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Characterization of polycyclic aromatic hydrocarbons in environmental samples by selective fluorescence quenching

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Abstract

Selective fluorescence quenching is used to profile polycyclic aromatic hydrocarbons (PAHs) in samples of environmental origin. After separation by high-efficiency capillary liquid chromatography, the PAHs are detected by laser-induced fluorescence spectroscopy. Nitromethane is added to selectively quench the fluorescence of alternant PAHs, whereas diisopropylamine is added to quench nonalternant PAHs. The chromatograms in the absence and presence of fluorescence quenching are evaluated by means of the product moment correlation method to quantify the statistical similarities and differences. This method is demonstrated by application to three samples: a standard mixture of 16 priority pollutants, a coal-derived fluid, and a contaminated soil. The correlation coefficients (r) are typically 0.99 or higher for samples that are identical in origin, 0.90–0.50 for closely related samples, and less than 0.50 for samples that are distinctly unrelated. This method can be used to confirm with high statistical confidence the cause or source of an event with environmental impact, such as an oil leak or spill, contamination or waste by-products from petroleum fuel production and processing, etc.

Keywords: Fluorescence; Fluorescence quenching; Environmental analysis; Polycyclic aromatic hydrocarbons; Nitromethane; Diisopropylamine

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) consist of fused aromatic rings arranged in varied isomeric configurations. Some of these isomers are benign, whereas many others have potent biological activity. Indeed, PAHs form the largest known class of chemical carcinogens and mutagens [1,2], 16 of which have been identified by the US Environmental Protection Agency (US EPA) as priority pollutants. Based on their structure, PAHs can be divided into two subclasses: alternant and nonalternant. To distinguish between these classes, it is helpful to label each carbon atom in the aromatic structure, alternately skipping an atom between labels. Alternant PAHs possess a structure in which no two atoms of the same type (labeled or unlabeled) are adjacent. Examples include anthracene, pyrene, and other PAHs that consist solely of six-membered rings. Nonalternant PAHs have a structure in which such labeling results in two adjacent atoms of the same type. Examples include fluorene and fluoranthene, which contain one five-membered ring in addition to six-membered rings. Such subtle changes in structure can cause large differences in the physical, chemical, and toxicological properties of PAHs [3–5].

The importance in differentiating between these two classes for environmental analysis is that the

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distribution of isomers can be indicative of the formation and history of the sample. The most stable PAH isomers contain alternant, clustered arrangements of aromatic rings (e.g. pyrene), followed by angular arrangements (e.g. phenanthrene), and linear arrangements (e.g. anthracene). These isomers are preferentially formed under heating conditions that are very long in duration (i.e. geochemical phenomena). Nonalternant PAHs, however, are less stable and are often formed when there is a short period of very intense heat (i.e. combustion). Thus, the distribution of alternant and nonalternant PAHs in a sample is a unique and characteristic fingerprint. This fingerprint may be useful to identify the cause or source of an event with environmental impact, such as an oil leak or spill, contamination or waste by-products from petroleum fuel production and processing, etc.

1.1. Fluorescence quenching

Most PAHs are natively fluorescent because the delocalized electrons within the aromatic rings can be easily excited and the rigid structure does not allow for efficient vibrational relaxation. Depending upon the number and arrangement of aromatic rings, the resulting fluorescence spectra are highly characteristic of the PAH. For example, many alternant PAHs exhibit fluorescence spectra with well-defined vibrational fine structure, whereas most nonalternant PAHs have broad spectra with few structural features [6,7]. Additional information can be obtained by means of selective fluorescence quenching [5,8,9]. Such quenching can occur by two mechanisms: static and dynamic. Static quenching occurs when the ground-state fluorophore and ground-state quencher form a stable complex [8,9]. This new complex may exhibit different spectral characteristics than the fluorophore, resulting in a reduction in the fluorescence power at the selected excitation and emission wavelengths. Dynamic quenching occurs when an excited-state fluorophore collides with and transfers energy to a ground-state quencher [8,9]. The excited-state quencher subsequently returns to the ground-state via a nonradiative path (i.e. vibrational relaxation). Dynamic quenching is quantified by the Stern–Volmer equation:

$$\frac{P_f^0}{P_f} = 1 + K_q[Q] \quad (1)$$

In this equation, P_f^0 and P_f are the fluorescence power in the absence and presence, respectively, of the quencher at molar concentration $[Q]$. A graph of P_f^0/P_f versus $[Q]$ yields the Stern–Volmer quenching constant (K_q). This constant is characteristic of the fluorophore–quencher interactions and is a direct measure of the efficiency of the fluorescence quenching process.

Although a large number of quenchers for PAHs have been identified, very few of them have been characterized in sufficient depth and detail to permit their routine use in environmental applications [5]. Initial studies by Sawicki et al. [10] showed that nitromethane, which acts as an electron acceptor, selectively quenches the fluorescence of alternant PAHs. Subsequent studies by Acree et al. [11–13] demonstrated that this so-called "nitromethane selective quenching rule" is broadly applicable with only a few exceptions. A quantitative study by Ogasawara et al. [14] revealed that the Stern–Volmer quenching constants of nitromethane are 33–100 times greater for alternant than for nonalternant isomers. In contrast, recent investigations by Goodpaster and McGuffin [15] demonstrated that amines, which act as electron donors, are selective quenchers for nonalternant PAHs. The Stern–Volmer quenching constants of diisopropylamine are typically 15–45 times greater for nonalternant than for alternant isomers. In the present study, selective fluorescence quenching by nitromethane and diisopropylamine are combined with high-efficiency capillary liquid chromatography for detection of alternant and nonalternant PAHs in environmental samples. This approach is advantageous for the improvement of qualitative and quantitative analysis by removing potential interferences. In addition, PAH profiling may aid in the identification of sample origin, either for environmental and health-hazard documentation or for geological studies on soil sedimentation [16–18].

