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Spectroscopic Properties of Polycyclic Aromatic Compounds: Examination of Nitromethane as a Selective Fluorescence Quenching Agent for Alternant Polycyclic Aromatic Nitrogen Hetero-Atom Derivatives

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Nitromethane is examined as a selective fluorescence quenching agent for alternant polycyclic aromatic nitrogen hetero-atoms (PANHs). Fluorescence emission behavior is reported for 1-azapyrene, 2-azapyrene, 4-azapyrene, 4-azachrysene, 12-azabenzo[a]pyrene, phenanthro[9,10g]isoquinoline, phenanthro[2,3h]isoquinoline, phenanthro[3,2h]-isoquino-line, 2-azabenz[a]anthracene, 1-azabenz[a]anthracene, 9-azabenz[a]anthracene, dibenzo[c,i]phenanthro[1,10,9,8anmlk]phenanthridine, diphenanthro[9,10,1def;1′,10′,9′hij]phthalazine, and benz[de]isoquino[1,8gh]quinoline dissolved in acetonitrile or aqueous-acetonitrile solvent mixtures at various nitromethane concentrations. Results of these measurements show that nitromethane does quench fluorescence emission of ten of the solutes studied; however, phenanthro-[2,3h]isoquinoline, 9-azabenz[a]anthracene, benz[de]isoquino[1,8gh]-quinoline, and dibenzo[c,i]phenanthro[1,10,9,8anmlk]phenanthridine are notable exceptions.

Index Headings: **Fluorescence; Spectroscopic techniques.**

INTRODUCTION

Fluorescence spectroscopy is rapidly becoming an extremely versatile, sensitive experimental technique for identifying and quantifying numerous environmentally important polycyclic aromatic hydrocarbons (PAHs) and polycyclic aromatic nitrogen heterocycles (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 nitromethane[5-9] 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 nonpolar solvents, since electronic interactions between a solvent dipole and an excited PAH/PANH solute 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.[10-21]

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_v^*, 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_v^* 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_v 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.[22,23]

The emission spectrum of many polycyclic aromatic compounds consists of several major vibronic bands labeled I, II, etc., in progressive order. Previous measurements[10-21] 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 73 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 probes are nearly complete, we have decided to redirect our experimental efforts to a comprehensive examination of selective fluorescence quenching agents. On the basis of limited fluorescence measurements for perylene, dibenzo[b,k]-

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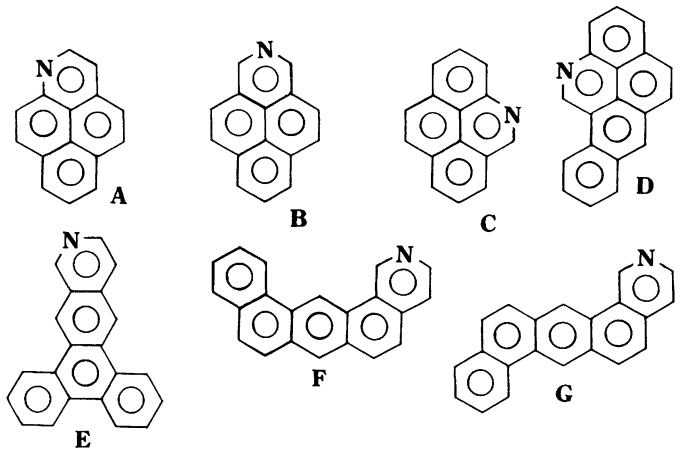

FIG. 1. Molecular structures of PANH solutes: (A) 1-azapyrene; (B) 2-azapyrene; (C) 4-azapyrene; (D) 12-azabenz[a]pyrene; (E) phenanthro[9,10g]isoquinoline; (F) phenanthro[2,3h]isoquinoline; and (G) phenanthro[3,2h]isoquinoline.

