH values several of these materials exhibit measurable fluorescence. Guanine fluoresces in aqueous solutions between pH 1 and 13 and even in 5 *N* sulfuric acid, with maximum fluorescence intensity at pH 11. Adenine fluoresces maximally at pH 1 but has negligible fluorescence at pH 7 and thymine fluoresces only in alkaline medium.

The fluorescence and absorption characteristics of pyrimido[5,4-d]pyrimidine-2,4,6,8-(1H,3H,5H,7H)-tetrone ("oxyhomouric acid") XXXVII was examined at various pH values (49). This compound fluoresces



XXXVII XXXVIII XXXIX

strongly in alkali and in acid solution. The curve showing relative fluorescence intensity at various pH values shows two maxima, one at pH 9 and one at pH 14. These maxima were ascribed to the monoionic form, XXXVIII, and the triionic form, XXXIX.

The troughs in the pH–fluorescence intensity curve were ascribed to the diionic and the completely ionized forms, on the basis of the pH values at which the changes occurred.

IV. CONCENTRATION EFFECTS

Increases in concentration of fluorescent solutions usually result in quenching at high concentrations and this is often accompanied by wave length shifts. Concentration effects can be ascribed to various phenomena including reabsorption of emitted light, "true" concen-

tration quenching, dimerization or aggregation, and miscellaneous experimental and instrumental factors. The examination of concentration effects is of importance because of the information obtained in this manner about solute–solute and solute–solvent interactions. Furthermore, the effects of concentration have to be taken into account in the measurement of quantum yields of fluorescence and relative fluorescence intensities.

A. CONCENTRATION QUENCHING

A detailed discussion of the mechanism of fluorescence quenching is beyond the scope of the present review but some aspects of the subject merit attention. Thus, in order to place concentration quenching in proper perspective a brief classification of various types of quenching follows.

Two principal types of quenching are recognized: (a) inner quenching or intramolecular quenching and (b) intermolecular quenching. Intramolecular quenching depends upon the properties of the fluorescent molecule itself and occurs by internal conversion, already referred to in section II. This type of quenching is, of course, independent of the environment, *i.e.*, solvent, temperature, or concentration.

Intermolecular quenching implies that some effects in the surroundings of the fluorescent molecule facilitates radiationless transitions resulting in quenching. The quantum yield of fluorescence will be dependent upon temperature, concentration, solvent, etc. There are two subgroups in this type of quenching: (i) dynamic quenching and (ii) static quenching.

Dynamic quenching, as the term implies, depends upon the rate of diffusion in the solution, *i.e.*, the quantum yield of fluorescence will be viscosity- and temperature-dependent. Also, since the number of intermolecular collisions determines the extent of quenching, the quantum yield of fluorescence will depend upon concentration; at high concentrations there is strong dynamic quenching. A special case of dynamic quenching concerns energy transfer interactions such as those involved in sensitized fluorescence described in section VII.

Static quenching is ascribed to association or aggregation of the fluorescent compound. The quantum yield of fluorescence is independent of the viscosity of the solution but changes in absorption and fluorescence spectra are brought about by changes in concentration. Several examples of this type of concentration quenching are described below.

True concentration quenching is often confused with the associated effects, *e.g.*, reabsorption; actually, it occurs only when there is a sharp decrease in quantum yield of fluorescence with increasing concentration (27, p. 19) *i.e.*, in measurements of concentration quenching the determination of the quantum yield of fluorescence, alone, has physical significance.

The change in efficiency of fluorescence is expressed by the Stern–Volmer equation

$$\frac{Q_0}{Q} = 1 + K_S C \tag{6}$$

where

Q_0 = efficiency at infinite dilution
Q = efficiency at concentration C (moles/l.)
K_S = self-quenching or the concentration quenching constant.

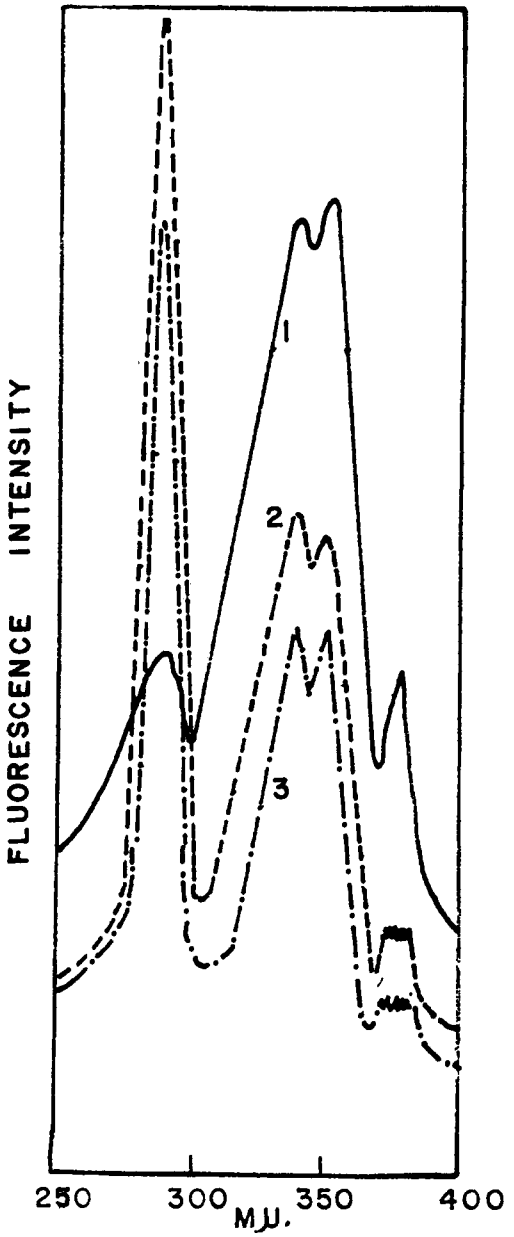


Fig. 3.—Fluorescence excitation spectra of 6-methylbenz(a)-anthracene: 1. ——, 10 γ/ml. (sensitivity 1.0); 2. – – –, 1.0 γ/ml. (sensitivity 0.10); 3. –·–·, 0.05 γ/ml. (sensitivity 0.01); solvent cyclohexane (163).

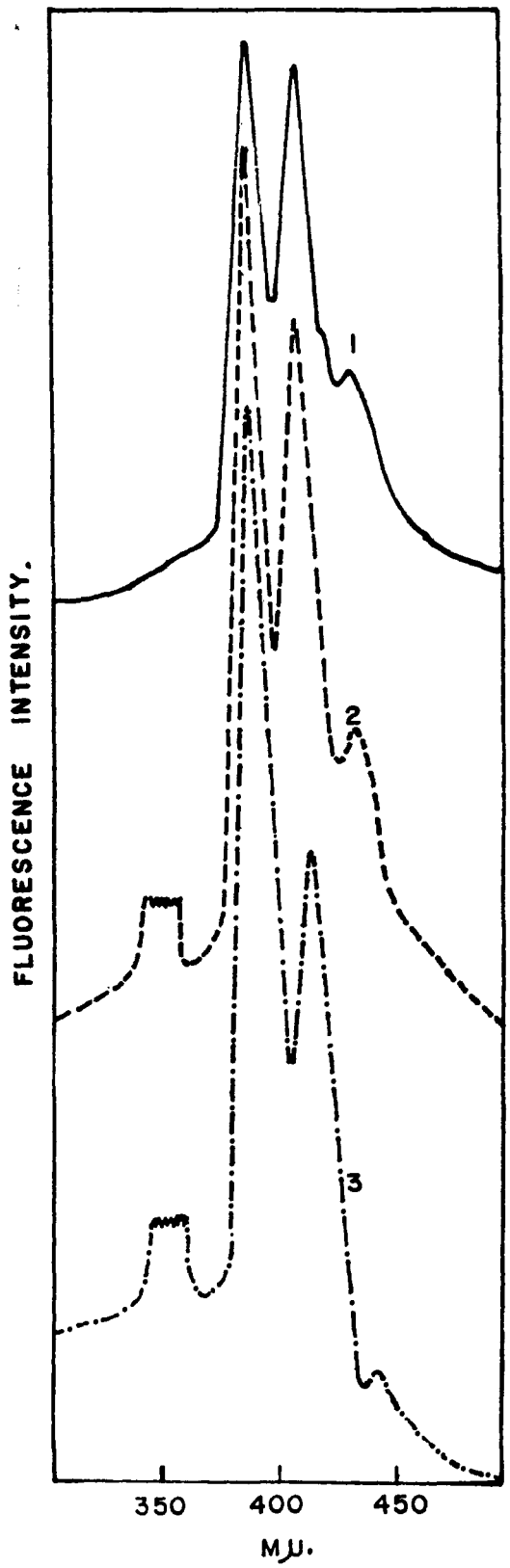


Fig. 4.—Fluorescence emission spectra of 6-methylbenz(a)-anthracene: 1. ——, 10 γ/ml. (sensitivity 1.0); 2. - - -, 1.0 γ/ml. (sensitivity 0.10); 3. -•--•, 0.05 γ/ml. (sensitivity 0.01); solvent cyclohexane (163).