2. Experimental section

2.1. Materials and methods

Three samples containing a mixture of PAHs have been chosen for analysis, all of which are certified reference materials (Table 1). The first sample is a

Table 1
Concentration of polycyclic aromatic hydrocarbons in certified reference materials[a]

Polycyclic aromatic hydrocarbons	Classification	Standard EPA 610 (μg/ml)	Coal-derived fluid SRM 1597 (μg/ml)	Contaminated soil CRM104-100 (μg/ml))
Anthracene	Alternant	100.1	87.4	1.44
Fluoranthene	Nonalternant	200.2	278	24.6
Pyrene	Alternant	99.9	204	15.0
Benz[*a*]anthracene	Alternant	100.0	85.3	7.98
Chrysene	Alternant	100.0	62.0	8.60
Benzo[*b*]fluoranthene	Nonalternant	200.0	(53.1)	(9.69)
Benzo[*k*]fluoranthene	Nonalternant	100.0	(33.6)	(5.10)
Benzo[*a*]pyrene	Alternant	100.1	82.9	5.09
Dibenz[*a*,*h*]anthracene	Alternant	200.0	NA[b]	(1.55)
Indeno[1,2,3-*cd*]pyrene	Nonalternant	100.1	52.1	4.46
Benzo[*ghi*]perylene	Alternant	200.0	46.5	3.58

[a] Concentrations given in parentheses are not certified values.
[b] NA: not available.

standard (EPA 610, Supelco) that contains 16 PAHs classified as priority pollutants by the US EPA. The second sample is an extract of Wheeling Pittsburgh medium crude coke oven tar (SRM 1647, National Institute of Standards and Technology), which is a natural combustion-related mixture of PAHs [19]. Both of these samples were used as received.

The third sample is a contaminated soil/sediment from the southern branch of the Elizabeth River near Norfolk, VA (CRM104-100, Resource Technology Corporation). This sample required extraction of the PAHs according to US EPA Method 3540c (Soxhlet Extraction) prior to analysis. The solvents used in the Soxhlet apparatus were pesticide-grade acetone and hexane (Baxter Healthcare, Burdick and Jackson Division) mixed in a 1:1 (v/v) ratio. After reflux for 24 h, the yellow extract was transferred to a Kuderna–Danish evaporator (Kontes) and the volume was reduced from 400 to 8 ml over a 2 h period. The resulting sample was then transferred, via a Pasteur pipette, in small aliquots to a conical vial. The sample was evaporated to dryness with a nitrogen stream, resulting in 0.63 g of solid material from 10.23 g of soil. The solid was reconstituted in 1.0 ml of pesticide-grade methylene chloride (Baxter Healthcare, Burdick and Jackson Division).

Two quenchers were chosen for these studies based upon their previously reported selectivity for alternant and nonalternant PAHs. Nitromethane (EM Science) was volumetrically diluted with pesticide-grade methanol (Baxter Healthcare, Burdick and Jackson Division) to yield a 2% (v/v) solution. Diisopropylamine (Aldrich) was volumetrically diluted with pesticide-grade acetonitrile (Baxter Healthcare, Burdick and Jackson Division) to yield a 50% (v/v) solution.

2.2. Instrumentation

Each of the samples was analyzed on the system shown in Fig. 1, which was constructed and characterized in house [20]. A reciprocating piston pump (Beckman Instruments, Model 114M) was used to deliver the methanol mobile phase at a nominal flow rate of 1.0 μl/min. The sample was introduced by means of a valve with a fixed volume of 1.0 μl (Valco Instruments, Model ECI4W1), which was subsequently split 1:42 to provide an injection volume of approximately 24 nl. The sample constituents were then separated on a fused-silica capillary column (Hewlett-Packard, 200 μm i.d., 320 μm o.d., 1.5 m length) that was packed with a 5 μm octadecylsilica stationary phase (Shandon, Hypersil C18, 115,000 theoretical plates), as described previously [21]. The column was immersed within a water bath maintained at 24 °C to minimize the effect of temperature fluctuations on the separation. The column effluent was combined and thoroughly mixed with the quencher solution, which was delivered by a syringe pump (PE/Applied Biosystems, Model 140) at a nominal flow rate of 1.0 μl/min.