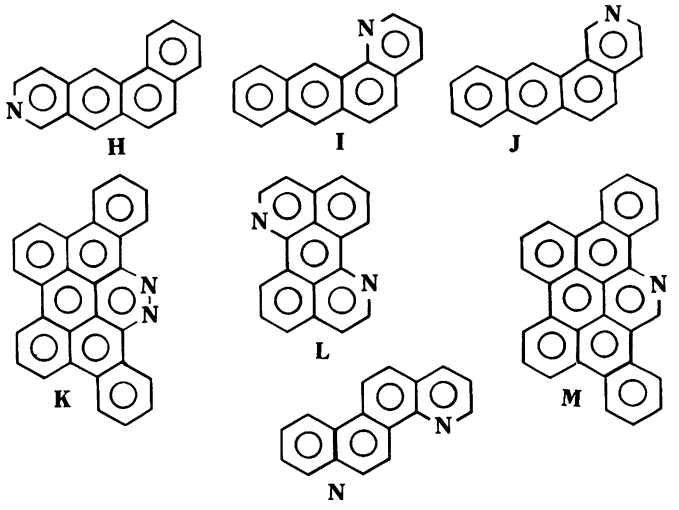

FIG. 2. Molecular structures of PANH solutes: (H) 9-azabenz[a] anthra cene; (I) 1-azbenz[a]anthracene; (J) 2-azabenz[a]anthracene; (K) diphenanthro[9,10,1def;1′,10′,9′hij]phthalazine; (L) benz[de]isoquino[1, 8gh]quinoline; (M) dibenzo[c,i]phenanthro[1,10,9,8anmlk]phenanthridine; and (N) 4-azachrysene.

chrysene, dibenzo[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 Zander[6] noted that nitromethane and nitrobenzene 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. The authors failed to investigate the hetero-atom analogs or methyl-substituted derivatives. For this reason, we report the effect that nitromethane has on the fluorescence emission of 1-azapyrene (1-AzPy), 2-azapyrene (2-AzPy), 4-azapyrene (4-AzPy), 12-azabenzo[a]pyrene (12-AzBPy), phenanthro[9,10g]isoquinoline (9,10-PIQ), phenanthro[2,3h]-isoquinoline (2,3-PIQ), phenanthro[3,2h]isoquinoline (3,2-PIQ), 2-azabenz[a]anthracene (2-AzBA), 9-azabenz-[a]anthracene (9-AzBA), 1-azabenz[a]anthracene (1-AzBA), 4-azachrysene (4-AzCh), dibenzo[c,i]phenanthro-[1,10,9,8anmlk]phenanthridine (DBPP), diphenanthro[9, 10,1def;1′,10′,9′hij]phthalazine (DPP), and benz[de]isoquino[1,8gh]quinoline (BIQQ). The various molecular structures are depicted in Figs. 1 and 2. These 14 solutes are classified as alternant PANHs because every alternant carbon and nitrogen atom in the aromatic ring system can be "starred." Nonalternant PAHs/PANHs, on the other hand, would have at least one pair of adjacent starred atoms.[24,25] Also included is a discussion of both primary and secondary inner-filtering artifacts associated with quenching determinations, and new experimental fluorescence results for DBPP, DPP, and BIQQ dissolved in nonelectrolyte organic solvents of varying solvent polarity and acidity.

MATERIALS AND METHODS

The various PANHs were synthesized and purified by procedures described in the literature.[26-37] 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 cm^{-1} ≤ 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 using 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 332 nm (1-AzPy), 338 nm (2-AzPy), 331 nm (4-AzPy), 364 nm (12-AzPy), 300 nm (9,10-PIQ), 330 nm (3,2-PIQ), 300 nm (2,3-PIQ), 300 nm (4-AzCh), 340 nm (1-AzBA), 340 nm (2-AzBA), 300 nm (9-AzBA), 410 nm (BIQQ), 350 nm (DBPP), and 400 nm (DPP) in a quartz 1-cm^2 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 PANH fluorescence spectra, depicted in Figs. 3–6, represent a single scan which was then solvent blank corrected and verified by repetitive measurements.