Boaz and Rollefson (12) discussed the various aspects of deviation from this equation. In an extensive study of the effects of concentration and solvents on quantum yields of fluorescence, Melhuish (108) also reported on deviations from the Stern–Volmer equation; attention was drawn in this work to the necessity for corrections for reabsorption in the measurement of quantum yields and the application of the Stern–Volmer equation.

B. REABSORPTION OF FLUORESCENCE AND RELATIVE FLUORESCENCE INTENSITIES

Bowen (27, p. 19) gave a concise but clear discussion of the geometry of fluorescence instrumentation in the vicinity of the sample cell in relation to reabsorption of fluorescence. In concentrated solutions the fluorescence emitted is partly reabsorbed on passing through the solution; this is particularly noticeable for the shorter wave length maxima where there is a significant overlap between fluorescence emission and absorption bands. This effect of reabsorption is demonstrated in Fig. 3 and 4 for the fluorescence excitation and emission spectra of 6-methylbenz(a)anthracene (163).

In view of the various concentration effects observed it is customary in quantitative fluorescence analysis to obtain as standards, concentration–relative fluorescence

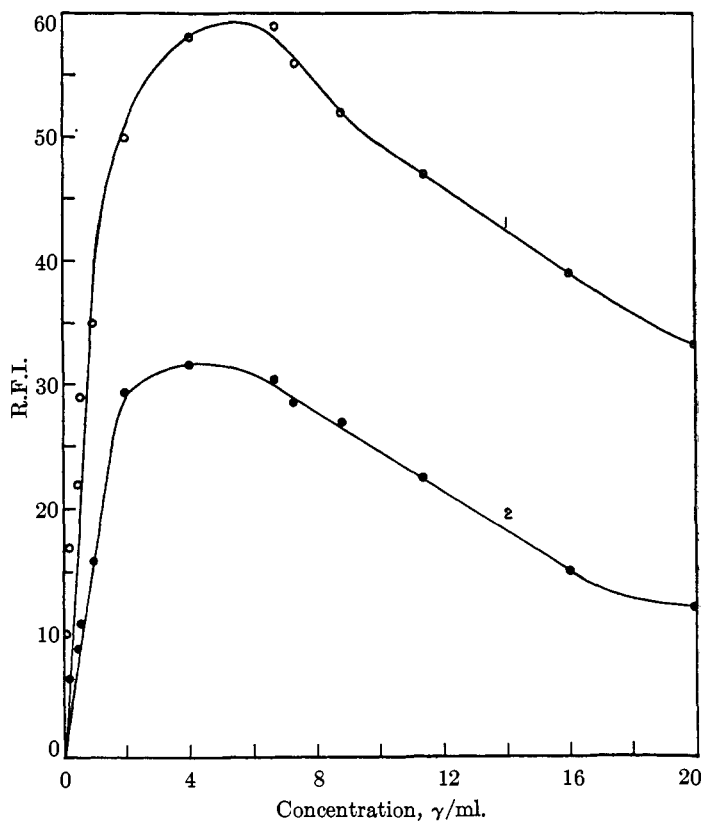


Fig. 5.—Effect of concentration on the fluorescence intensity of dibenz(a,h)anthracene in benzene: 1. 1 × 1-cm. Farrand mirrored cell; 2. standard 1 × 1-cm. cell. Excitation at 297 mμ. Relative fluorescence intensities for 397 mμ fluorescence emission maximum (162).

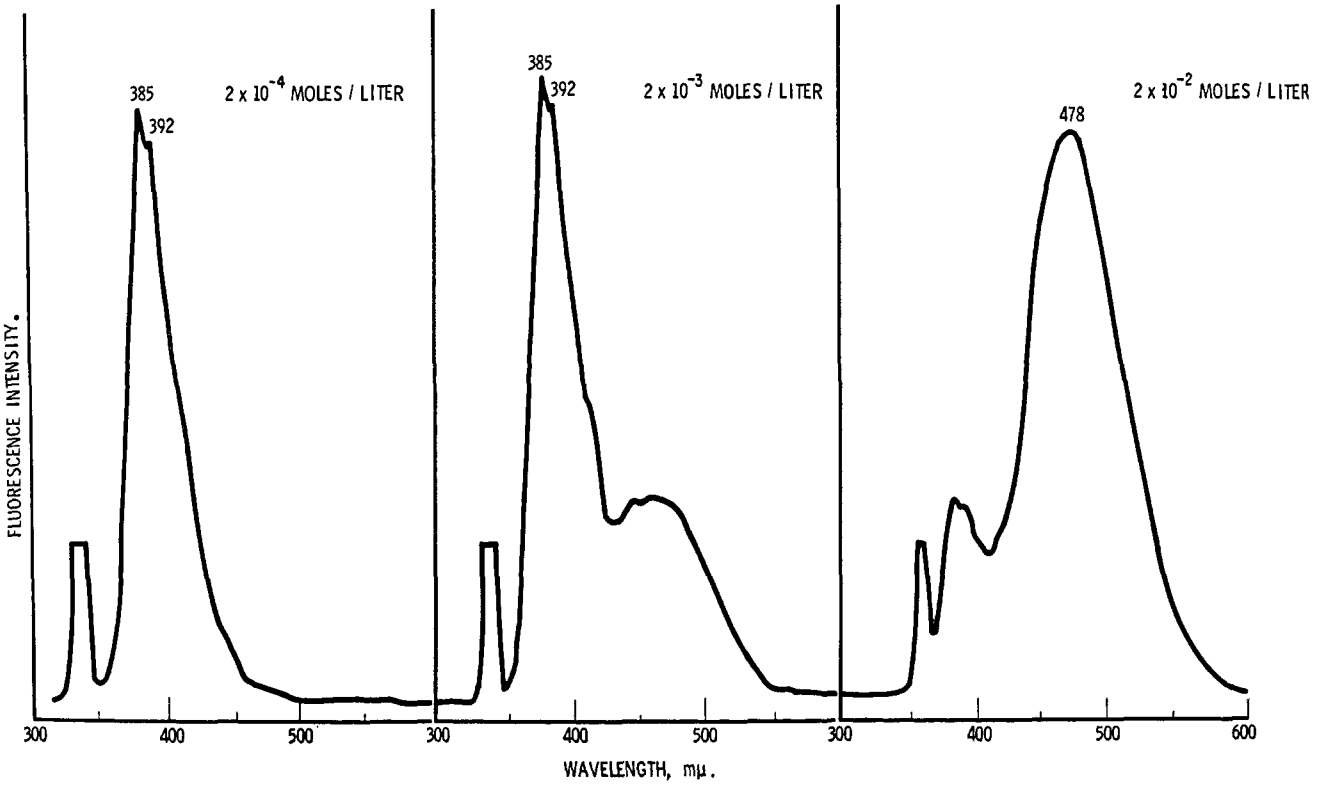


Fig. 6.—Shift in fluorescence emission maxima of pyrene with increasing concentration; solvent, cyclohexane (162).

intensity curves. The general appearance of such curves is shown in Fig. 5 for dibenz(a,h)anthracene. The curve for benzo(a)pyrene has a very similar appearance (154). However, Wheelock (172) found that certain coumarin derivatives show the same fluorescence intensities over wide ranges in concentrations. The exact relationship between concentration and relative fluorescence intensity will differ widely from one compound to the next (36, 142, 154, 163, 172).