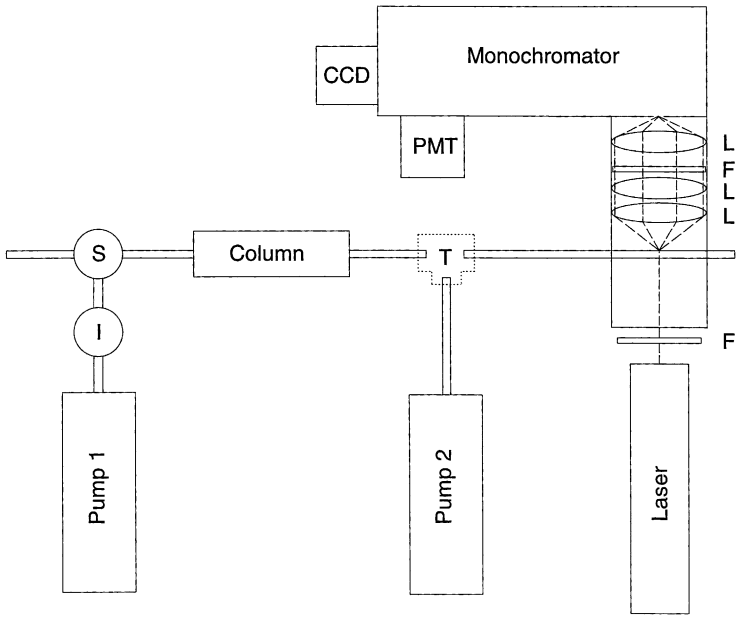

Fig. 1. Schematic diagram of the experimental system for capillary liquid chromatography with laser-induced fluorescence and fluorescence quenching detection (I: injection valve; S: splitting tee; T: mixing tee; L: lens; F: filter; CCD: charge-coupled device; PMT: photomultiplier tube).

The PAHs were then detected by laser-induced fluorescence in a fused-silica capillary flow cell (Polymicro Technologies, 75 μm i.d., 360 μm o.d, 1.0 m length), which minimized the interference from trivial processes such as primary or secondary absorption, refractive index effects, etc. [22]. This flow cell was irradiated with a helium–cadmium laser (Melles Griot, Model 3074-40M, 325 nm, 32 mW). Fluorescence emission was collected orthogonal to the incident radiation and was collimated and filtered to remove stray light. The resulting emission was then refocused onto the entrance slit of a 0.34 m Czerny–Turner monochromator (Instruments SA, Model 340E, 300 groove/mm grating, 10 nm/mm reciprocal linear dispersion) and detected by a charge-coupled device (Instruments SA, Model (A)TECCD-2000x800-7, 15 μm pixels). This system provided a wavelength range of 300 nm and a resolution of 0.15 nm. Instrument control and data acquisition were achieved by using a commercially available electronic interface (Instruments SA, Model CCD 2000) and the associated software (Instruments SA, Spectramax for Windows, Version 3.1).

2.3. Data analysis

Data from the chromatographic separation of each sample were integrated over the wavelength range of 350–564 nm by using the Spectramax software. These chromatograms were used for visual display (Figs. 2–4), for discernment of fluorescence quenching behavior and, where possible, for identification of the individual PAHs. For correlation, it was necessary to normalize the abscissa such that the known PAHs had the same retention times in each chromatogram. The resulting chromatograms were then exported as ASCII files into the statistical analysis software (Jandel, SigmaStat, Version 1.02). The chromatograms were correlated with one another, in a point-by-point manner, using the product moment correlation method [23,24]. This method is useful to establish the extent of association or similarity between two chromatograms, both of which are regarded as independent variables. This parametric method assumes that the association (if any) is linear and that the residuals are normally distributed with constant variance. The resulting scatter plot shows the relationship between the relative