DISCUSSION OF SOLVENT POLARITY AND PROTONATION RESULTS

Representative fluorescence emission spectra of dibenzo[c,i]phenanthro[1,10,9,8anmlk]phenanthridine, diphenanthro[9,10,1def;1′,10′,9′hij]phthalazine, and benz[de]isoquino[1,8gh]quinoline dissolved in *n*-hexadecane, butyl acetate, dichloromethane, and dimethyl sulfoxide are depicted in Figs. 3 and 4. The four nonelectrolyte solvents were judiciously selected so as to encompass the entire range of solvent polarity, from the nonpolar *n*-hexadecane hydrocarbon to the moderately polar butyl acetate and dichloromethane solvents to the very

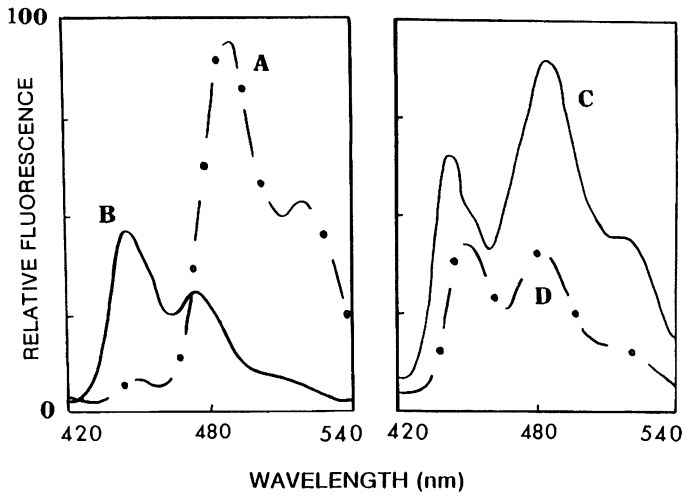

FIG. 3. Fluorescence emission spectra of diphenanthro[9,10,1def;1',10',9'hij]phthalazine dissolved in [*A*(——●——)] *n*-hexadecane; [*B*(——)] dichloromethane; [*C*(——)] butyl acetate; and [*D*(——●——)] dimethyl sulfoxide. In dimethyl sulfoxide major emission bands occur at about 449 and 482 nm.

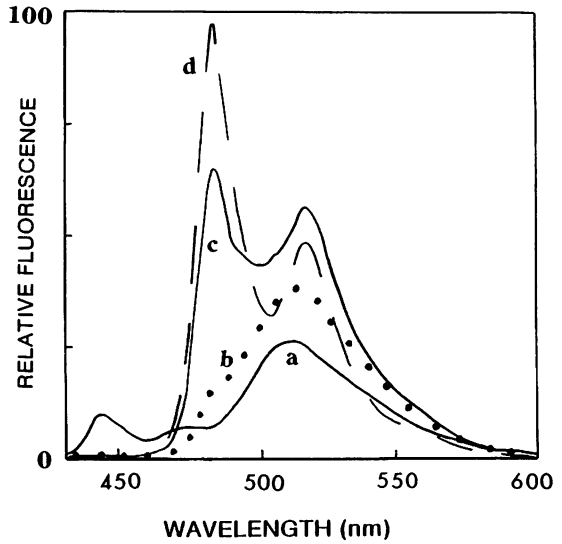

FIG. 5. Fluorescence emission spectra of benzo[de]isoquino[1,8gh]-quinoline dissolved in 2,2,2-trifluoroethanol at concentrations of $HClO_4$ of (*a*) neat trifluoroethanol; (*b*) 1 pasteur pipet drop; (*c*) 3 pasteur pipet drops; and (*d*) 5, 6, 7, and 8 pasteur pipet drops of $HClO_4$-trifluoroethanol solution. Curves *b* and *d* are believed to correspond to the mono- and diprotonated forms of BIQQ, respectively.

