Spectroscopic Investigation of Fluorescence Quenching Agents: Effect of Nitromethane on the Fluorescence Emission Behavior of Select Cyclopenta-PAH, Aceanthrylene, and Fluorene Derivatives

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Nitromethane is examined as a selective fluorescence quenching agent for "alternant" alkyl-substituted polycyclic aromatic hydrocarbons (PAHs). Fluorescence emission behavior is reported for 11*H*-benz[bc]aceanthrylene, 4*H*-cyclopenta[def]phenanthrene, 4*H*-cyclopenta[def]chrysene, 13*H*-dibenzo[a,g]fluorene, 13*H*-dibenzo[a,i]fluorene, 4*H*-benzo[b]cyclopenta[mno]chrysene, 4*H*-cyclopenta[pqr]picene, 7*H*-dibenzo[c,g]fluorene, 9*H*-benz[6,7]indeno[1,2]phenanthrene, 4*H*-benzo[b]cyclopenta[jkl]triphenylene, 13*H*-dibenz[bc,k]aceanthrylene, 13*H*-dibenz[bc,l]aceanthrylene, and 4*H*-benzo[def]cyclopenta[mno]chrysene dissolved in organic solvents of varying polarity and in acetonitrile or aqueous-acetonitrile solvent mixtures at various nitromethane concentrations. Results of these measurements show that nitromethane quenches fluorescence emission of the thirteen solutes studied, which is in complete agreement with what would be expected on the basis of the fact that all solutes are "alternant" polycyclic aromatic hydrocarbons.

Index Headings: Fluorescence; Molecular structure; Spectroscopic techniques.

INTRODUCTION

Fluorescence spectroscopy is rapidly becoming an extremely versatile, sensitive experimental technique for identifying/quantifying numerous environmentally important polycyclic aromatic hydrocarbons (PAHs) and polycyclic aromatic nitrogen hetero-atoms (PANHs). PAH/PANH identification and quantification in unknown mixtures require accurate fluorescence emission intensity measurements and availability of a large spectral data file for comparing the unknown's spectrum against PAH/PANH standards. Kalman filtering and Gaussian or other curve-fitting methods,¹⁻⁴ along with selective photochemical quenching agents such as nitromethane⁵⁻¹⁰ and 1,2,4-trimethoxybenzene,⁹ may be needed to uncouple overlapping spectra if more than one fluorescent species is present. To prevent misidentification, the data file should include both polar and non-polar solvents, since electronic interactions between a solvent dipole and an excited PAH/PANH solutes can lead to spectral distortions, wavelength shifts and/or intensity ratio variations, as was the case with many of the polycyclic aromatic compounds examined previously.¹¹⁻²²

Solvent-induced fluorescence spectral changes can be rationalized qualitatively in a relatively straightforward

manner. Excitation promotes the PAH/PANH solute from a ground state of low dipole moment to one of the vibrational levels of the first electronic excited state, S_0^* , with an accompanying electron distribution in the surrounding solvent molecules. Insufficient time exists, however, for solvational-sphere molecules to physically reorient with the new PAH/PANH dipole moment. Relaxation from the vibrationally excited S_0^* level to the excited S_0^* level occurs whenever solvent molecules rotationally reorient to a more stable dipole configuration during the excited state's lifetime. Emission of the fluorescence photon returns both the PAH/PANH molecule to the ground S_0 state and solvational molecules to their initial electronic configuration. Subsequent rotation of solvent molecules to the ground-state dipole orientation restores the system to its original state. Transition probabilities and energy separations between the different energy levels vary with each solute-solvent pair, and give rise to observed intensity ratio changes and emission wavelength shifts.^{23,24}