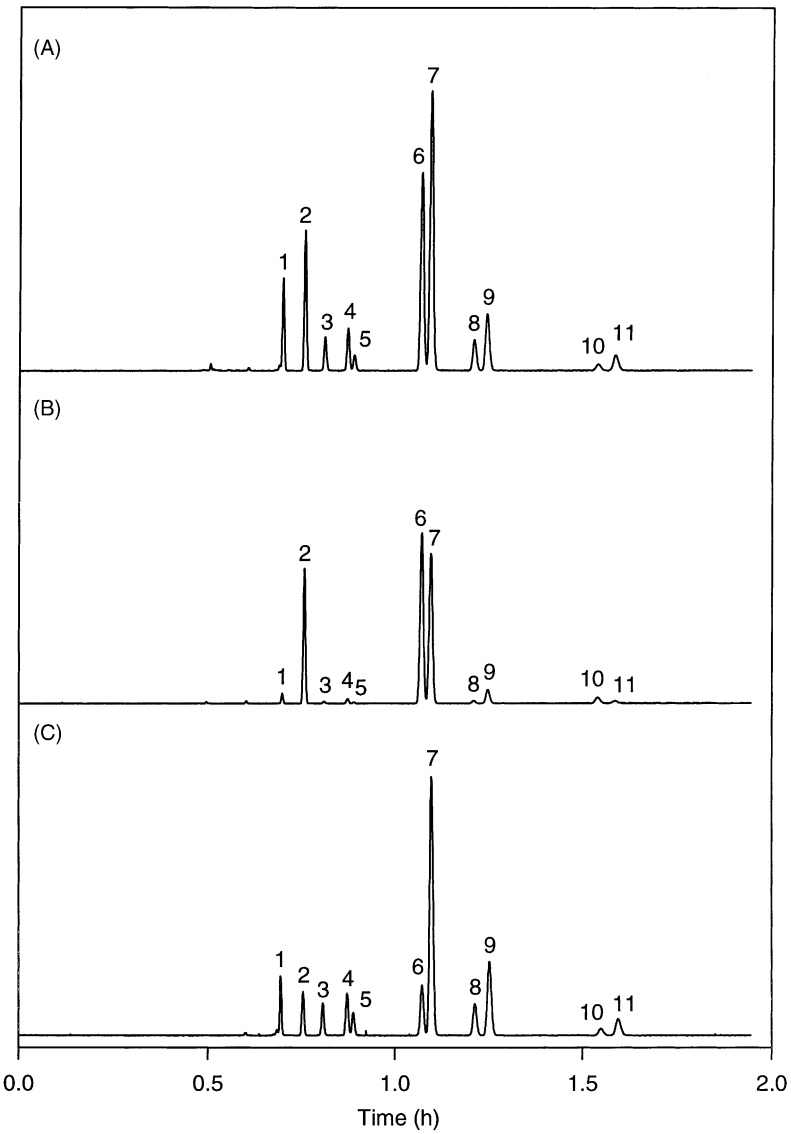

Fig. 2. Chromatogram of standard PAHs (EPA 610) without (A) and with fluorescence quenching by nitromethane (B) and diisopropylamine (C). Column: 1.5 m × 200 μm i.d. fused-silica capillary, packed with 5 μm Shandon Hypersil C18. Mobile phase: methanol, 1.0 μl/min, 24 °C, with post-column addition of (A) methanol, 1.0 μl/min; (B) 2% (v/v) nitromethane in methanol, 1.0 μl/min; (C) 50% (v/v) diisopropyl amine in acetonitrile, 1.0 μl/min. Laser-induced fluorescence detection: 325 nm excitation, 350–564 nm emission. Solutes: (1) anthracene, (2) fluoranthene, (3) pyrene, (4) benz[*a*]anthracene, (5) chrysene, (6) benzo[*b*]fluoranthene, (7) benzo[*k*]fluoranthene, (8) benzo[*a*]pyrene, (9) dibenz[*a*,*h*]anthracene, (10) indeno[1,2,3-*cd*]pyrene, (11) benzo[*ghi*]perylene.

fluorescence power or concentration of the PAHs in the two samples. The correlation coefficient (r) quantifies the degree of similarity, and the corresponding P-value expresses the statistical reliability of the results. This same approach can be used to examine the correlation of fluorescence spectra in order to verify the identity and purity of PAHs in the chromatograms.

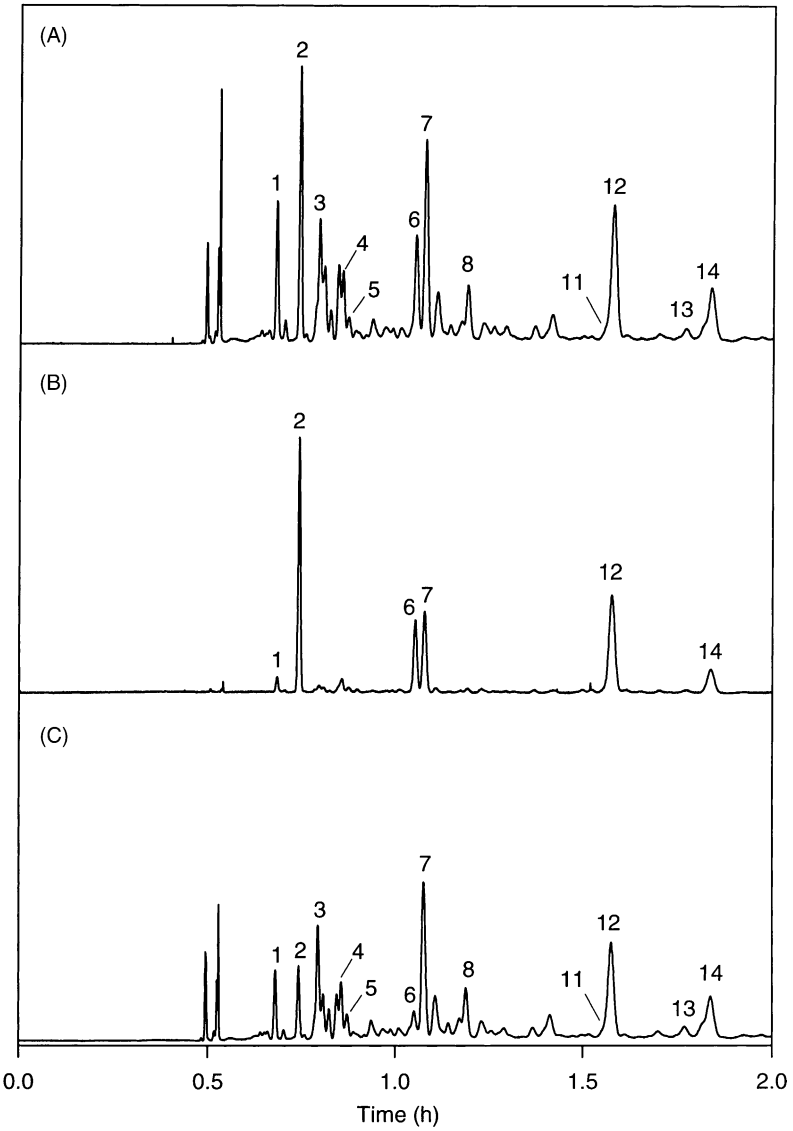

Fig. 3. Chromatogram of PAHs in a coal-derived fluid (SRM 1597) without (A) and with fluorescence quenching by nitromethane (B) and diisopropylamine (C). Solutes: (12) unknown, possibly dibenzofluoranthene or naphthofluoranthene isomer, (13) dibenzo[*def*,*mno*]chrysene, (14) unknown, possibly dibenzofluoranthene or naphthofluoranthene isomer. Other experimental conditions and solutes as described in Fig. 2.

