

# Fluorescent Polycyclic Aromatic Hydrocarbons as Probes for Studying the Impact of Colloids on Pollutant Transport in Groundwater

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■ A fluorescence-quenching method was developed to assess the hydrophobic organic pollutant binding potential of organic colloids (OC) in unaltered natural waters. This method allows (1) direct assessment of the importance of OC-enhanced pollutant transport for environmental samples under in situ water chemistry conditions, without requiring the isolation of OC or separation of equilibrated phases; (2) testing of chemicals that suffer substantial wall losses from aqueous solutions; and (3) examination of unstable water samples such as anoxic samples. Our experiments show that some OC (Aldrich humic acids) fully quench OC-associated perylene fluorescence, but others (bovine serum albumin) do not. This implies that fluorescence-quenching results collected for a single [OC] or over a limited [OC] range provide only a lower limit estimate of the potential for OC association. Tests with groundwater, recharged with secondarily treated sewage and containing OC at  $\sim 1$  mg of C/L, showed temporal variation in the ability of OC present to quench or bind perylene.

## Introduction

Organic colloids or inorganic colloids with organic coatings (both referred to herein as OC) may enhance the transport of hydrophobic organic pollutants in groundwater (1-4). For such facilitated transport to be significant, not only must OC be present and mobile in the subsurface, but they must also be sufficiently capable of sorbing hydrophobic organic contaminants to augment the mobile load. Literature evidence suggests OC exist in uncontaminated groundwaters at approximately ppm levels (5-8) and in contaminated groundwaters at levels up to hundreds of ppm (9, 10). Some examples suggest that at least a portion of this OC may be mobile in groundwater. Reinhard (9) reported milligram per liter quantities of OC (MW 500-10 000) in groundwater, necessarily derived from a wastewater reclamation facility 140 m upgradient, as these macromolecules were chlorinated. Robertson et al. (10) detected up to 780 mg/L tannin and lignin in groundwater 900 m downgradient of a waste pulp liquor disposal pit, apparently traveling at the same rate as conservative constituents in the contaminant plume.

This paper focuses on the issue of sorption of hydrophobic organic pollutants to OC. For the purposes of this study, OC are operationally defined as organic matter that will not pass through a 500 molecular weight cutoff (MWCO) ultrafiltration membrane. Such matter may include humic and fulvic acids, other macromolecules such as proteins or carbohydrates, microorganisms, or organic coatings on inorganic colloids. The first objective of this study was to develop a nonintrusive method that could be used to determine the extent of sorptive interactions between hydrophobic organic compounds and unaltered groundwater OC. After testing with model OC, the technique was applied to groundwater samples. These were obtained from a site contaminated by the recharge of secondarily treated sewage and known to contain suspended colloids (2). The results allow assessment of the potential impact of colloid-facilitated transport at this site.

## Fluorescence Quenching in Whole Water Samples.

The key to using fluorescence to determine OC-water partitioning behavior, and directly assessing the importance of OC association in natural waters, is the observation that the fluorescence of a polycyclic aromatic hydrocarbon (PAH) is quenched on association with OC (11-14). This allows a distinction between dissolved PAH and OC-sorbed PAH without having to separate equilibrated phases. However, to date fluorescence-quenching studies have been performed after the OC were removed from the soil or natural water of interest. Isolation and concentration steps could change the character of these colloidal sorbents or may collect only a fraction of the OC sorbents present. Further, since pH, ionic strength, or specific cations may affect the magnitude of the observed  $K_{oc}$  (14-17), if the isolated OC are not rediluted with ultrafiltrate with in situ pH and ion conditions, the relevant  $K_{oc}$  value may be incorrectly estimated. To avoid such difficulties, we use a relatively simple adaptation of the fluorescence-quenching method to provide direct assessment of OC-enhanced transport for a given pollutant using whole groundwater samples under in situ conditions.