Recently the geometrical aspects of instrumentation were re-examined and an expression was derived to calculate fluorescence intensities at each wave length of emission for different concentrations (154). The formula derived, based on Beer's law, related the intensity of fluorescence at one wave length, intensity of the exciting light, concentration, and cell dimensions. In the small number of compounds examined, there was close correspondence between calculated and observed curves similar to the observed curve shown in Fig. 5 for dibenz-(a,h)anthracene. As expected, deviations become greater at higher concentrations.

C. DIMERIZATION

1. Pyrene

Förster (58) observed that pyrene in dilute solution (2×10^{-4} mole/l.) in benzene shows a violet fluorescence with maxima at 372, 384, and 392 mμ. As the concentration of the solution is increased (2×10^{-3} mole/l.) the intensity of these bands decrease and a new band appears at 478 mμ. At a concentration of 2×10^{-2} mole/l. only the 478 mμ band is observed. This effect is illustrated in Fig. 6 for various concentrations of pyrene in cyclohexane (162). This shift is not accompanied by any change in the ultraviolet absorption maxima and is observed also with alcohols or aliphatic hydrocarbons as solvents. The possibility of photochemical reactions were excluded by Förster and it was concluded that a dimer is formed between a molecule in the ground state and one in the excited state, and that the long wave length fluorescence is due to this dimer. Since no change is observed in the ultraviolet absorption spectrum such associated molecules (or dimers) do not exist in the ground state. It is expected that the excitation energy is not localized in either of the halves of the dimer but is divided equally, *i.e.*, there are two mesomeric forms of the dimer

$$\begin{array}{lcl} \mathrm{Py} + [\mathrm{Py}]^* \rightarrow \mathrm{Py}^*\,\mathrm{Py} \rightleftarrows \mathrm{Py}\,\mathrm{Py}^* & & (7) \\ \quad\swarrow \qquad\qquad\qquad\quad \downarrow & & \\ \mathrm{Py} + h\bar{\nu} \qquad\quad \mathrm{PyPy} + h\bar{\nu} & & \\ \qquad\qquad\qquad\quad \downarrow & & \\ \qquad\qquad\qquad\quad \mathrm{Py} + \mathrm{Py} & & \end{array}$$

In further studies on this association phenomenon in pyrene, Förster (58) examined the effect of solvent viscosity. Since the formation of the excited dimer must occur during the very brief lifetime of the excited single molecule its rate of formation will depend upon the rate

of diffusion of the monomer in the solvent; one would therefore expect that with increasing viscosity the rate constant for dimer formation will decrease. This is borne out by comparison of the fluorescence of pyrene at various concentrations in pentane and in viscous paraffin oil. In the latter solvent the fluorescence change takes place at higher concentrations.

A more extensive study of this solvent viscosity effect in the excited state dimerization of pyrene was carried out by Kasper (77). These results are summarized in Table X. The concentration at which the fluorescence

TABLE X

EFFECT OF SOLVENT VISCOSITY ON EXCITED STATE DIMERIZATION OF PYRENE (77)

Solvent	Viscosity, poise	Concentration, moles/l. 1×10^{-3} [a]
Pentane	0.229	0.30
Heptane	.409	.50
Isooctane	.540	.55
Methyl alcohol	.584	.65
Benzene	.650	1.50
Cyclohexane	.970	0.75
Ethyl alcohol	1.190	0.85
Isopropyl alcohol	2.390	1.10
Decalin	2.400	1.00
n-Butyl alcohol	2.950	1.60
Paraffin oil	12.80	12.00

[a] Concentration at which fluorescence intensities of both components are reduced to one-half of the respective maximum values.

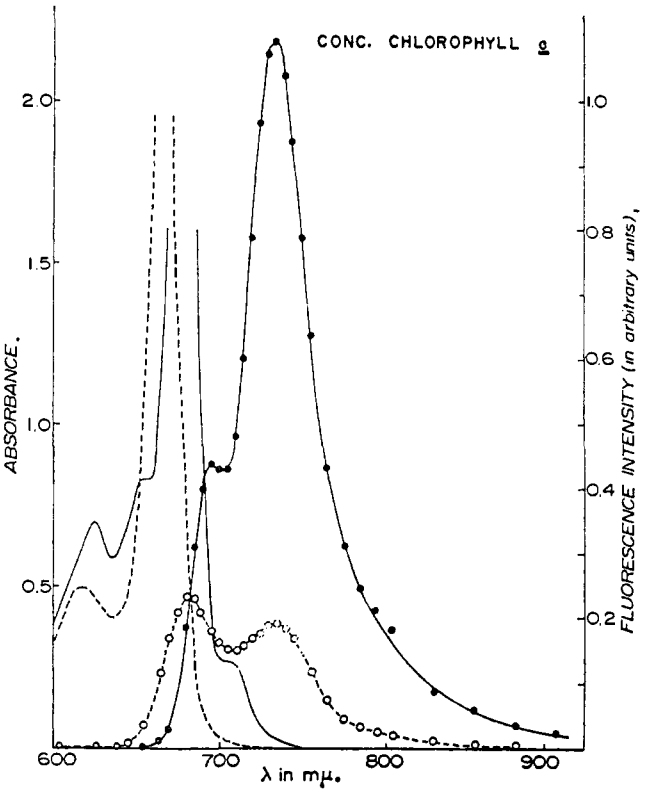


Fig. 7.—Spectra of concentrated solutions of chlorophyll-a, 8×10^{-3} mole/l. in ethyl alcohol: - - - -, absorption spectrum at 25°; —— absorption spectrum at −196°; —O—O—, fluorescence emission spectrum at 25°; —●—●—, fluorescence emission spectrum at −196° (146).

of the two species is reduced to one-half their maximum values is increased with increasing viscosity. The sole exception is benzene, which was the only aromatic hydrocarbon solvent used. The effect of elevated temperatures on the fluorescence emission of the dimer was also examined (57), and it was shown that the dimer dissociates on heating.

A concentration effect similar to that observed for pyrene was noticed also for 3-chloropyrene (58) and for benzo(a)pyrene (4).

2. *Chlorophyll*

Extensive studies have been carried out on the fluorescence of chlorophyll (32, 34, 35, 146) in connection with the mechanism of action of chlorophyll in the photosynthetic process. Thus it has been suggested that chlorophyll has long-lived excited states in which absorbed light energy can be stored for photochemical action. Of particular interest here is the observed appearance of an intense long wave length band in the emission spectrum of concentrated ethanolic solutions of chlorophyll-a and -b at liquid nitrogen temperature. The absorption and fluorescence spectra of chlorophyll-a and -b are shown in Fig. 7 and 8. Although there is some disagreement in the reported maxima it is clear that a minor long wave length band in dilute solutions at both room temperature and at −196° becomes the most intense peak in concentrated ethanolic solutions at −196°. If this peak is ascribed to the fluorescence of a chlorophyll dimer, as is suggested by these workers (32, 146), then it should be noted that this dimer is already present in dilute solution at room temperature.

The visible absorption spectrum of chlorophyll-a was measured at temperatures from −196° to room temperature in dilute and concentrated solutions (146). Concentrated solutions showed a shoulder at 705 mμ on the main peak which occurs at 676 mμ. This shoulder was not observed in dilute solutions and is probably associated with the long wave length fluorescence emission peak. A difference spectrum was plotted from the visible absorption spectra of dilute (10^{-5} mole/l.) and concentrated (3×10^{-2} mole/l.) solutions of chlorophyll-a in ethanol (35). This difference spectrum with maxima at 648 and 682 mμ was ascribed to the absorption of the dimer of chlorophyll-a (35).