The emission spectrum of many polycyclic aromatic compounds consists of several major vibronic bands labeled I, II, etc., in progressive order. Previous measurements¹¹⁻²² revealed that pyrene, benzo[ghi]perylene, ovalene, coronene, benzo[a]coronene, naphtho[2,3a]coronene, benzo[e]pyrene, naphtho[8,1,2abc]coronene, dinaphtho[8,1,2abc;2',1',8'klm]coronene, dibenzo[def,p]chrysene, phenanthro[5,4,3,2efghi]perylene, benzo[rst]pentaphene, 1-azabenz[a]anthracene, 2-azabenz[a]anthracene, 12-azabenz[a]pyrene, phenanthro[2,3h]isoquinoline, and phenanthro[3,2h]isoquinoline exhibit probe character, as evidenced by systematic variation of emission intensity ratios with solvent polarity. Interestingly, only 25 of the 76 compounds studied to date behave in this fashion. Various emission intensity ratios of perylene, dibenzo[bc,ef]coronene, benzo[a]pyrene, benzo[pqr]naphtho[8,1,2bcd]perylene, dibenzo[fg,ij]pentaphene, 1-azapyrene, 2-azapyrene, 4-azapyrene, and several other PAHs/PANHs remained essentially constant, irrespective of solvent polarity.

Now that studies on PAH/PANH benzenoid probes are nearly complete, we have decided to redirect our experimental efforts to more practical applications, such as a comprehensive examination of selective fluorescence quenching reagents. On the basis of limited fluorescence measurements for perylene, dibenzo[b,k]chrysene, di-

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FIG. 1. Molecular structures of PAH solutes: (A) 11*H*-benzo[bc]aceanthrylene; (B) 4*H*-cyclopenta[def]phenanthrene; (C) 4*H*-cyclopenta[def]chrysene; (D) 13*H*-dibenzo[a,g]fluorene; (E) 13*H*-dibenzo[a,i]fluorene; (F) 4*H*-benzo[b]cyclopenta[mno]chrysene; (G) 4*H*-cyclopenta[pqr]picene; (H) 13*H*-dibenz[bc,l]aceanthrylene; and (I) 13*H*-dibenz[bc,k]aceanthrylene.

benzo[h,rst]pentaphene, naphtho[1,2b]fluoranthene, indeno[1,2,3cd]pyrene, and 10,11-(perinaphthylene)-fluoranthene dissolved in a binary aqueous-acetonitrile solvent mixture, Blümer and Zander⁶ noted that nitrobenzene and nitromethane selectively quenched fluorescence emission of only the so-called "alternant" polycyclic aromatic hydrocarbons. Emission intensities of the three nonalternant PAHs (e.g., naphtho[1,2b]fluoranthene, indeno[1,2,3cd]pyrene, and 10,11-(perinaphthylene)-fluoranthene) were unaffected. Additional polycyclic aromatic hydrocarbons need to be examined in order to ascertain which PAHs (if any) are exceptions to the selective quenching rule. For this reason, we report the effect that nitromethane has on the fluorescence emission of 11*H*-benzo[bc]aceanthrylene (BAA); 4*H*-cyclopenta[def]phenanthrene (CyPh); 4*H*-cyclopenta[def]chrysene (CyCh); 13*H*-dibenzo[a,g]fluorene (DB_{ag}F); 13*H*-dibenzo[a,i]fluorene (DB_{ai}F); 4*H*-benzo[b]cyclopenta[mno]chrysene (B_bCyCh); 4*H*-cyclopenta[pqr]picene (CyPi); 7*H*-dibenzo[c,g]fluorene (DB_{cg}F); 9*H*-benz[6,7]indeno[1,2l]phenanthrene (BIPh); 4*H*-benzo[b]cyclopenta[jkl]triphenylene (BCyTP); 13*H*-dibenz[bc,k]aceanthrylene (DBAA-1); 13*H*-dibenz[bc,l]aceanthrylene (DBAA-2); and 4*H*-benzo[def]cyclopenta-

FIG. 2. Molecular structures of PAH solutes: (J) 4*H*-benzo[b]cyclopenta[jkl]triphenylene; (K) 7*H*-dibenzo[c,g]fluorene; (L) 9*H*-benz[6,7]indeno[1,2l]phenanthrene; and (M) 4*H*-benzo[def]cyclopenta[mno]chrysene.