As derived by Gauthier et al. (12), the ratio of PAH probe fluorescence in the absence of OC quencher,  $F_0$ , (e.g., ultrafiltered groundwater) to that observed in the presence of OC,  $F$ , (e.g., untreated groundwater sample) can be used to determine the  $K_{oc}$  of the PAH probe:

$$\frac{F_0}{F} = \frac{[PAH_T]}{[PAH_d]} = (1 + K_{oc}[OC]) \quad (1)$$

where  $[PAH_T]$  is the total PAH mass per unit volume of sample,  $[PAH_d]$  is the dissolved PAH concentration of the sample, and  $K_{oc}$  is the OC-groundwater partition coefficient for the PAH probe. Gauthier et al. (12) measured the fluorescence ratio as a function of [OC] to determine  $K_{oc}$  as the resulting slope.  $K_{oc}$  can be determined from a single fluorescence ratio if the [OC] of the sample is known (eq 1). The fluorescence ratio provides a direct assessment of OC-enhanced "solubility" even in the absence of [OC] estimates, as the ratio represents the total (OC-associated plus dissolved) mobile load relative to the truly dissolved load. For a given groundwater sample, the OC concentration cannot be adjusted without altering the sample by concentration or dilution. Therefore, to observe a decrease in fluorescence, the PAH probe used must be carefully chosen. If an estimate of the sample's [OC] is available, the proper PAH can be chosen on the basis of the compound's hydrophobicity. For example, to observe a  $[PAH_T]/[PAH_d]$  ratio of at least 2.0, the PAH probe must have a  $K_{oc} \geq [OC]^{-1}$ . If the [OC] is unknown, a suite of PAH probes with a range of tendencies to sorb can be used to maximize the chance of observing quenching between the extremes of fully and insignificantly quenched. If there is no observable difference in PAH fluorescence between the ultrafiltered and unaltered samples ( $F_0/F = 1$ ), significant association with OC is probably not occurring for the probe compound of interest (with the caveat concerning quenching efficiency discussed below); and by implication there would be no association of OC with other

nonpolar pollutants of similar hydrophobicity. Hence the observed fluorescence ratio is a direct indication of the potential importance of colloid-enhanced transport for both the PAH probe under investigation and pollutants of similar hydrophobicity.

**Considerations in Applying Fluorescence Quenching to Unaltered Samples.** Use of this method with unaltered samples complicates the analysis and requires that a few concerns be addressed to obtain valid data. These concerns include (1) the possibility that other types of quenchers exist in the natural sample, (2) the need to account for sorption of the PAH probes to experimental vessels in a rigorous way for samples containing low levels of OC requiring the use of very hydrophobic probes and (3) the possibility that the quenching efficiency of OC for PAH probes may not be 100% for all types of OC capable of binding PAH in natural samples. Provided that only OC are removed by the ultrafilter, other quenchers such as oxygen, aromatic amines, metals, or heavy atoms like  $I^-$  will be accounted for by using the sample ultrafiltrate to obtain  $F_0$ . The other two concerns are discussed below.

**(A) PAH Probe Partitioning to Glass Vessels.** Preliminary experiments indicated that for very hydrophobic probes, such as perylene, substantial wall losses occurred. Gauthier et al. (12) dealt with the possibility of PAH sorption to cuvette walls by preequilibrating cuvettes with aqueous PAH probe solutions. This approach assumes that PAH sorbed to the cuvette wall does not desorb as the dissolved PAH concentration in the cuvette decreases on PAH/OC association. We do not believe this is a good assumption. Sorption to experimental vessel walls can be handled in a more rigorous way by either equilibrium or kinetic methods. Both approaches rely on the assumption that the PAH probe itself, rather than OC-associated probe, is partitioning to the vessel surface.