polar dimethyl sulfoxide, which is the most polar solvent considered in the present investigation. Examination of the three figures reveals that these PANH solutes fluoresce strongly and have several resolvable emission bands in the 390–540 nm spectral region. DPP initially appeared to exhibit solvent polarity behavior as evidenced by a changing emission intensity ratio, but upon much closer examination it was noted that there existed no correlation between the observed ratios and solvent polarities. For example, I/II band intensity ratios for *n*-hexadecane and acetonitrile were I/II = 0.09 and I/II = 0.32, respectively, despite the fact that these two solvents are situated at opposite ends of the PAH solvent polarity scales.[10-21,38] Acetonitrile should behave similarly to dimethyl sulfoxide, and as shown in Fig. 3, the two major emission bands of DPP have approximately the same intensity in dimethyl sulfoxide. Not too much significance is placed on the smaller 0.09–0.40 DPP values. To keep the larger second peak on scale, one is forced to work with a small first fluorescent signal. Small changes or uncertainties in the emission intensity of a weak signal can lead to a relatively large change in the calculated ratio. Emission intensity ratios for BIQQ and DBPP remained essentially constant in the fifteen solvents examined, irrespective of solvent polarity. Estimated uncertainties in the numerical intensity ratios for BIQQ and DBPP are believed to be *circa* ±0.07 or less on the basis of replicate measurements for select solvents. The

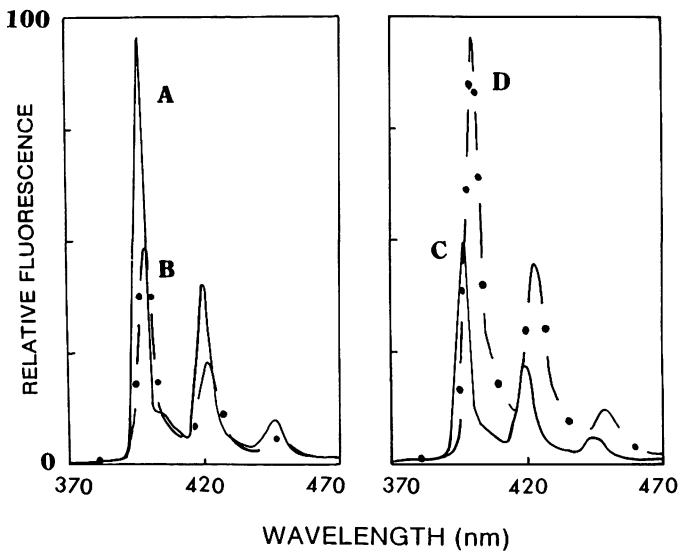

FIG. 4. Fluorescence emission spectra of dibenzo[c,i]phenanthro-[1,10,9,8anmlk]phenanthridine dissolved in [*A* (——)] *n*-hexadecane; [*B*(——●——)] dichloromethane; [*C* (——)] butyl acetate; and [*D* (——●——)]dimethyl sulfoxide. In butyl acetate major emission bands occur at about 397, 420, and 446 nm.

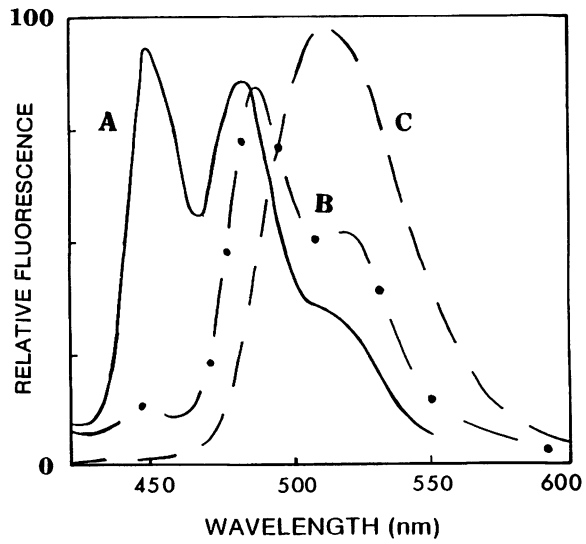

FIG. 6. Fluorescence emission spectra of the neutral (*A*, in dimethyl sulfoxide) and protonated (*C*, in $HClO_4$-trifluoroethanol) forms of diphenanthro[9,10,1def;1',10',9'hij]phthalazine. Curve *B* was recorded in trifluoroethanol, and shows only partial protonation. Protonation of the nitrogen hetero-atom results in loss of emission fine structure accompanied by a redshift in emission wavelengths.