3. *Dyes*

The fluorescent dye 1,1′-diethyl-2,2′-pyridocyanine iodide shows in its room temperature absorption spectrum in methyl alcohol two peaks, *viz.*, at 357 and 486 mμ (92). The 486 mμ peak has a shoulder at *ca.* 460 mμ. At −196° in an isopropyl alcohol–isopentane glass

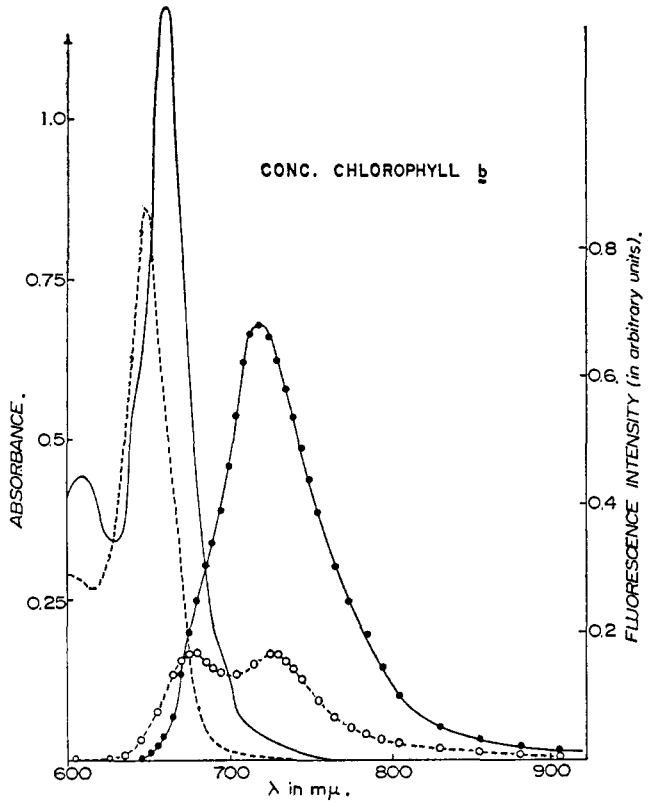
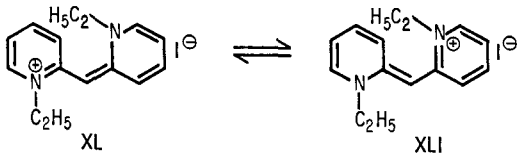


Fig. 8.—Spectra of concentrated solutions of chlorophyll-b in ethyl alcohol: - - - -, absorption spectrum at 25°, 5×10^{-3} mole/l.; ——, absorption spectrum at −196°, 5×10^{-3} mole/l.; —O—O—, fluorescence emission spectrum at 25°, 7×10^{-3} mole/l.; —●—●—, fluorescence emission spectrum at −196°, 7×10^{-3} mole/l. (146).

the long wave length peak is split into two distinct peaks with maxima at 462 and 495 mμ. When the absorption spectrum was measured at a higher concentration at −196°, the 462 mμ peak was shifted to 455 mμ while the 495 mμ peak remained unchanged. From these absorption spectra it was concluded that two species are present at −196° and the absorption spectra of the two species were derived from the low temperature absorption spectra by difference and by extrapolation. From a comparison of the spectra of the two species and by a quantum mechanical treatment it was concluded (92) that the aggregation process involves dimerization only.

The dye shows three peaks in its fluorescence emission spectrum at −196°, *viz.*, at 540, 600, and 650 mμ. As the concentration of the solution was increased the long wave length maxima became more intense at the expense of the 540 mμ maximum. This maximum was ascribed to that of the monomer whereas the 600 and 650 mμ maxima were ascribed to the dimer. Dimerization occurred even in very dilute solution and it was not possible to obtain a fluorescence spectrum for the monomer only. However, by changing the excitation wave length from 470 mμ, at which the monomer absorbs intensely, to 435 mμ, at which the dimer absorbs intensely, the intensity of the dimer fluorescence emission peaks at 600 and 650 mμ were increased while that of the 540 mμ peak decreased.

The formation of the dimer was explained on the basis of its greater stability compared to the monomer. The resonance of the monomeric dye cation would confer one-half of a positive charge on each nitrogen atom, XL and XLI. The anion can be adjacent to a full positive charge only by polarization of the dye, in which case resonance stabilization is lost. In the dimer, on the other hand, the two dye cations can be sandwiched, as shown in Fig. 9, in such a manner that each iodide ion will be between and adjacent to two half-charges.



Several early investigators have drawn attention to the fact that the quantum yield of fluorescence of many dyes remains constant throughout the greater part of the fluorescence excitation spectrum but drops off sharply at the long wave length end. In other words, when these compounds are excited with light of wave lengths at the long wave length end of the fluorescence excitation spectrum, they are capable of dissipating electronic excitation more successfully, resulting in decreased fluorescence emission, than when light of shorter wave lengths is used for excitation. This phenomenon has been studied more extensively in recent years (59, 90) and it has been established that it can be explained on the basis of the formation of nonfluorescent dimers. Also, recent work suggests that the efficiency of fluorescence declines on both ends of the fluorescence excitation peaks, and this decline is markedly solvent dependent. At low concentrations it is hardly noticeable and it becomes more pronounced as the concentration is increased. Lavorel (90) compared the relative absorption of the dye monomers (A_m) and the quantum yield of fluorescence (ϕ) for thionine and fluorescein, as a function of wave length at a number of concentrations. It was assumed that in dilute solutions (about 10^{-6} mole/l.) only monomer is present. In the case of fluorescein no

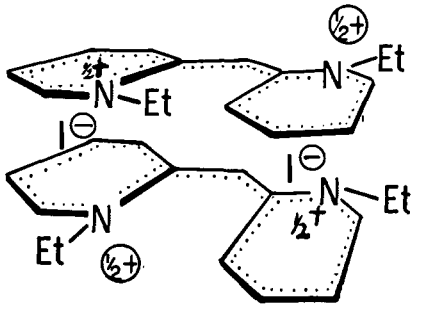


Fig. 9.—Dimer of 1,1'-diethyl-2,2'-pyridocyanine iodide.

change in the fluorescence efficiency curve or the absorption spectrum were found up to 10^{-3} mole/l. At higher concentrations changes were found in both values, A_m and ϕ, and these changes followed a parallel course with a minimum on the short wave length side, a maximum at the absorption peak, and a large drop on the long wave length side of this peak. The equilibrium constant K for the reaction

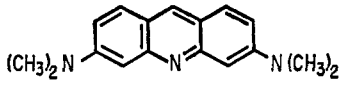
$$\text{Dimer} \rightleftarrows 2 \text{ Monomer} \qquad (8)$$

was calculated and from this value a tentative absorption spectrum for the dimer of fluorescein was arrived at. Two absorption bands were found for the dimer between 400 and 520 mμ in which region the monomer has only one absorption band. Using data obtained in an earlier study (126) the same series of calculations were made for thionine and here also two dimer bands were observed, whereas the monomer showed only one band.

The presence of two dimer bands was explained by Förster (53, p. 230) who pointed out that the dipoles of the two molecules forming the dimer could be either parallel to each other or antiparallel thus giving rise to two absorption bands. Dispersion forces are held responsible for the dimerization in Förster's theory. The position of the long wave length second absorption band of the dimer is such that it will overlap with the fluorescence band of the monomer. Transfer of electronic excitation from an excited monomer to a dimer in the ground state would then seem more likely than transfer to another monomer resulting in decreased fluorescence (59) and since it occurs with increasing concentration the net result is concentration quenching. Since the wave lengths of fluorescence emission remain essentially unchanged it has to be concluded that the fluorescence is due to the monomer only, *i.e.*, the dimer is nonfluorescent.