In the equilibrium approach, the mass balance equation is modified to allow for PAH partitioning to the vessel walls as well as to OC. Defining the equilibrium partition coefficient for the vessel wall as follows

$$K_w = \frac{[\text{PAH-wall}]}{[\text{PAH}_d](\text{SA}/V)} \quad (\text{mL}/\text{cm}^2) \quad (2)$$

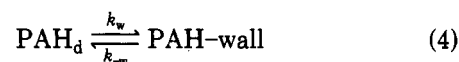
where  $[\text{PAH-wall}]$  is the mass of PAH associated with the wall per unit volume of sample, and  $\text{SA}/V$  is the surface area to volume ratio of the vessel estimated from its geometry, the mass balance equation can be simplified and rearranged to

$$\frac{[\text{PAH}_T]}{[\text{PAH}_d]} = 1 + K_{oc}[\text{OC}] + K_w(\text{SA}/V) \quad (3)$$

$K_w$  can be obtained from a series of fluorescence measurements on wall-equilibrated aliquots of the sample following sequential exposure of the sample to new surfaces. If probe molecules are significantly lost to the vessel walls, the inverse of measured fluorescence, yielding measures of  $1/[\text{PAH}_d]$ , vs cumulative  $\text{SA}/V$  should decrease linearly with slope  $K_w/[\text{PAH}_T]$  and intercept  $(1 + K_{oc}[\text{OC}])/[\text{PAH}_T]$ . Note that  $[\text{PAH}_T]$  cannot be measured directly when wall loss is substantial, but this quantity can be estimated by extrapolation of data from the ultrafiltered sample to  $\text{SA}/V = 0$ . If both the ultrafiltered and unaltered samples are spiked with the same quantity of PAH, then this estimate of  $[\text{PAH}_T]$  is valid for the OC-containing vessel as well. Substituting this value of  $[\text{PAH}_T]$  into the expressions for the slope and the intercept of the unaltered sample yields values for  $K_w$  and  $(1 + K_{oc}[\text{OC}])$ .

An alternative approach is to use observations of the kinetics of wall loss. This approach requires the additional

assumption that the kinetics of PAH partitioning to OC is fast relative to the kinetics of PAH adsorption to the vessel wall. Assume that the sorption process can be represented as



where  $k_w$  and  $k_{-w}$  are first-order forward and backward rate constants for wall adsorption. Fluorescence as a function of time is described by

$$F = \frac{k_{-w}F_0'}{k_w + k_{-w}} + \frac{k_wF_0'}{k_w + k_{-w}}e^{-(k_w + k_{-w})t} \quad (5)$$

where  $F_0' = [\text{PAH}_T] - [\text{PAH-OC}]$  at time zero (18). A nonlinear least-squares curve-fitting program can be used to determine  $k_w$ ,  $k_{-w}$ , and  $F_0'$  from fluorescence measurements obtained as a function of time. The ratio of fluorescence values extrapolated to  $t = 0$  for the ultrafiltered sample relative to that for the unaltered sample allows calculation of the quantity  $K_{oc}[\text{OC}]$ .  $K_w$  can be estimated from the ratio of rate constants and vessel  $\text{SA}/V$ .

**(B) Efficiency of Static Quenching by OC.** The analysis thus far has also relied on the assumption that only the  $[\text{PAH}_d]$  species is quantified by measured fluorescence. This assumption is valid if static quenching of PAH fluorescence by OC results in complete quenching of PAH fluorescence. If this is not the case, the formulation must incorporate the possibility that the OC-associated PAH contributes to the observed fluorescence. Ignoring wall losses for the purposes of this derivation and assuming only one type of OC exists in the sample

$$F = F_0[(\text{fraction dissolved}) + (\text{fraction sorbed})\phi] \quad (6)$$

where  $\phi$  is the fluorescence quantum yield of OC-bound PAH. In this case, the fluorescence ratio reflects

$$\frac{F}{F_0} = \frac{1 + \phi K_{oc}[\text{OC}]}{1 + K_{oc}[\text{OC}]} \quad (7)$$

For groundwaters with a given quantity of OC of unknown composition,  $\phi$  cannot be determined directly. If, however, the groundwater OC are concentrated,  $\phi$  and  $K_{oc}$  can be determined by observing fluorescence as a function of  $[\text{OC}]$ . Unfortunately, it is uncertain how the concentration procedure might affect the sorbent properties of the OC or how estimation of the  $K_{oc}$  under other than *in situ* conditions may affect the magnitude of the product,  $K_{oc}[\text{OC}]$ . We have examined this issue indirectly by using model OC (Aldrich humic acids and bovine serum albumin, BSA), as discussed later.