three PANHs are thus labeled as nonprobe molecules in order to be consistent with our past classification scheme.[10-21]

Polycyclic aromatic nitrogen heterocycles are susceptible to protonation, particularly in the more acidic environments.[19,20,39-44] Protonation of the nitrogen lone electron pair by a hydrogen ion often results in the loss of emission fine structure accompanied by a sizeable red-shift in emission wavelength(s). Degree of protonation should be reflected by solvent acidity and PANH basicity. Figures 5 and 6 document that 2,2,2-trifluoroethanol only partially protonates BIQQ, DPP, and DBPP (not shown). Both the neutral (440–450 nm band in BIQQ; 485–495 nm band in DPP) and protonated (broader 510–550 nm bands) species are present in the BIQQ and DPP spectra. Protonation is complete upon addition of perchloric acid. The smaller first emission bands disappeared, and in the case of BIQQ, a new spectral emission band appeared near 483 nm for the presumed diprotonated $PANH^{+2}$ cation. The four spectra in Fig. 5 were obtained by titrating drop-sized amounts of a $HClO_4$-trifluoroethanol solution into the original PANH-trifluoroethanol sample. Curve *d* corresponded to a constant spectra recorded after 5, 6, 7, and 8 drops of $HClO_4$-trifluoroethanol solution. On the basis of the fact that it was impossible to eliminate the 510–530 nm peak by adding $HClO_4$, we must conclude that the BIQQ dication really does have two emission bands in its fluorescence spectra. As expected, the protonation is completely reversible. Addition of sodium methoxide to a $HClO_4$-trifluoroethanol-PANH solution restored the original "unprotonated" PANH spectrum, though a slight loss in emission intensity was observed. DPP also possesses two nitrogen hetero-atoms along the external perimeter of the aromatic ring system; however, only a single broad band was noted upon addition of $HClO_4$. In all likelihood, the broad 510–550 nm band belongs to the DPP monocation. The second protonation step should be significantly suppressed because it places "repelling positive charges" on two adjacent nitrogen atoms. 1,2,7,8-Tetraazacoronene (AzCo) is the sole multi-nitrogen polycyclic aromatic compound that we have attempted to study thus far. Unfortunately, fluorescence signals for AzCo were much too weak in many of the solvents examined to permit accurate determination of emission wavelengths and intensity ratios, even after spectral averaging of fifty repetitive scans.[20]

DISCUSSION OF FLUORESCENCE QUENCHING RESULTS

From an analytical perspective, identification and quantification of unknown PAH/PANH mixtures require accurate fluorescence emission intensity measurements and availability of 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 isomeric 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 fluorometric analysis affords a viable alternative, but again the method is extremely time-consuming whenever large numbers of isomeric PAHs/PANHs are present. Blümer and Zander[6] 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. No attempt was made to study polycyclic aromatic hetero-atoms or methyl-substituted PAHs/PANHs.

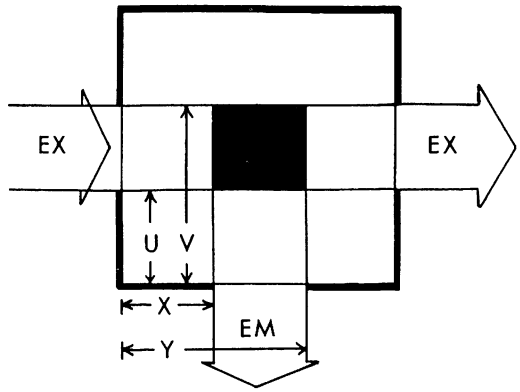

FIG. 7. Typical cell configuration for right-angle fluorometry. Window parameters (x,y) and (u,v) are determined by masking apertures or some other limiting aperture in emission and excitation beam, respectively.