D. AGGREGATION OF ACRIDINE ORANGE

Acridine orange, XLII, has been used extensively in the staining of biological tissues (159, 166) for the



XLII

fluorescence analysis of tissue sections. The observed metachromatic fluorescence was ascribed to a concentration effect, *i.e.*, cells which absorb a smaller amount of dye fluoresce green; those which absorb higher concentrations fluoresce yellow or orange or red (182). The metachromatic effect has been observed and studied in pure solutions of acridine orange as a function of temperature, solvent, and concentration (88, 133, 151, 179, 180, 181, 182). Measurements of the fluorescence emission spectra were carried out at concentrations from 8×10^{-2} to 1×10^{-5} mole/l. solutions of the acridine orange cation in 95% ethyl alcohol (182). Two excitation wave lengths were used for these measurements; in one series the excitation wave length was at 365–366 mμ, and in another series at 546 mμ. Between concentrations of 1×10^{-3} and 1×10^{-5} mole/l. the fluorescence emission bands occur at 508, 543, and 576 mμ. With increasing concentrations the relative intensities of the long wave length fluorescence maxima increased regularly. At higher concentrations, $>2 \times 10^{-3}$ mole/l., a band at 600–605 mμ becomes more prominent and at concentrations greater than 5×10^{-3} mole/l. a new maximum appears at 620–630 mμ (corrected value 629 mμ (182)); the shorter wave length fluorescence maxima appear at these concentrations as inflections on the long wave length maximum, or at higher concentrations not at all.

When the 546 mμ band was used for excitation a broad structureless fluorescence emission band appears with a maximum between 625 and 630 mμ. For these measurements 95% ethyl alcohol was used as solvent and fluorescence was measured at $-183°$ and concentrations from 8×10^{-2} to 1×10^{-4} mole/l. The long wave length maximum is identical in shape and position with that observed by excitation at 365–366 mμ at high concentrations ($>10^{-2}$ mole/l.). Zanker (182) concludes that these red fluorescence bands must be ascribed to the same excited state and that it corresponds to an associated form of the acridine orange cation.

Schmillen measured the fluorescence decay time of acridine orange hydrochloride in aqueous and ethanolic solutions and of the free base in benzene (133). The decay time was found to be independent of concentration below 1×10^{-4} mole/l. for the aqueous solution and 1×10^{-2} mole/l. for the alcoholic solutions. Above these concentrations the rate of decay decreases rapidly with increasing concentration pointing to the formation of an associated state of the acridine orange cation at higher concentrations. The aggregation of acridine orange and other dyes has also been observed when these dyes are bound to polymers of biological origin (28, 67, 69, 74, 111, 112, 144, 148) and to synthetic polymers (8). Recently the term *stacking* was introduced (28, 148) to indicate such interaction between adjacent dye molecules when they are ordered in cardpack fashion on the polymer; *stacking tendency* refers to the strength of such interaction and this varies with the nature of the dye and the polymer.

Steiner and Beers (144) measured the absorption and fluorescence spectra of acridine orange in aqueous solution at pH 6.7 in the presence of increasing concentrations of adenosine monophosphate and also with polyadenylic acid. The dye concentration was kept low (6.7×10^{-6} mole/l.) in order to keep the dye predominantly in the monomer form (182). The acridine orange spec-

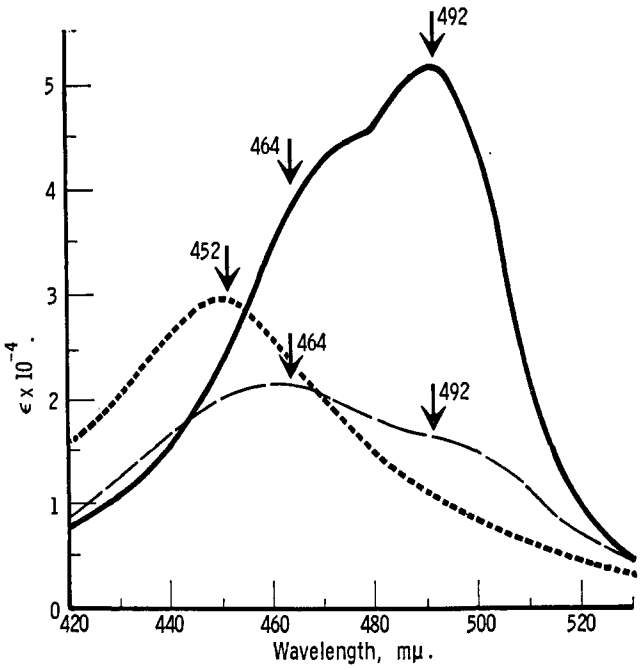


Fig. 10.—Absorption spectra of acridine orange bound to desoxyribonucleic acid: 1. ——, acridine orange, 2×10^{-5} mole/l. at pH 6.7 in cacodylate buffer (148); 2. ...., acridine orange–desoxyribonucleic acid in 1:1 complex, pH 6.7 in cacodylate buffer (148); 3. . . . ., acridine orange, 9.1×10^{-2} mole/l. at pH 6.0 in citrate–phosphate buffer (179).

tra were not affected by the addition of adenosine monophosphate. In the case of polyadenylic acid, however, marked effects were observed. Two of the many absorption bands of acridine orange (182) were involved in these changes. These are the last two bands at the long wave length end of the absorption spectrum for which the maxima 465 and 495 mμ were observed (144). The positions of these bands are actually at 451.5 mμ (at 9.1×10^{-2} mole/l.) and 490.2 mμ (at 10^{-7} to 10^{-8} mole/l.) and at intermediate wave lengths at the concentrations between these limits (179) due to the appearance of one band and disappearance of the other with changing concentration as described above. Steiner and Beers (144) found that their 495 mμ band in the absorption spectrum shows a decrease in intensity relative to the 465 mμ band with increasing concentrations of polyadenylic acid. When the mole ratio of acridine orange to polyadenylic acid (as adenosine monophosphate units) reaches 1:1 the change in relative intensity of the two bands discontinues and remains constant up to a mole ratio of 1:2. The dye, when bound to the polymer in the 1:1 complex and up to a ratio of 1:2, shows in its absorption spectrum the maximum associated with the dye aggregate, *i.e.*, at 465 mμ due to interaction between neighboring bound dye molecules. When the mole ratio reaches 1:70 the 495 mμ band increases in intensity again. Similar absorption changes occur with desoxyribonucleic acid (148) as shown in Fig. 10 together with that of acridine orange in solution at various concentrations.

The absorption changes noted by Steiner and Beers (144) were accompanied by a quenching of the fluorescence of acridine orange. The fluorescence emission maximum was given as 550 mμ (144) but actually a number of bands are involved (179, 182), as already described above. The quenching of fluorescence reaches a maximum at a mole ratio of 1:1. Above this concentration the fluorescence intensity increases again and at a mole ratio of 1:70 it is very nearly the same as that of the dye solution alone, *i.e.*, fluorescence changes parallel absorption changes, at least insofar as the mole ratios are concerned. No indication was given as to shifts in fluorescence emission maximum which is expected if aggregation takes place.

The quenching of the fluorescence of acridine orange, atebrin, and other acridine dyes was observed also when increasing quantities of strandin were added to aqueous solutions of these dyes (67). The quenching phenomenon was the same as that observed by Steiner and Beers (144), *i.e.*, when fluorescence intensity was plotted against increasing polymer concentration a U-shaped curve was obtained. In this instance a minor shift, 5 mμ to longer wave length in the fluorescence emission maximum of the acridine orange was observed with increasing strandin concentration.

The site of binding of dye to polymer was not given much attention in some of these studies. Steiner and Beers (144) did point out that when polyadenylic acid was pretreated with formaldehyde none of the spectral shifts observed in their experiments could be seen. Since formaldehyde treatment would block the 6-amino group of adenine they assumed that the adenine ring is involved in the interaction process. This interpretation is not clear, in fact it is much more likely that dye cation attaches itself to the anionic phosphoric acid group of polyadenylic acid or desoxyribonucleic acid. This type of binding was suggested by Oster (118). Doubtless the true picture is much more complicated and other views have been expressed recently (91).

It was pointed out (64) that the Crick and Watson structure for desoxyribonucleic acid allows the planar portion of the dye molecule to lie between the successive layers of purine and pyrimidine bases. The positively charged nitrogen of a dye such as acridine orange would then be oriented toward the phosphoric acid group. This arrangement will permit, without change in configuration of the polymer, optimum electrostatic interaction and van der Waals bonding.

Regardless of the site of attachment however, it is expected that the interaction between adjacent dye molecules on the polyelectrolyte would occur by the same electronic forces which are operative in aggregated dyes in solution (28). As more sites are added

to the system by the addition of more polyelectrolyte, the bound dye, which is in equilibrium with a small amount of the free dye in solution, will distribute itself among the available sites and the spectrum will change again and become similar to that of the dye alone in dilute aqueous solution.