FIG. 3. Fluorescence emission spectra of 11*H*-benz[bc]aceanthrylene dissolved in [A (—○—)] *n*-hexadecane; [B (—)] butyl acetate; [C (—)] dichloromethane; and [D (—●—)] dimethyl sulfoxide. In dimethyl sulfoxide major emission bands occur at about 392, 415, and 439 nm.

[mno]chrysene (B_aCyCh). The various molecular structures are depicted in Figs. 1 and 2. These thirteen aromatic compounds are classified as alternant PAHs because every alternant carbon atom in the aromatic ring system(s) can be "starred." Nonalternant PAHs, on the other hand, would have at least one pair of adjacent starred atoms.^{25,26} Also included is a discussion of both primary and secondary inner-filtering artifacts associated with quenching determinations, and new experimental fluorescence results for the thirteen solutes dissolved in nonelectrolyte organic solvents of varying polarity.

MATERIALS AND METHODS

The various PAH solutes were synthesized and purified by procedures described in the chemical literature.²⁷ Stock solutions were prepared by dissolving the solutes in dichloromethane. Small aliquots of the stock solutions were transferred into test tubes, allowed to evaporate, and diluted with the solvent of interest. Final solute concentrations were sufficiently dilute to minimize inner-filtering artifacts. Solvents were of HPLC, spectroquality, or AR grade, purchased commercially from either Aldrich or Fisher Scientific, and the resulting solutions were optically dilute (absorbances $\text{cm}^{-1} \leq 0.01$) at all wavelengths investigated, except for the quenching study, where the nitromethane concentration was continually increased to allow examination of inner-filtering artifacts.

Absorption spectra were recorded on a Bausch and Lomb Spectronic 2000 and a Hewlett-Packard 8450A photodiode array spectrophotometer in the usual manner with a 1-cm quartz cuvette. The fluorescence spectra were run on a Shimadzu RF-5000U spectrofluorometer with the detector set at high sensitivity. Solutions were excited at 350 nm (BAA), 320 nm (CyPh), 320 nm (CyCh), 340 nm (DB_{ag}F), 320 nm (DB_{ai}F), 330 nm (B_bCyCh), 330 nm (CyPi), 300 nm (BCyTP), 400 nm (DBAA-1), 320 nm

FIG. 4. Fluorescence emission spectra of 4H-cyclopenta[pqr]picene dissolved in [A (—•—)] *n*-hexadecane; [B (—)] butyl acetate; [C (—•—)] dichloromethane; and [D (—)] dimethyl sulfoxide. In dimethyl sulfoxide major emission bands occur at about 377, 397, 421, and 448 nm.

(DBAA-2), 300 nm (DB_{cg}F), 290 nm (BIPh), and 320 nm (B_dCyCh) in a 1-cm² quartz cuvette. All fluorescence data were accumulated at 19°C, ambient room temperature, with excitation and emission slit width settings of 15 nm and 3 nm, respectively. The fluorescence spectra, depicted in Figs. 3–5, represent a single scan which was then solvent blank corrected and verified by repetitive measurements.

RESULTS AND DISCUSSION

Representative fluorescence emission spectra of 11H-benz[bc]aceanthrylene, 4H-cyclopenta[pqr]picene, and 4H-cyclopenta[def]phenanthrene dissolved in *n*-hexadecane, butyl acetate, dichloromethane, and dimethyl sulfoxide are depicted in Figs. 3–5. These four nonelectrolyte solvents were judiciously selected so as to encompass the entire range of solvent polarity, from the non-polar *n*-hexadecane hydrocarbon to the moderately polar butyl acetate and dichloromethane solvents to the very polar dimethyl sulfoxide, which is the most polar solvent considered in the present investigation. Examination of the spectral data reveals that 4H-benzo[b]cyclopenta[jkl]triphenylene, 4H-cyclopenta[def]phenanthrene, 4H-benzo[def]cyclopenta[mno]chrysene, 13H-dibenz[bc,k]-aceanthrylene, 11H-benz[bc]aceanthrylene, 4H-benzo[b]cyclopenta[mno]chrysene, 4H-cyclopenta[pqr]picene, 13H-dibenz[bc,l]aceanthrylene, and 4H-cyclopenta[def]chrysene do exhibit two-, three- and/or four-band emission fine structure similar to that observed for many of the PAH6 benzenoids studied previously. Of the nine solutes named, only the latter five showed modest probe character, as evidenced by systematic variation of emission intensity ratios with solvent polarity. Calculated emission band ratios for 11H-benz[bc]aceanthrylene (BAA; I/II) and 4H-benzo[b]cyclopenta[mno]chrysene (B_dCyCh; I/II) ranged from ~BAA = 1.80 and B_dCyCh = 1.48 in cyclohexane to BAA = 1.33 and B_dCyCh = 1.0