Utilization of selective quenching agents 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 cm^{-1} ≤ 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. 7). Only fluorescence emission originating from the center interrogation zone of the sample cell is actually collected. Attenuation of the excitation beam before reaching the region viewed by the fluorescence detection optics (pre-filter 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:[45-47]

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

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. 7. Equation 1 strictly applies to monochromatic light, which from an experimental stand-

TABLE I. Effect of nitromethane concentration on the fluorescence emission intensities of select alternant polycyclic aromatic nitrogen heteroatoms.

PANH solute		F^{obs}[a]	A cm^{-1}[b]	f_{prim}[c]	F^{corr}
2-Azapyrene		983.7	0.000	1.000	983.7
(Emission: 376 nm)		58.3	0.196	1.253	73.1
		16.1	0.376	1.541	24.8
1-Azapyrene		996.0	0.000	1.000	996.0
(Emission: 369 nm)		275.1	0.138	1.172	332.5
		111.8	0.309	1.427	159.6
		53.7	0.470	1.717	92.2
		26.0	0.617	2.033	53.0
4-Azapyrene		860.9	0.000	1.000	860.9
(Emission: 371 nm)		101.1	0.218	1.285	129.9
		28.0	0.465	1.708	47.8
		4.2	0.700	2.237	9.3
4-Azachrysene		326.3[d]	0.000	1.000	326.3
(Emission: 363 nm)		61.1	0.566	1.918	117.2
12-Azabenzo[a]pyrene		207.6	0.000	1.000	207.6
(Emission: 407 nm)		101.5	0.008	1.009	102.5
		61.6	0.042	1.050	64.7
		45.0	0.066	1.079	48.6
		31.8	0.096	1.117	35.5
		24.5	0.123	1.152	28.2
1-Azabenz[a]anthracene		697.0	0.000	1.000	697.0
(Emission: 394 nm)		148.2	0.129	1.160	171.9
		66.8	0.272	1.368	91.3
		35.3	0.420	1.621	57.2
Phenanthro[3,2h]isoquinoline		467.6	0.001	1.000	467.6
(Emission: 392 nm)		243.4	0.207	1.269	308.9
		95.9	0.612	2.022	193.9
		58.0	0.844	2.639	153.2
Benz[de]isoquino[1,8gh]quinoline		844.5[d]	0.000	1.000	844.5
(Emission: 439 nm)	(5 drops)	887.7	0.000	1.000	887.7
	(10 drops)	873.2	0.000	1.000	873.2
Dibenzo[c,i]phenanthro[1,10,9,8anmlk]phenanthridine		152.3[d]	0.000	1.000	152.3
(Emission: 397 nm)	(3 drops)	143.0	0.202	1.262	180.5
	(10 drops)	68.3	0.736	2.331	159.2
Diphenanthro[9,10,1def;1′,10′,9′hij]phthalazine		254.7[e]	0.020	1.023	260.6
(Emission: 450 nm)	(4 drops)	130.8	0.014	1.016	132.9
	(10 drops)	85.2	0.014	1.016	86.6
Phenanthro[9,10g]isoquinoline		549.6[d]	0.000	1.000	549.6
(Emission: 382 nm)		201.4	0.539	1.859	374.4
		83.6	1.115	3.601	301.2
9-Azabenz[a]anthracene		174.8[d]	0.000	1.000	174.8
(Emission: 396 nm)		94.4	0.524	1.827	172.5
		48.8	1.052	3.350	163.6
2-Azabenz[a]anthracene		256.7[d]	0.000	1.000	256.7
(Emission: 389 nm)		149.2	0.127	1.158	172.7
	(3 drops)	66.8	0.398	1.581	105.5
Phenanthro[2,3h]isoquinoline		307.9[d]	0.000	1.000	307.9
(Emission: 393 nm)		153.3	0.571	1.929	295.6
		83.1	1.136	3.688	306.6

[a] After the initial intensity reading, unless otherwise specified, each successive value corresponds to addition of one pasteur pipet drop of nitromethane to the PANH dissolved in aqueous acetonitrile (20:80 by volume).
[b] Absorbance of the solution measured at the excitation wavelength of the PANH under investigation.
[c] Primary inner-filtering correction factors based upon $x = u = 0.45$ cm and $y = v = 0.55$ cm.
[d] Solvent used was acetonitrile.
[e] Solvent used was dimethyl sulfoxide.

point is never achievable, even with the finest spectrofluorometers having small spectral bandpasses. Yappert and Ingle[47] derived a more rigorous mathematical treatment for non-monochromatic excitation and emission beams.