## Methods

**Apparatus.** Fluorescence measurements were obtained on a Perkin-Elmer LS-5 spectrofluorometer with slit widths set at 3 nm (ex)/10 nm (em). Fluorescence data were collected at the following wavelength pairs (nm) (ex/em): 434/466, 250/350, 340/460, 492/519, and 605/600. These wavelengths monitor perylene, phenanthrene, OC, rhodamine 110, and light scattering, respectively. The wavelength pairs were chosen to maximize each fluorophore signal and minimize background interference.

Absorbance measurements were obtained on a Beckman DU-7 spectrophotometer. Data were collected at the following wavelengths (nm): 519, 492, 466, 460, 434, 350, 340, and 250 to allow for inner-filter-effect correction of fluorescence data.

**Materials. (A) Test Solutions.** Concentrated stock solutions of PAH were made up in methanol. Experimental solutions for fluorescence studies were spiked with 10–25  $\mu\text{L}$  of the stock PAH solution per 20–50 mL of test

**Table I. Groundwater Chemistry**

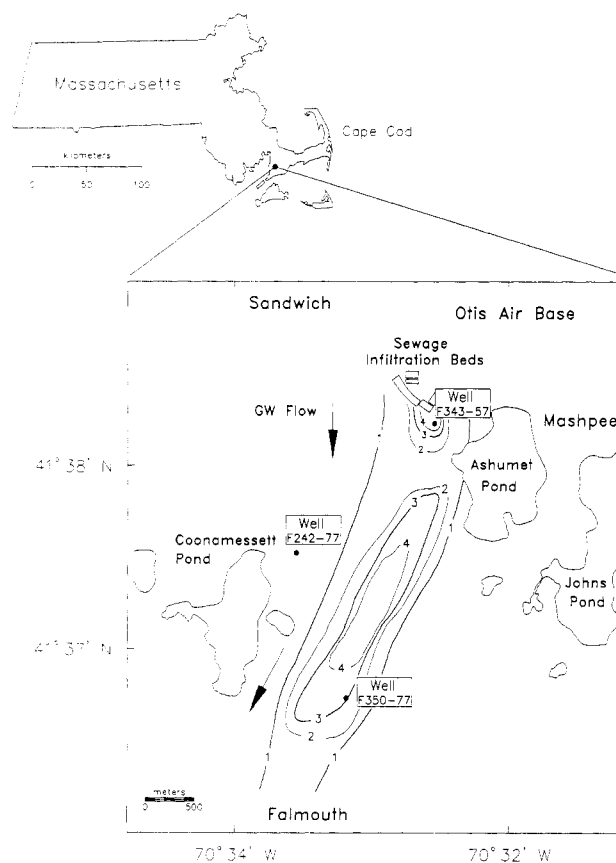
sample	pH	$E_h$ , mV	O <sub>2</sub> , ppm	conductivity, $\mu$ S	[TOC], mg of C/L	[OC], <sup>a</sup> mg of C/L	laser light scattering, counts/s
well F242-77							
Oct 87	5.2	+120	5.0	84	0.8	0.1	120
Apr 88	4.7	+130	5.1	77	1.1	0.2	34
well F350-77							
Oct 87	5.5	+65	0.7	330	2.5	1.4	240
July 88	5.6	+70	1.7	210	2.6	1.3	140
well F343-57							
Apr 88	6.4	-70	0.7	450	2.9	1.1	380
July 88	6.3	-80	1.0	300	3.3	1.5	590

<sup>a</sup> Established as the decrease in TOC after ultrafiltration.