V. CHARGE TRANSFER COMPLEXES

Although the formation of molecular complexes have been known for many years (3), it is only recently that these materials have been subjected to more extensive investigation and interpretation (2, 105). Mulliken (115, 116) discussed the theory of complexes of this type and indicated that a *charge transfer complex* is formed between two molecules one of which behaves as an electron donor, D, and another as the acceptor, A. The complex will be a resonance hybrid of two structures, an uncharged form and an ionic structure in which an electron has been transferred from D to A.

$$DA \rightleftarrows D^+ A^- \qquad (9)$$

Mulliken's charge transfer theory was the subject of a recent review (105).

In complexes of this type one or more intense new bands are observed in the visible or near-ultraviolet absorption spectrum and these are called the *charge transfer bands*. The charge transfer band is associated with a transition from the ground state which is mostly the uncharged form to an excited state in which the ionized form predominates. The formation of charge transfer complexes can be interpreted in terms of interaction between the π electrons of the donor and the acceptor (31). The molecular orbital method has been applied to the formation of charge transfer complexes (45, 46) and has been reviewed (149). The main interaction in the formation of charge transfer complexes is between the filled (bonding) orbitals of the donor and the empty (antibonding) orbitals of the acceptor which results in a net transfer of negative charge from the donor to the acceptor.

Dewar and Lepley (45) suggest that, since very little charge is transferred in the ground state and since much of their stability may be due to back-coördination, *i.e.*, interactions between the empty orbitals of the donor and filled orbitals of the acceptor, the term *τ-complex* may be more appropriate.

The absorption spectra of charge transfer complexes formed between aromatic hydrocarbons as donors and various acceptors, including 1,3,5-trinitrobenzene (11, 45), tetracyanoethylene (31, 46), iodine (9, 10, 31), and tropylium ion (48), have been examined and have been used in some instances in the calculation of molecular orbital parameters (45, 46). Not all of the acceptors give fluorescent complexes; the complexes obtained from aromatic hydrocarbons and styphnic acid or picric acid, for example, are nonfluorescent (127). However, other acceptors do give fluorescent complexes and the fluorescence and absorption characteristics of a number of these complexes have been examined (39, 127).

Reid (127) observed a general parallelism of the phosphorescence spectra of uncomplexed aromatic hydrocarbons and the emission spectra of the aromatic hydrocarbon–trinitrobenzene complexes and the possibility arose that the emission observed was actually the phosphorescence of the uncomplexed donor. Therefore it was necessary to establish beyond doubt that the emission observed is fluorescence and not phosphorescence.

An extensive examination was carried out on the charge transfer spectra—absorption and fluorescence—

TABLE XI

CHARGE TRANSFER ABSORPTION AND EMISSION SPECTRA FOR AROMATIC HYDROCARBONS WITH VARIOUS ACCEPTORS[a]

Donor	Acceptor[b]	Absorption maximum, mμ	Emission maximum, mμ
Benzene	1	385 (46),[d] 384 (110)[e]	—
	4	305 sh (48)	—
Durene	1	480 (110)[e]	—
	2	485	667
	3	441	614
	5	357	513
	6	340	461
Naphthalene	1	550 (110),[e] 560 (46)[d]	—
	2	483	641
	3	446	578
	4	430 (48)	—
	5	377, 365 (11)	513
	6	360	444
	7	348	435
Anthracene	4	530 (48)	—
	5	463, 460 ± 5 (45)[c]	610
	6	426	526
	7	417	521
Phenanthrene	1	540 (46)[d]	—
	2	467	676
	3	426	633
	4	425 sh (48)	—
	5	373, 376 ± 5 (45)[c]	521
	6	351	465
	7	341	444
Benz(a)anthracene	1	748 (46)[d]	—
	2	588	741
	5	446, 450 ± 10 (45)[c]	588
	6	422	510
	7	400	500

[a] Except where otherwise indicated, charge transfer bands are from reference 39; these spectra were obtained in *n*-propyl alcohol–isopentane at −190°. [b] Acceptors are numbered as follows: 1, tetracyanoethylene; 2, tetrachlorobenzoquinone; 3, 2,5-dichloroquinone; 4, tropylium ion as tropylium fluoroborate in 1,2-dichloroethane solution; 5, 1,3,5-trinitrobenzene; 6, tetrachlorophthalic acid anhydride; 7, 2,4,6-trichlorobenzene-1,3,5-tricarboxylic acid. [c] Absorption spectra were obtained on potassium bromide pellets of the complexes (178). [d] Chloroform as solvent at room temperature. [e] Methylene dichloride as solvent at 22°.

for dilute solutions of a number of aromatic hydrocarbons in *n*-propyl ether–isopentane at −190° (39). These and other pertinent data are summarized in Table XI. The charge transfer absorption and emission spectra shift to shorter wave length with decreasing electron affinity of the acceptor. From these data it was reasonable to conclude that the observed emissions are charge transfer fluorescence rather than phosphorescence. If these emissions were phosphorescence then it should be little affected in complexes between different acceptors and the same donor. However, if the emission is really the reverse of the charge transfer absorption, then, in order to maintain the mirror-image relationship, it should move to longer wave length with increasing electron affinity of the acceptor. That the latter is indeed the case is very clear from the results obtained by Czekalla, Briegleb, and Herre (31, 39) most of which constitute the compilation shown in Table XI. A similar conclusion was drawn earlier by Bier (11) who noticed a mirror-image symmetry of his charge transfer absorption spectra with the emission spectra obtained by Reid (127). It is noticeable that there is a complete lack of fine structure in both absorption and emission spectra in solution, however, some complexes in suspension at −190° did exhibit fine structure (39) and the observed fine structure of the anthracene–trinitrobenzene complex reported earlier (127) may well have been due to precipitation of the solid at −180°.

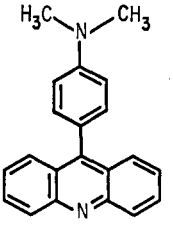
Measurements of the rate of decay of emission in some complexes were examined in the solid state (41, 42) and it was concluced that fluorescence emission does occur in addition to phosphorescence. Some attention has been devoted also to the phosphorescence of aromatic hydrocarbon–acceptor complexes (40, 105, 106, 114). McGlynn (106) concluded that absorption in the charge transfer band is followed by fluorescence emission and to some extent by intersystem crossing which results in the triplet state of the hydrocarbon; the latter is responsible for the observed phosphorescence.

It has been pointed out (46) that the charge transfer absorption bands of complexes between aromatic hydrocarbons and acceptors such as trinitrobenzene, picric acid, iodine, chloranil, and dichloromaleic acid anhydride often lie in the same region where bands due to normal $\pi \rightarrow \pi^*$ transitions of the donors and the acceptors occur. The result is that in such cases it is difficult to determine the exact wave lengths of the charge transfer bands. When tetracyanoethylene was used as acceptor the charge transfer bands were found at longer wave length than with other acceptors and more accurate wave length measurements could be made (46). The fluorescence of charge transfer complexes of the latter type have not as yet been examined but are expected to occur in the near-infrared.

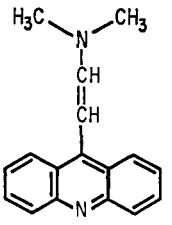
The absorption and fluorescence spectra of tryptophane and indole were measured at −196° in aqueous medium in the presence of various acceptors (63). The charge transfer absorption band of the tryptophane–trinitrobenzene complex, for example, occurs at 380 mμ and the fluorescence emission of the complex at 465 mμ (63).

Merrifield and Phillips (110) determined the equilibrium constants of complex formation between aromatic hydrocarbons and tetracyanoethylene and found that the values of the equilibrium constants vary with the nature of the solvent used. This observation they ascribed to a competitive acceptor–solvent complex formation.

Recently, the term charge-transfer has also been applied to cases where charge is transferred from one part of a molecule to another part in the same molecule, *i.e.*, *intramolecular* charge transfer (184, 185, 186). McGlynn (105) points out that such transitions may be more appropriately termed *charge relocalization transitions*. Transitions of this type, exemplified by 9-substituted acridines XLIII and XLIV,



XLIII



XLIV

result in new long wave length absorption bands and the electronic structure of these materials was examined extensively by absorption and fluorescence polarization measurements (184, 185, 186).