FIG. 5. Fluorescence emission spectra of 4H-cyclopenta[def]phenanthrene dissolved in [A (—)] *n*-hexadecane; [B (—•—)] dichloromethane; [C (—•—)] butyl acetate; and [D (—)] dimethyl sulfoxide. In dimethyl sulfoxide major emission bands occur at about 347, 355, 364, and 383 nm.

in dimethyl sulfoxide. Actual numerical values for the five PAHs that exhibited any signs of probe behavior are tabulated in Table I. Estimated uncertainties in the measured intensity ratios are believed to be on the order of ± 0.04 (or less) on the basis of replicate determinations. The remaining compounds studied possessed one broad, fairly unsymmetrical emission band (likely two or more unresolvable bands) in the 350–600 nm spectral region.

Although BAA, B_dCyCh, CyPi, DBAA-2, and CyCh do show modest probe character, their dynamic ranges are significantly smaller than those of pyrene (1.37), benzo[ghi]perylene (0.98), and ovalene (2.08).¹¹ Better solvent discrimination is permitted by the three PAH6 benzenoids. Furthermore, benzo[ghi]perylene and ovalene have excitation wavelengths into the visible region, and are thus less prone to inner-filtering artifacts arising from solvent absorption of excitation radiation. Presently there appears to be no compelling reason for us to ever recommend using the five cyclopenta-PAH derivatives in place of pyrene, benzo[ghi]perylene, and ovalene in solvent polarity determinations.

From an analytical perspective, identification and quantification of unknown PAH/PANH mixtures require accurate fluorescence emission intensity measurements and availability of a large spectral data file for comparing the unknown's spectrum against PAH/PANH standards. Mixtures of environmental/industrial importance rarely contain a single component. The majority of mixtures commonly encountered contain several isomeric pairs or structurally similar PAHs/PANHs, which emit in approximately the same spectral regions. Kalman filtering and Gaussian or other curve-fitting techniques theoretically allow uncoupling of overlapped spectra. Such methods become less reliable, however, as the number of mixture components increases. High-performance liquid chromatographic (HPLC) separation prior to fluorimetric analysis affords a viable alternative, but again the method is extremely time-consuming

TABLE I. Ratios of fluorescence emission intensities of 11*H*-benz[bc]aceanthrylene, 4*H*-benz[b]cyclopenta[mno]chrysene, 4*H*-cyclopenta[pqr]picene, 13*H*-dibenz[bc,l]aceanthrylene, and 4*H*-cyclopenta[def]chrysene in various organic solvents.

Organic solvent	Py ^a	BAA ^b	B ₆ CyCh ^c	CyPi ^d	DBAA-2 ^e	CyCh ^f
Cyclohexane	0.58	1.80	1.48	0.42	1.83	0.72
2,2,4-Trimethylpentane	0.59	1.80	1.47	0.41		
<i>n</i> -Hexadecane	0.60	1.73	1.41	0.43	1.82	0.75
Carbon tetrachloride		1.61	1.37	0.69		
Dibutyl ether	0.84	1.68	1.33	0.53		
Benzene	1.05	1.46	1.26	0.69	1.60	0.86
2-Propanol	1.09	1.53	1.16	0.64		
Chloroform	1.25	1.37	1.17	0.79		
Butyl acetate	1.35	1.54	1.25	0.69	1.62	0.82
Methanol	1.35	1.51	1.19	0.72	1.58	0.86
Dichloromethane	1.35	1.37	1.15	0.77	1.49	0.91
Acetonitrile	1.79	1.39	1.08	0.83		
Dimethyl formamide	1.81	1.36	1.06	0.90	1.43	1.08
Dimethyl sulfoxide	1.95	1.33	1.00	0.95	1.44	1.09
Dynamic range	1.37	0.57	0.48	0.54	0.40	0.37

^a Experimental Py values are taken from Dong and Winnik³³ and are based upon the properties of pyrene.