3. Results and discussion

In this study, fluorescence and fluorescence quenching are explored as a means of profiling the alternant and nonalternant PAHs in environmental samples. For each of the samples, a high-efficiency separation of the PAHs was achieved by capillary liquid chromatography using methanol as the mobile phase. The chromatograms were recorded first with laser-induced fluorescence detection over a wavelength range of 350–564 nm. Subsequently, the chromatograms were recorded under the same conditions with the addition of 2% nitromethane or 50% diisopropylamine as quenchers.

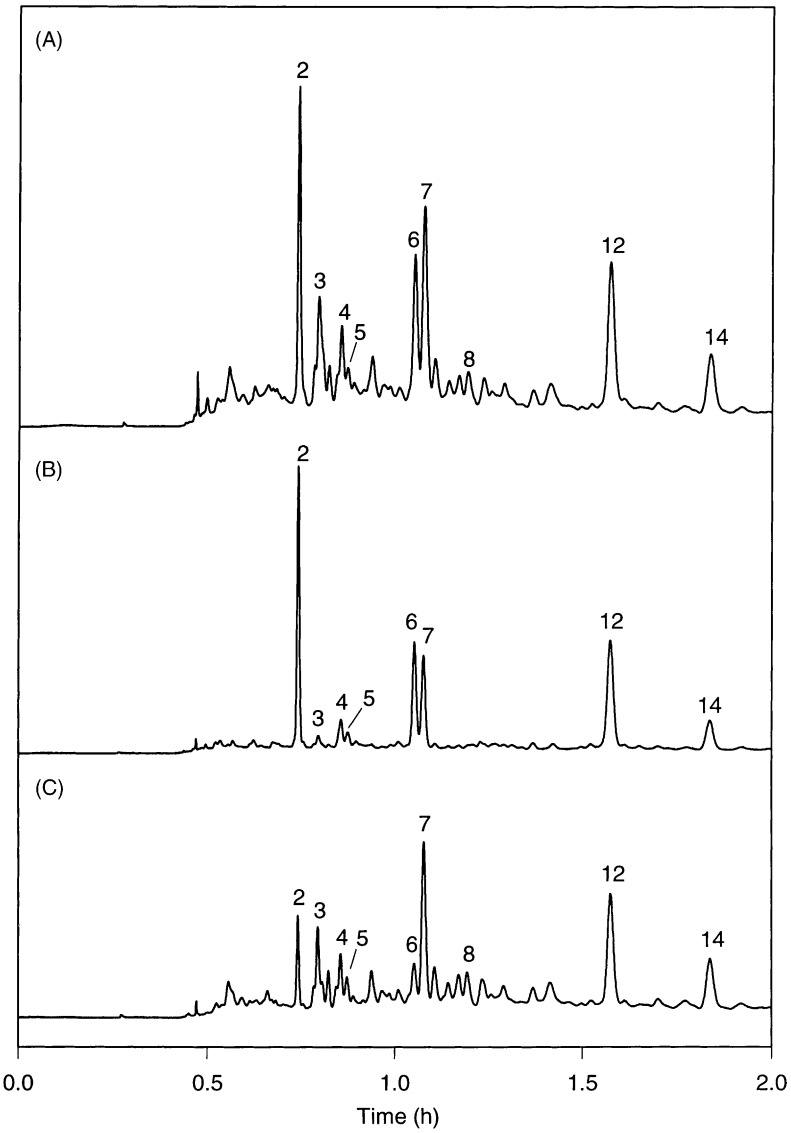

Fig. 4. Chromatogram of PAHs in a contaminated soil (CRM104-100) without (A) and with fluorescence quenching by nitromethane (B) and diisopropylamine (C). Other experimental conditions and solutes as described in Figs. 2 and 3.

This experimental approach provides a wide range of information that can be used to identify the individual PAHs, including chromatographic retention time, fluorescence emission spectra, and Stern–Volmer quenching constants. In addition, it provides many ways to uniquely profile the distribution of PAHs in the sample, including chromatograms at individual fluorescence wavelengths, chromatograms at integrated fluorescence wavelengths, chromatograms with fluorescence quenching of alternant PAHs by nitromethane, and chromatograms with fluorescence quenching of nonalternant PAHs by diisopropylamine. The results for each sample are discussed in detail in the following sections.

3.1. Standard (EPA 610)

A chromatogram of the standard (EPA 610) is shown in Fig. 2A. The identity of each PAH was confirmed by comparison of the retention time and fluorescence spectrum with authentic standards [7,25]. Of the sixteen known components in this sample, only eleven are fluorescent with excitation at 325 nm and emission at 350–564 nm. Several of the smaller PAHs, including naphthalene, acenaphthylene, acenaphthene, fluorene, and phenanthrene, are not excited efficiently by the helium–cadmium laser. The PAHs ranging from anthracene to benzo[*ghi*]perylene are readily detected in spite of the relatively small mass injected (2.4–4.8 ng). The fluorescence power of each PAH is a function of the concentration (Table 1), as well as the molar absorptivity and quantum efficiency at the selected wavelengths for excitation and emission.