Primary inner-filtering can often be ignored in PAH/PANH 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 agent 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. To determine whether selective quenching really occurred, one must multiply observed emission intensities, F^{obs} by the inner-filtering correction factor, f_{prim},

in order to eliminate the undesired effects from this chemical artifact. Failure to correct the observed intensities may lead to erroneous conclusions concerning PAH/PANH identification (alternant vs. nonalternant), particularly if excitation wavelengths of 300 nm or less are employed. Many PAHs/PANHs have excitation wavelengths in the 300–320 nm spectral region, and a few drops of nitromethane (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},[46]

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

contains the sample transmittance (T) across the entire cell pathlength (b) at the emission wavelength. Transmittances at the two interrogation zone boundaries, $T_{\text{at } v/b}$ and $T_{\text{at } u/b}$, 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 appear in the 370–500 nm spectral region where nitromethane's absorbance is greatly diminished. Readers are reminded that only a few drops of quenching agent are used in this type of experiment.

The corrected fluorescence emission intensity is given by

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

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. Calculation 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. These particular values led to consistent F^{corr} signals for quinine sulfate solutions, which were inner-filtered by differing concentrations of potassium dichromate.[48]

Numerical values of the fluorescence emission intensities (both F^{obs} and F^{corr}) and solution absorbances (A cm^{-1}) are summarized in Table I for the 14 alternant PANH solutes studied. Careful examination reveals that F^{obs} does decrease with increasing nitromethane concentration, though not all of the intensity reduction is attributed to fluorescence quenching. A significant fraction of the observed intensity reduction results from primary inner-filtering artifacts. This is particularly true in the case of the four PANH solutes (4-AzCh, 9,10-PIQ, 2,3-PIQ, and 9-AzBA) having an excitation wavelength of 300 nm. Multiplication of the observed fluorescence intensities of 2,3-PIQ and 9-AzBA by f_{prim} increases the values of $F^{corr} \approx 300$ and $F^{corr} \approx 168$, respectively, which is approximately equal to the initial emission intensities before addition of nitromethane. Similar conclusions can be drawn from the experimental data for BIQQ and DBPP. The "apparent" increase in F^{obs} for DBPP and BIQQ, noted after addition of a few pasteur pipet drops of nitromethane, is likely the result of nitromethane redissolving a very small amount of PANH which was still adsorbed onto the test tube or cuvette walls. Large polycyclic aromatic compounds are not very soluble in water, and we have tried to eliminate any solubility artifacts by substituting acetonitrile for the aqueous-acetonitrile binary solvent mixture recommended by Blümer and Zander.[6]

Our experimental results clearly show that nitromethane does not quench the fluorescence emission of 2,3-PIQ, 9-AzBA, BIQQ, and DBPP. At the moment we are at a loss to explain why these particular four PANH solutes are exceptions to the selective quenching observations previously noted by several research groups.[5-9,20,49] Recall that nitromethane is suppose to selectively quench the fluorescence emission of alternant PAHs as opposed to nonalternant PAHs. Polycyclic aromatic nitrogen hetero-atoms can have their π-orbitals and nonbonding orbitals reversed, and this possibility might perhaps partly explain the unusual quenching behavior of 2,3-PIQ, 9-AzBA, BIQQ, and DBPP. Additional measurements are currently underway to further study the effect of nitromethane, nitrobenzene, and 1,2,4-trimethoxybenzene on the fluorescence emission behavior of still more alternant and nonalternant PAHs, as well as alkylated polycyclic aromatic hydrocarbons, to determine to what extent previously reported quenching observations hold for substituted PAHs.

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