^b Defined as the ratio of band I (at 385–392 nm)/band II (at 408–415 nm) for 11*H*-benz[bc]aceanthrylene.

^c Defined as the ratio of band I (at 390–400 nm)/band II (at 415–425 nm) for 4*H*-benz[b]cyclopenta[mno]chrysene.

^d Defined as the ratio of band I (at 373–378 nm)/band II (at 387–395 nm) for 4*H*-cyclopenta[pqr]picene.

^e Defined as the ratio of band I (at 395–401 nm)/band II (at 422–427 nm) for 13*H*-dibenz[bc,l]aceanthrylene.

^f Defined as the ratio of band I (at 360–365 nm)/band II (at 378–384 nm) for 4*H*-cyclopenta[def]chrysene.

whenever large numbers of isomeric compounds are present. Blümer and Zander⁶ recommended that nitromethane and/or nitrobenzene could be added to an aqueous-acetonitrile (20:80 percent by volume) binary mobile phase to selectively suppress fluorescence signals of alternant PAHs. Emission intensities of nonalternant PAHs would remain unchanged.

Utilization of selective quenching reagents can significantly simplify observed emission spectra. To prevent misidentification, experimentally determined spectra must be free of chemical and instrumental artifacts that might unexpectedly reduce emission intensities. Inner-filtering is a major problem associated with obtaining correct fluorescence data, which assumes that the sample is optically dilute ($A \text{ cm}^{-1} \leq 0.01$) at all analytical wavelengths. Most commercial instruments employ right-angle fluorometry, which reduces stray radiation by placing the emission detector at 90° with respect to the incoming excitation beam (see Fig. 6). Only fluorescence emission originating from the center interrogation zone of the sample cell is actually collected. Attenuation of the excitation beam before it reaches the region viewed by the fluorescence detection optics (pre-filtering region) and through the interrogation volume element is denoted as primary inner-filtering. The correction factor, f_{prim} , for primary inner-filtering is given by the following expression:^{28–30}

$$f_{\text{prim}} = \frac{F^{\text{corr}}}{F^{\text{obs}}} = \frac{2.303A(y-x)}{10^{-Ax} - 10^{-Ay}} \quad (1)$$

where F^{corr} and F^{obs} refer to the corrected and observed fluorescence emission signal, respectively; A is the absorbance per centimeter of pathlength at the excitation wavelength; and x and y denote distances from the boundaries of the interrogation zone to the excitation plane, as shown in Fig. 6. Equation 1 strictly applies to monochromatic light, which from an experimental standpoint is never achievable, even with the finest spectrofluorometers having small spectral bandpasses. Yappert and Ingle³⁰ derived a more rigorous mathematical treat-

ment for non-monochromatic excitation and emission beams.

Primary inner-filtering can often be ignored in solvent polarity experiments requiring determination of intensity ratios as the excitation wavelength remains constant (i.e., A in Eq. 1 remains constant). Emission intensities of both bands are thus affected by the same relative amount. Selective quenching studies are another matter, however, as absorption of the excitation beam by the quenching reagent would reduce emission intensities of every fluorophore having the given excitation wavelength. In the case of nitromethane, inner-filtering would reduce emission intensities of both alternant and nonalternant PAHs by the same relative amount. For determination of whether selective quenching really occurred, observed emission intensities, F^{obs} , must be multiplied by the inner-filtering correction factor, f_{prim} , in order to eliminate the undesired effects from this chemical artifact. Failure to correct the observed inten-

FIG. 6. Typical cell configuration for right-angle fluorometry. Window parameters (x , y) and (u , v) are determined by masking apertures or some other limiting aperture in emission and excitation beam, respectively.