A chromatogram of the standard with fluorescence quenching by nitromethane is shown in Fig. 2B. It is immediately evident that the nonalternant PAHs (fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and indeno[1,2,3-*cd*]pyrene) substantially retain their original fluorescence power. In contrast, the alternant PAHs are significantly quenched. This observation is consistent with the previously reported Stern–Volmer constants of 0.07 and 0.64 M^{-1} for the representative nonalternant PAHs fluoranthene and benzo[*b*]fluoranthene, and 94 and 61 M^{-1} for the representative alternant PAHs pyrene and benzo[*a*]pyrene [20]. It is also noteworthy in Fig. 2B that benzo[*k*]fluoranthene appears to be more highly quenched than the other nonalternant PAHs. This is consistent with differences in the electron-donating ability of the aromatic system to the nitromethane quencher [14,22]. The gas-phase ionization energy [26] of benzo[*k*]fluoranthene (8.167 eV) is substantially less than that of fluoranthene (8.466 eV) and benzo[*b*]fluoranthene (8.410 eV), which suggests that it is a better electron donor. It is, in fact, more similar to the alternant PAHs benz[*a*]anthracene (8.111 eV) and chrysene (8.261 eV), which is reflected in the quenching behavior.

A chromatogram of the standard with fluorescence quenching by diisopropylamine is shown in Fig. 2C. In general, the nonalternant PAHs are moderately quenched and the alternant PAHs are unaffected. This observation is consistent with the previously reported Stern–Volmer constants of 17.1 and 21.2 M^{-1} for the representative nonalternant PAHs fluoranthene and benzo[*b*]fluoranthene, and 1.2 and 0.47 M^{-1} for the representative alternant PAHs pyrene and benzo[*a*]pyrene [15]. Benzo[*k*]fluoranthene is an interesting exception to this general trend, as it is relatively unquenched by diisopropylamine. Its behavior, again, is more similar to the alternant PAHs benz[*a*]anthracene and chrysene than to the other nonalternant PAHs fluoranthene and benzo[*b*]fluoranthene.

3.2. Coal-derived fluid (SRM 1597)

A chromatogram of the coal-derived fluid (SRM 1597) is shown in Fig. 3A. The identity of each PAH was again confirmed by comparison of the retention time and fluorescence spectrum with authentic standards [7,25]. The concentrations of the known PAHs are summarized in Table 1. In addition, there are a large number of unidentified PAHs that elute in the time range of 0.75–1.5 h. As the fluorescence spectra of many of these PAHs have vibrational fine structure similar to alternant PAHs, we suggest that these may be methylated or other alkylated analogs. This is consistent with the combustion-related origin of this sample and is supported by a prior detailed analysis by gas chromatography with mass spectrometric detection, which was able to identify many methylated PAH isomers [19]. There are two additional large peaks that can be observed between 1.5 and 2.0 h. On the basis of the fluorescence spectra and the prior analysis [19,27], we suggest that these may be dibenzofluoranthene or naphthofluoranthene isomers. In addition, dibenzo[*def,mno*]chrysene (anthanthrene) can be identified unambiguously from the fluorescence spectrum [7].

A chromatogram of the coal-derived fluid with fluorescence quenching by nitromethane is shown in Fig. 3B. All of the known nonalternant PAHs are relatively unaffected and the alternant PAHs are quenched in the same manner as for the standard sample in Fig. 2B. The many unidentified PAHs are highly quenched by nitromethane, which is consistent with their tentative assignment as alkylated alternant isomers. Acree et al. [11,12] have reported that both alternant and nonalternant PAHs with alkyl substituents preserve the inherent quenching behav-

ior of the parent PAHs. In addition, the fluorescence power of the two PAHs tentatively assigned as dibenzofluoranthene or naphthofluoranthene isomers is largely retained, as would be expected for nonalternant PAHs. However, the partial quenching by nitromethane indicates that these PAHs are most similar to benzo[*k*]fluoranthene, which has significant alternant character. This observation is in agreement with the previous results of Tucker and Acree [28] for selected naphthofluoranthenes. Finally, we note that the qualitative and quantitative analysis for all of the alternant PAHs has been substantially improved by fluorescence quenching with nitromethane in Fig. 3B.

A chromatogram of the coal-derived fluid with fluorescence quenching by diisopropylamine is shown in Fig. 3C. All of the known alternant PAHs are relatively unaffected and the nonalternant PAHs are quenched in the same manner as for the standard sample in Fig. 2C. The unidentified PAHs that have been tentatively assigned as alkylated alternant isomers are not significantly quenched by diisopropylamine, as would be expected. The PAHs tentatively assigned as dibenzofluoranthene or naphthofluoranthene isomers are slightly quenched. Although this behavior is unexpected for nonalternant PAHs, it is fully consistent with the behavior of benzo[*k*]fluoranthene. Finally, because of the complexity of this sample with many alternant PAHs, the chromatogram is not greatly simplified by fluorescence quenching with diisopropylamine. However, some minor improvement in qualitative and quantitative analysis may be obtained.