VI. Low Temperature Fluorescence Spectra

It is well known that the maxima of ultraviolet and visible absorption spectra are much better resolved and detailed when measured at low temperature than when measured at room temperature. The same situation prevails in the measurement of fluorescence emission spectra, and low temperatures become indispensable in the measurement of phosphorescence spectra. In this section some of the phenomena encountered in the measurement of fluorescence spectra at low temperature will be discussed.

The measurement of the fluorescence spectra of aromatic hydrocarbons at low temperatures, usually at −180° or −196° in aliphatic hydrocarbon solvents, has received considerable attention (4, 13, 14, 15, 20, 21, 72, 79, 119, 120, 136, 137, 138, 139, 140, 155, 176, 177). The excellent resolution obtained in these spectra are illustrated in Fig. 11 and 12 showing the fluorescence emission spectra of dibenzo(ghi,pqr)-

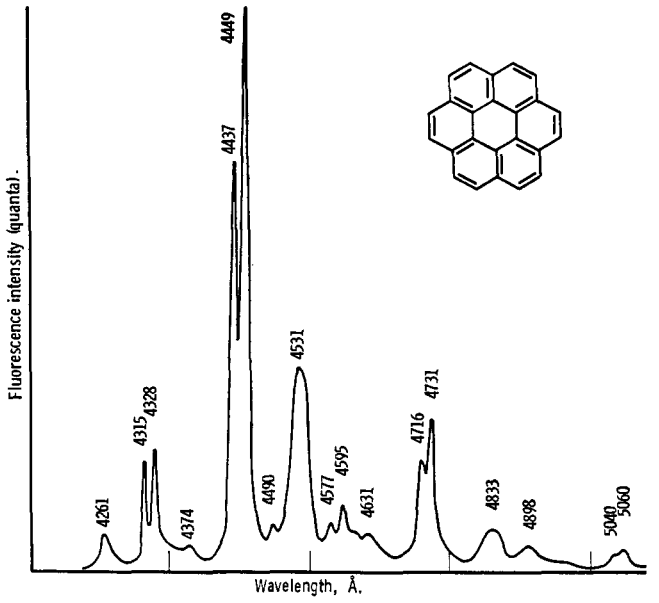


Fig. 11.—Fluorescence emission spectrum of dibenzo(ghi,pqr)-perylene at −180° in heptane (20).

perylene and benzo(g,h,i)perylene in aliphatic hydrocarbon glasses at −180° (20). When pure aliphatic hydrocarbons were used as solvents, *e.g.*, *n*-pentane, *n*-hexane, or *n*-heptane, the solvent was crystalline at −180° (21, 137) whereas in slightly impure solvents or in mixed solvents, glasses were obtained (21). When the same spectra shown in Fig. 11 and 12 were measured in pure solvents and recorded photographically 45 maxima were observed in the fluorescence emission of dibenzo(ghi,pqr)perylene (21) compared to the 17 bands shown in Fig. 11; 74 maxima appeared in the fluorescence emission of benzo(g,h,i)perylene compared to 28 shown in Fig. 12 (21).

There was no sign in the fluorescence emission spectra of dibenzo(ghi,pqr)perylene of the bands which are

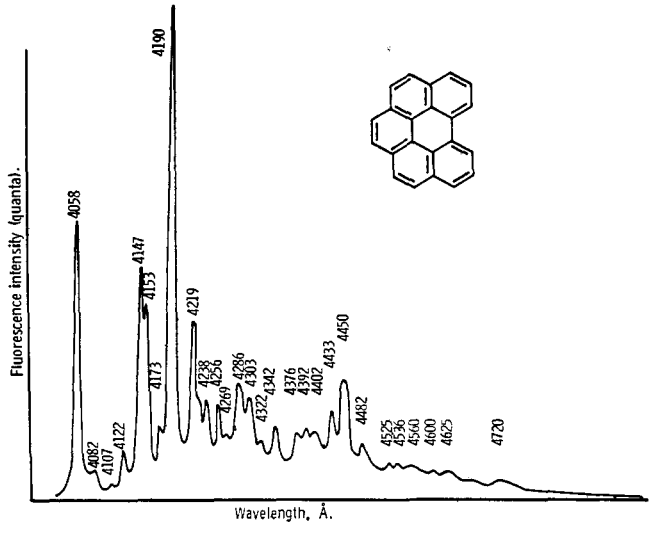


Fig. 12.—Fluorescence emission spectrum of benzo(g,h,i)-perylene at −180° in hexane (20).

ascribed to the solid compound, *i.e.*, it exists in true solid solution in the frozen hydrocarbon solvent. It was concluded that dibenzo(ghi,pqr)perylene molecules fit readily into the crystal lattices of the paraffin hydrocarbons without deformation of the lattice (21). In changing solvent from C_5 to C_9 straight chain aliphatic hydrocarbons, a change in the intensity of the O—O fluorescence emission band (426 mμ) of dibenzo-(ghi,pqr)perylene was observed, and these changes were ascribed to differences in the crystal structure of the solvents (21). In the case of dibenzo(ghi,pqr)-perylene and other hydrocarbons the sharpest and best resolved spectra were observed with hexane as solvent; benzo(g,h,i)perylene, however, exhibited the best resolution with *n*-pentane as solvent and this behavior is exceptional. When long-chain aliphatic hydrocarbons such as penta- or hexadecane are used as solvents diffuse spectra are obtained similar to that obtained with mixtures of short-chain aliphatic hydrocarbons.

Bolotnikova (13, 14) carried out a systematic study on the relationship between the degree of resolution and detail of the fluorescence spectra of aromatic hydrocarbon in several crystalline aliphatic hydrocarbon solvents. The linear condensed hydrocarbons naphthalene, anthracene, and naphthacene show their best resolved emission spectra in *n*-pentane, *n*-heptane, and *n*-nonane, respectively. Anthraquinone and 9,10-dichloroanthracene exhibit their best resolved spectra in *n*-hexane and in *n*-heptane. Bolotnikova (14) suggests that the length of the naphthalene molecule is very close to the length of the pentane molecule, and the anthracene molecule to the heptane molecule, etc. It would seem then that in the formation of the solid solution the aromatic hydrocarbon fits into the lattice of the crystalline aliphatic hydrocarbon in such a manner that the long axes are parallel. When the aromatic hydrocarbons are packed in this manner the amount of solute–solute and solute–solvent interaction is small at the low concentrations used; hence the well defined spectra.

The fluorescence emission spectrum of perylene in hexane was measured at −180° (21) and at −196° (119). Forty-five maxima between 443 and 551 mμ, were recorded photographically (119) compared to diffuse spectra obtainable at room temperature. Small differences occur in both wave length and intensity of the maxima with different aliphatic hydrocarbon solvents.

Although the work described above has established the desirability of measuring fluorescence at −196° in crystalline solids this is not always feasible because of solubility limitations. Several solvent mixtures which give transparent glasses at low temperature are available. The various useful mixtures have recently been tabulated (134).

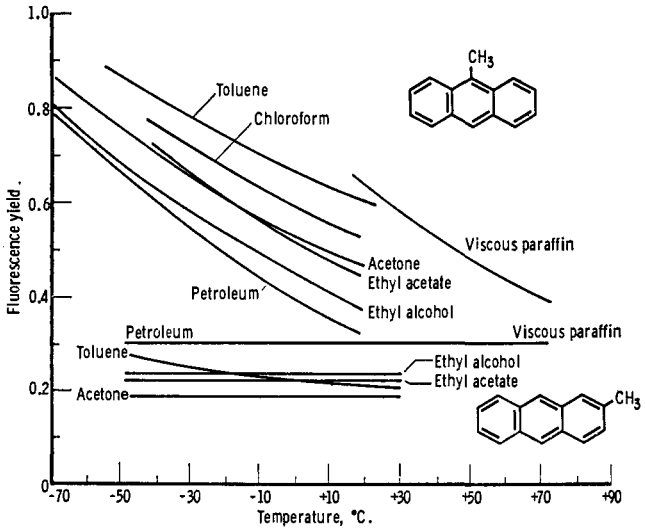


Fig. 13.—Effect of temperature on the fluorescence yield of 9- and 2-methylanthracene in various solvents (24).