FIG. 7. Fluorescence emission spectra of 4*H*-benzo[*b*]cyclopenta-[*jkl*]triphenylene (left-hand) and 13*H*-dibenzo[*bc,k*]aceanthrylene (right-hand) dissolved in acetonitrile at various nitromethane concentrations. From top to bottom, the curves correspond to 0 and 1 drop of nitromethane for BCyTP, and 0, 3, and 10 drops of nitromethane for DBAA-1.

sities may lead to erroneous conclusions regarding PAH identification (alternant vs. nonalternant), particularly if excitation wavelengths of 300 nm or less are employed. Many of the PAHs have excitation wavelengths in the 300–320 nm spectral region, and a few drops of nitro-

methane (or nitrobenzene) give solutions of appreciable absorbances.

Secondary inner-filtering results from absorption of large quantities of emitted fluorescence, and the correction factor, f_{sec} ,

$$f_{sec} = \frac{F_{corr}}{F_{obs}} = \frac{(v - u)(1/b) \ln T}{T_{at v/b} - T_{at u/b}} \quad (2)$$

contains the sample transmittance (T) across the entire cell pathlength (b) at the emission wavelength.²⁹ Transmittances at the two interrogation zone boundaries, $T_{at v/b}$ and $T_{at u/b}$, are calculated from the measured absorbances at the emission wavelength via the Beers-Lambert law. Remember that v/b and u/b now serve as the new cell pathlengths.

For PAH/PANH solvent polarity determinations, secondary inner-filtering is a primary concern if the solution preferentially absorbs one of the PAH/PANH emission bands, thus leading to different transmittances (T values in Eq. 2) at the various emission wavelengths and erroneously low intensity ratios. Selective quenching experiments are not generally affected by secondary inner-filtering artifacts. PAH/PANH emission bands often appear in the 370–550 nm spectral region where nitromethane's absorbance is greatly diminished. Readers are reminded that only a few drops of quenching reagent are used in this type of experiment.

The corrected fluorescence emission intensity is given by

$$F_{corr} = f_{prim} f_{sec} F_{obs} \quad (3)$$

TABLE II. Effect of nitromethane concentrations on the fluorescence emission intensities of select alternant polycyclic aromatic hydrocarbons.

PAH Solute	F_{obs}^a	$A \text{ (cm}^{-1}\text{)}^b$	f_{prim}^c	F_{corr}
11 <i>H</i> -Benz[<i>bc</i>]aceanthrylene (emission: 389 nm)	388.8 50.4 21.9	0.002 0.079 0.159	1.003 1.095 1.201	339.5 55.2 26.4
4 <i>H</i> -Cyclopenta[<i>def</i>]phenanthrene (emission: 362 nm)	112.3 Zero	0.000 0.247	1.000 1.329	112.3 Zero
4 <i>H</i> -Cyclopenta[<i>def</i>]chrysene (emission: 360 nm)	758.5 60.1	0.000 0.284	1.000 1.384	758.5 83.3
13 <i>H</i> -Dibenzo[<i>a,g</i>]fluorene (emission: 381 nm)	688.8 35.5	0.000 0.111	1.000 1.136	688.8 40.4
13 <i>H</i> -Dibenzo[<i>a,i</i>]fluorene (emission: 384 nm)	484.0 25.3	0.000 0.259	1.000 1.347	484.0 34.1
4 <i>H</i> -Benzo[<i>b</i>]cyclopenta[<i>mno</i>]chrysene (emission: 398 nm)	885.4 217.8 42.5	0.000 0.086 0.426	1.000 1.104 1.633	885.4 240.5 69.4
4 <i>H</i> -Cyclopenta[<i>pqr</i>]picene (emission: 395 nm)	299.7 25.0	0.000 0.452	1.000 1.682	299.7 42.1
7 <i>H</i> -Dibenzo[<i>c,g</i>]fluorene (emission: 386 nm)	611.0 90.8	0.001 0.444	1.001 1.667	611.8 151.4
9 <i>H</i> -Benz[6,7]indeno[1,2]phenanthrene (emission: 394 nm)	664.3 29.4	0.000 0.123	1.000 1.152	664.3 33.8
4 <i>H</i> -Benzo[<i>b</i>]cyclopenta[<i>jkl</i>]triphenylene (emission: 397 nm)	566.0 28.9	0.000 0.986	1.000 3.106	566.0 89.6
13 <i>H</i> -Dibenzo[<i>bc,k</i>]aceanthrylene (emission: 460 nm)	783.0 365.2 156.4	0.000 0.000 0.000	1.000 1.000 1.000	783.0 365.2 156.4
13 <i>H</i> -Dibenzo[<i>bc,l</i>]aceanthrylene (emission: 398 nm)	635.6 242.4 69.8	0.000 0.260 0.860	1.000 1.349 2.688	635.6 326.9 187.7
4 <i>H</i> -Benzo[<i>def</i>]cyclopenta[<i>mno</i>]chrysene (emission: 400 nm)	537.3 176.8 21.7	0.000 0.102 0.168	1.000 1.125 1.214	537.3 198.9 26.3