3.3. Contaminated soil (CRM104-100)

A chromatogram of the contaminated soil (CRM-104-100) is shown in Fig. 4A. The identity of each PAH was again confirmed by comparison of the retention time and fluorescence spectrum with authentic standards [7,25]. The concentrations of the known PAHs are summarized in Table 1. Like the coal-derived fluid, this sample has numerous PAHs in the time range of 0.75–1.5 h that appear to be alkylated alternant isomers. Moreover, the PAHs that have been tentatively identified as dibenzofluoranthene or naphthofluoranthene isomers are also present.

Chromatograms of the contaminated soil with fluorescence quenching by nitromethane and diisopropylamine are shown in Figs. 4B and C, respectively. The alternant and nonalternant PAHs are quenched in the expected manner, as described above for the standard and coal-derived fluid.

3.4. Statistical correlation analysis

In order to demonstrate the utility of fluorescence and fluorescence quenching for profiling PAHs in environmental samples, the chromatograms were correlated by using the product moment method [23,24]. The complete chromatograms were correlated, point-by-point, which provides a more detailed comparison than the use of peak maxima or peak areas alone. The correlation graphs for three representative cases are shown in Fig. 5. When samples are derived from exactly the same origin, the relative fluorescence power or concentration of PAHs in each sample is identical. The resulting scatter plot (Fig. 5A) shows a high degree of correlation. Accordingly, the correlation coefficients (r) for identical or replicate samples are typically 0.99 or higher. When samples are of similar or related origin, many of the same PAHs may be present but at different concentrations. This results in an intermediate degree of correlation (Fig. 5B), with typical values of r in the range of 0.90–0.50. Finally, when samples are of distinctly unrelated origin, the disparate distribution of PAHs will result in little or no correlation (Fig. 5C), with typical values of r less than 0.50. In all cases, valid conclusions can be drawn about the identity or origin of the samples when the P-value for the product moment correlation is less than 0.05 (95% CL).

Table 2 summarizes the results of the product moment correlation for the three samples examined with fluorescence detection alone. It is apparent that there is little correlation between the standard and the coal-derived fluid or contaminated soil, despite the common PAHs found in each sample. As many of the PAHs in the more complex samples are not found in the standard sample, these samples present the unique challenge of profiling with limited information for which this correlation method is well suited. The coal-derived fluid and contaminated soil show an intermediate degree of correlation ($r = 0.877$), which is consistent with their combustion-related origin and the similar appearance of their chromatograms in Figs. 3A and 4A.