The effect of temperature on the fluorescence yield of anthracene and derivatives in a series of solvents has been examined (24, 25, 26). The concentration of the solute was on the order of 10^{-4} mole/l. and the temperatures ranged between $-90°$ and $+90°$. Both increases and decreases in fluorescence yield were observed with increase in temperature. Anthracene, for example, showed a decrease of fluorescence efficiency with increasing temperature in solvents as widely different as hexane, acetic acid, triethylamine, and mesitylene. In dioxane, thiophene, and chloroform there was an increase in fluorescence efficiency with increasing temperature. Toluene and *p*-xylene showed maxima in the fluorescence efficiency *vs.* temperature curves (26). Anthracene derivatives substituted in the *meso*-positions show high yields of fluorescence at low temperature and rapid decreases in fluorescence yield with increasing temperature in a series of solvents.

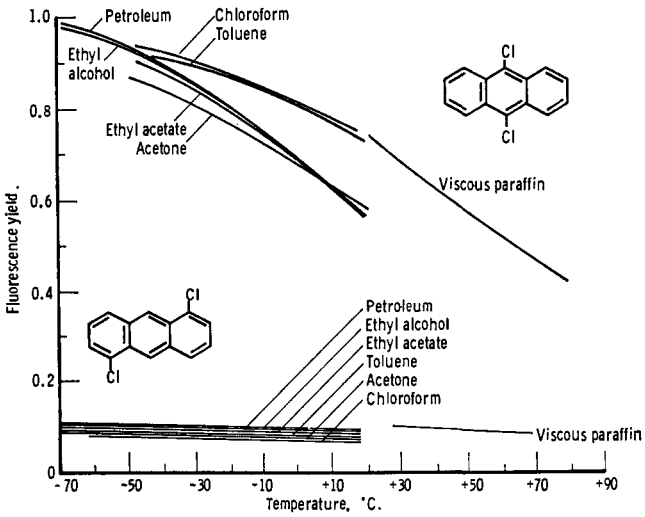


Fig. 14.—Effect of temperature on the fluorescence yield of 9,10- and 1,5-dichloroanthracene in various solvents (24).

9-Cyano- and 9,10-diphenylanthracene, however, showed high fluorescence yields over the entire temperature range studied (24). In other substituted anthracenes the fluorescence efficiency is low and there is only slight dependence on temperature. These effects are illustrated in Fig. 13 and 14 (24).

Bowen and Sahu (24) concluded from their studies that there are two processes by which loss of excitation energy can occur. One process is essentially independent of temperature and is probably associated with the singlet–triplet conversion. The temperature-dependent process, such as occurs in *meso*-substituted anthracenes was associated with a direct transition from the excited to the ground state.

The room temperature absorption and fluorescence spectra of 1- and 2-naphthol and several dihydroxynaphthalenes, already discussed under pH effects above, were recently examined also at $-196°$ (71). Two solvent glasses were used: ether–isopentane–alcohol, 8:3:5 (EPA) and ether–ethyl alcohol–ammonia, 10:9:1 (EAA). With EAA as solvent a shift of fluorescence maxima to longer wave length occurs relative to the same spectra in EPA, due to the existence of ionized forms in EAA glass. In the case of some dihydroxynaphthalenes the resolution improved considerably when their spectra were measured at $-196°$ compared to their room temperature spectra. This was particularly noticeable for 2,3-, 1,5-, and 2,7-dihydroxynaphthalene. Under the same conditions 1- and 2-naphthol and 1,3-, 1,6-, 2,6-, and 1,4-dihydroxynaphthalene still exhibited relatively diffuse spectra at $-196°$.

All the compounds examined showed a shift in fluorescence emission maxima to shorter wave length on lowering the temperature to $-196°$. These maxima are given in Table XII. This blue shift was explained

TABLE XII

BLUE SHIFTS IN FLUORESCENCE EMISSION OF NAPHTHALENE DERIVATIVES WITH LOWERING OF TEMPERATURE (IN EPA AND EAA) (71)

Compound	Fluorescence emission maxima (mμ) Room temperature	−196°
1-Naphthol	365	345
2-Naphthol	361	355
1,3-Dihydroxynaphthalene	379	372
1,4-Dihydroxynaphthalene	425	419
1,5-Dihydroxynaphthalene	339, 352	338, 351
1,6-Dihydroxynaphthalene	366	363
2,3-Dihydroxynaphthalene	339	338
2,6-Dihydroxynaphthalene	360	358
2,7-Dihydroxynaphthalene	348	347

(71) by the existence of a Franck–Condon state (6) at low temperature. At room temperature the solvent molecules can rapidly adjust to the equilibrium excited state of the solute and fluorescence occurs from the equilibrium excited state, rather than from the

Franck–Condon state. At low temperature reorientation of solvent molecules will require longer time intervals and emission will be observed from the Franck–Condon state. Because of the differences in energy between the Franck–Condon state and the equilibrium excited state, shifts in fluorescence maxima are observed as indicated in Table XII above. On the other hand, energy differences between the ground state and the Franck–Condon state should be independent of the rigidity of the medium so that spectral shifts in the absorption spectra are not expected with lowering of temperature to −196°. The absorption spectra of the same series of compounds at room temperature in ethyl alcohol were substantially the same as those in EPA and EAA glasses at −196° (71).

VII. Energy Transfer and Sensitized Fluorescence

Bowen and Brocklehurst (19) examined the transfer of electronic energy in dilute solutions of binary mixtures of aromatic compounds. In order for this phenomenon to be observed it is necessary that in a mixture of compounds A and B in which energy is transferred from A to B, A absorbs the exciting radiation whereas B absorbs very little or no exciting radiation. The fluorescence emission spectrum of B has to occur at a longer wave length than that of A. Several such binary mixtures were examined, *e.g.*, 9-phenylanthracene and 9,10-dichloroanthracene. These compounds were mixed in equal molar ratios at various concentrations between 10^{-2} and 10^{-3} mole/l. and the fluorescence of the mixtures was examined. The combined fluorescence efficiency decreased with increasing concentration because of concentration quenching. The fluorescence emission due to 9-phenylanthracene (*i.e.*, the absorbing component, A) decreased with increasing concentration whereas that of the B component, 9,10-dichloroanthracene, increased with increasing concentration. Another system examined by these workers was 1-chloroanthracene which has a low fluorescence efficiency and perylene, which has a high fluorescence efficiency. The combined emission rises rapidly with increasing concentration. The fluorescence emission due to perylene increases rapidly whereas that due to 1-chloroanthracene decreases.

There is a possibility that transfer of energy may occur *via* emission from the A component, absorption of this emission by the B component, and then emission from the B component. This process, referred to as "trivial" energy transfer apparently occurs to a minor extent. From the appearance of the calculated and observed peaks it was concluded that for the greater part energy transfer occurs by a radiationless process involving direct transfer of electronic energy from the A to the B component (19, 22). Moreover, the transfer of energy occurs by a bimolecular interaction between an excited molecule of A and a molecule of B in the ground state. The reaction is not due to the formation of dimers (22, 56) and is independent of the viscosity of the solvent, *i.e.*, it is not diffusion-controlled (22, 56). This process of energy transfer is also referred to as *sensitized fluorescence* (19, 22, 56).

Moodie and Reid (113) measured the fluorescence of binary mixtures of aromatic hydrocarbons at −180° and found no change in their fluorescence spectra. They concluded that energy transfer did not occur with their systems in solution. It was noted, however, that this may have been due to the low concentration ranges (*ca.* 10^{-4} mole/l.) necessitated by the low temperature used. In the work of Bowen and Brocklehurst (19) concentrations of 10^{-2} to 10^{-3} mole/l. were used. Moodie and Reid (113) observed energy transfer in suspensions of one hydrocarbon in a solution of another, *e.g.*, a suspension of naphthacene in anthracene solution in ether–pentane–alcohol glass showed a broad intense fluorescence band instead of the usual, well resolved bands.

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