^a After the initial intensity reading, each successive value corresponds to addition of one drop of nitromethane unless otherwise noted.

^b Absorbance of the solution measured at the excitation wavelength of the PAH under investigation. The solvent is acetonitrile.

^c Primary inner-filtering correction factors based upon $x = u = 0.45 \text{ cm}$ and $y = v = 0.55 \text{ cm}$.

assuming that primary and secondary inner-filtering are independent processes. As a general rule of thumb, inner-filtering corrections work well for f_{prim} and f_{sec} values less than three. Calculations of each correction factor requires *a priori* knowledge of interrogation zone volume and dimensions. Realizing that most instrument manufacturers rarely supply information regarding sample compartment aperture slit widths, particularly for the less expensive spectrofluorometers and fluorescence detectors used in HPLC, we have elected to base f_{prim} and f_{sec} computations upon assumed values of $x = u = 0.45$ cm and $y = v = 0.55$ cm. Inner-filtering corrections calculated in this fashion differ by only a few percent from approximate values²⁴ based upon Eq. 4:

$$f_{prim} f_{sec} \approx \text{antilog}[0.5 A_{at \lambda_{ex}} + 0.5 A_{at \lambda_{em}}]. \quad (4)$$

Figure 7 depicts the fluorescence emission spectra of 4*H*-benzo[*b*]cyclopenta[*jk*]triphenylene (left-hand side) and 13*H*-dibenz[*bc,k*]aceanthrylene (right-hand side) dissolved in acetonitrile as a function of nitromethane concentration. Numerical values of F_{obs} , F_{corr} , and $A \text{ cm}^{-1}$ for BCyTP and DBAA-1 are summarized in Table II, along with representative experimental data for the remaining eleven alternant PAHs studied. For BCyTP, part of the observed emission intensity reduction results from primary inner-filtering as nitromethane absorbs an appreciable amount of the 300-nm excitation radiation. Multiplication of the observed signal by f_{prim} increases the value to $F_{corr} \approx 89$; however, this is still significantly below the initial emission intensity of $F_{corr} \approx 566$. Primary inner-filtering correction is not necessary in the case of DBAA-1 since the excitation wavelength ($\lambda_{ex} = 400$ nm) now occurs in the spectral region where nitromethane is optically dilute. Careful examination of Table II reveals that nitromethane does quench the fluorescence emission of all thirteen solutes investigated, which is in complete agreement with what would be expected on the basis of the fact that all solutes are "alternant" polycyclic aromatic hydrocarbons. Results from the present study, combined with previously reported observations,^{31,32} further suggest that nitromethane cannot be used to distinguish between different subclasses of alternant PAHs, such as PAH6 benzenoids vs. alkylated PAH6 benzenoids vs. nitrogen hetero-atom PAH6 benzenoids vs. "methylene-bridged" cyclopenta-PAH molecules.

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