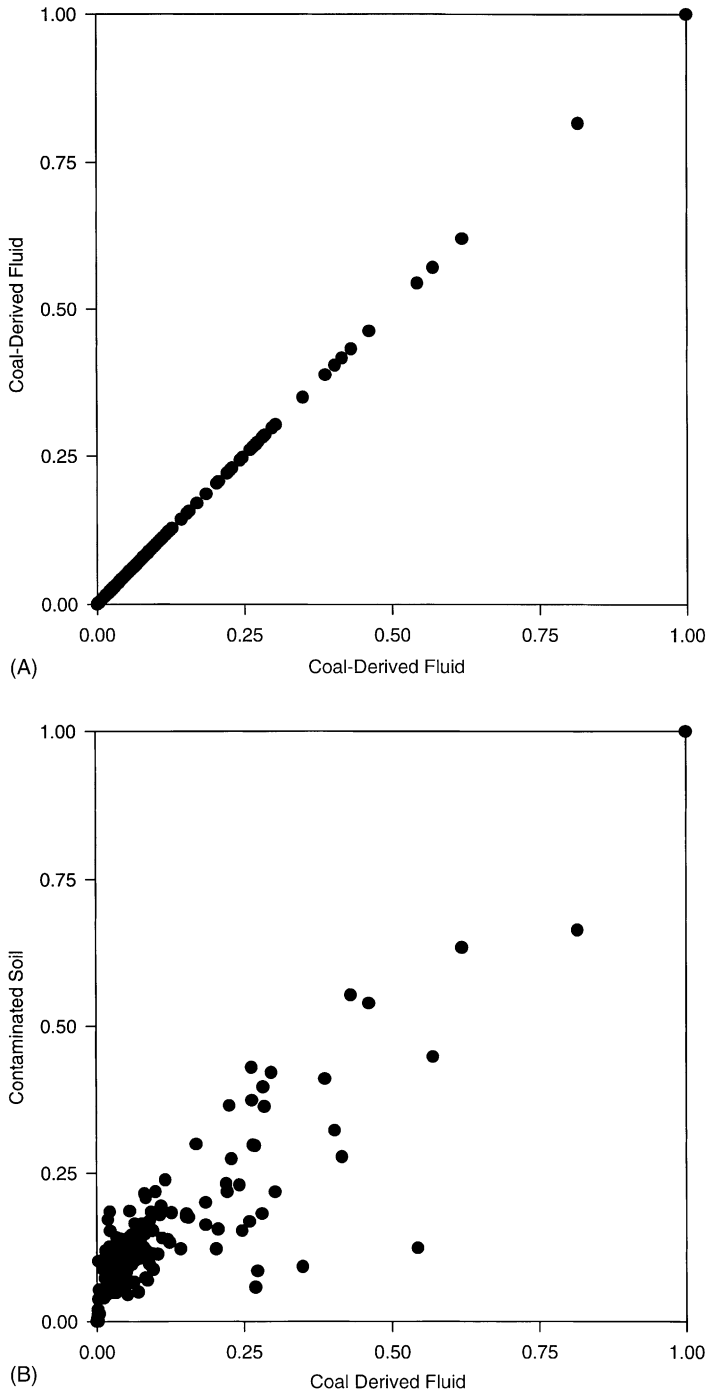

Fig. 5. Scatter plots demonstrating three differing degrees of product moment correlation. (A) Coal-derived fluid (Fig. 3A) vs. coal-derived fluid (Fig. 3A), $r = 1.000$, $P = 0.00 \times 10^{-4930}$; (B) contaminated soil (Fig. 4A) vs. coal-derived fluid (Fig. 3A), $r = 0.877$, $P = 2.83 \times 10^{-113}$; (C) coal-derived fluid (Fig. 3A) vs. standard (Fig. 2A), $r = 0.120$, $P = 0.0241$.

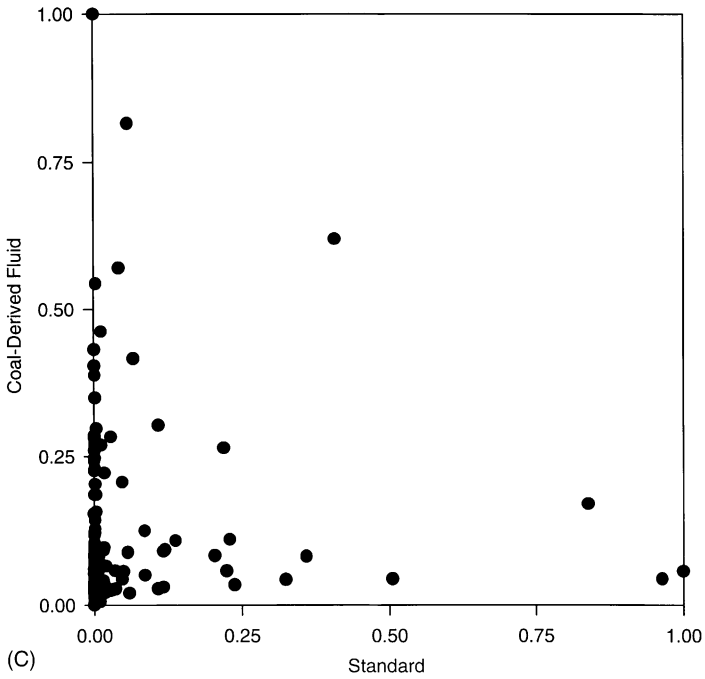

Fig. 5. (*Continued*).

This approach for PAH profiling becomes even more versatile and powerful when combined with selective fluorescence quenching. The results of the product moment correlation for the three samples with fluorescence quenching by nitromethane are summarized in Table 3. As the alternant PAHs are selectively quenched, this correlation discriminates on the basis of the distribution of nonalternant PAHs in the samples. When viewed on this basis, the standard is still distinctly different from the coal-derived fluid or contaminated soil. However, because of their common combustion-related origin, the coal-derived fluid and contaminated soil are highly correlated in nonalternant character ($r = 0.977$). The results of

Table 2
Correlation coefficient (r) of the product moment method for chromatograms obtained by using laser-induced fluorescence detection

Sample	Standard EPA 610	Coal-derived fluid SRM 1597	Contaminated soil CRM104-100
Standard EPA 610	1.000	0.120	0.214
Coal-derived fluid SRM 1597	0.120	1.000	0.877
Contaminated soil CRM104-100	0.214	0.877	1.000

Table 3
Correlation coefficient (r) of the product moment method for chromatograms obtained by using laser-induced fluorescence detection with quenching by nitromethane

Sample	Standard EPA 610	Coal-derived fluid SRM 1597	Contaminated soil CRM104-100
Standard EPA 610	1.000	0.111	0.144
Coal-derived fluid SRM 1597	0.111	1.000	0.977
Contaminated soil CRM104-100	0.144	0.977	1.000

Table 4
Correlation coefficient (r) of the product moment method for chromatograms obtained by using laser-induced fluorescence detection with quenching by dusopropylanune

Sample	Standard EPA 610	Coal-derived fluid SRM 1597	Contaminated soil CRM104-100
Standard EPA 610	1.000	0.104	0.146
Coal-derived fluid SRM 1597	0.104	1.000	0.884
Contaminated soil CRM104-100	0.146	0.884	1.000

the product moment correlation with fluorescence quenching by diisopropylamine are summarized in Table 4. These results confirm that the dissimilarities between the coal-derived fluid and the contaminated soil lie in the distribution of alternant PAH isomers ($r = 0.884$). This approach has been successfully applied to a variety of other petroleum-based samples, including gasoline, motor oils, petrolatum jellies, etc.

4. Conclusions

In summary, fluorescence and selective fluorescence quenching appear to provide complementary information for profiling PAHs in complex samples. The single wavelength or total fluorescence emission offers broad-based information about the PAH distribution. In contrast, fluorescence quenching by nitromethane allows selective discrimination of the nonalternant PAHs and quenching by diisopropylamine allows selective discrimination of the alternant PAHs. Only when all of these profiles indicate a high degree of correlation can it be confidently concluded that two environmental samples are of the same origin.

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