solution. The resulting spiked test solutions contained combinations of the following: 0.3–1  $\mu$ g/L perylene (Aldrich, 99%, Gold Label), 26–65  $\mu$ g/L phenanthrene (Eastman), 10–26  $\mu$ g/L rhodamine 110 (Kodak, Laser-grade), and 200–500 mg/L methanol (EM Science, Omnisolve glass distilled). We do not believe such low levels of methanol ( $5 \times 10^{-3}$  volume fraction) will affect PAH aqueous activities (19–21) enough to cause observable effects on PAH sorption by OC. Note the resulting perylene concentration of some test solutions exceeded the reported solubility of 0.4  $\mu$ g/L (22). Substantial losses to glass walls were expected, so some samples were overspiked to maximize the fluorescent signal remaining after equilibration. For most experiments, perylene, phenanthrene, and rhodamine 110 were spiked concurrently. The fluorescence spectra of these probes do not overlap substantially. Partition coefficients observed for perylene to OC were indistinguishable in the presence and absence of the other two probes. Some test solutions received only methanol. These solutions allowed for background subtraction of fluorescence due to sample components. Rhodamine 110 was included in test solutions to normalize probe responses to account for slight differences in spike level. Rhodamine 110 has a strong fluorescence signal at wavelengths that do not interfere with the PAH used in this study and is soluble enough that sorption to glass walls and OC was not expected.

**(B) Samples.** Initial equilibrium-approach experiments were carried out using distilled water and 5 mg/L Aldrich humic acid solutions (Techgrade, Aldrich, Milwaukee, WI) at pH 4.5. Aldrich humic acids were used as received. Subsequent kinetic experiments were carried out with Norganic water (Millipore Corp., Bedford, MA) at pH 7, and 1.3 mg/L Aldrich humic acid solutions at pH 7. For these experiments Aldrich humic acid was cleaned prior to use by repeated dissolution in base, precipitation in acid, centrifugation, redissolution, and filtration through a 100-nm Nuclepore filter. For experiments involving BSA, distilled water dilutions of a 1 g/L bovine serum albumin stock were used.

Groundwater samples used in experiments were collected from three U.S. Geological Survey monitoring wells located near Otis Air Base, Cape Cod, MA. At this site, recharge of secondarily treated sewage to a sand and gravel aquifer for more than 50 years (23) has created an extensive plume of "dissolved" organic carbon (DOC, Figure 1), a portion of which is expected to be OC (Table I). Well F242-77 is outside the plume, and so relatively low levels of DOC and OC would be expected. Well F343-57 is near the contamination source, where higher levels of OC were anticipated. Well F350-77 is in the middle of a detergent plume. Half of the total DOC at the latter well consists of detergents (24); we expected a portion of the remainder



**Figure 1.** Map showing the location of infiltration beds and monitoring wells sampled. Location of DOC plume contours (in mg of C/L) are from Thurman et al. (24). Arrows indicate the general direction of groundwater flow.

to consist of OC. Groundwater samples were obtained from the three wells with a submersible gear-driven pump (Fultz Pump Inc., Lewistown, PA) at very low flow rates (100 mL/min) to minimize shearing of aquifer material. Samples were pumped to the surface through polypropylene tubing and collected in glass Luerlok syringes or in argon-filled biological oxygen demand (BOD) bottles in argon-filled zipper-lock bags. Samples were stored in the dark at slightly below groundwater temperature. Subsequent sample transfers were made in a glovebox in which commercial grade argon (<2 ppm O<sub>2</sub>) was used to maintain an inert atmosphere. The pH,  $E_h$ , conductivity, dissolved oxygen (DO), and laser light scattering intensity (N4 submicron particle analyzer, Coulter Electronics, Hialeah, FL) of the samples were measured while sampling in the field, on subsampling in the laboratory, and after fluorescence-quenching studies were conducted to determine whether groundwater samples maintained their in

situ conditions through storage and experimentation. These parameters are reported for each well in Table I. For a given sample, these parameters were found to remain constant throughout storage and experimentation.

**(C) Glassware.** Fluorescence-quenching experiments were carried out in 100-mL round-bottom flasks with ground-glass stoppers or 50-mL glass Luerlok syringes. Reverse-phase separation equilibrations were carried out in 20-mL glass syringes. Quartz cuvettes (1 cm × 1 cm × 4 cm) were used for both fluorescence and absorbance measurements. Flasks and cuvettes were cleaned for use by soaking in concentrated chromic/sulfuric acid followed by rinsing with distilled water. Glass syringes were cleaned by soaking in persulfate solution (5 g/L potassium persulfate and 0.5% phosphoric acid in distilled water) at 90–100 °C for at least 1 h. This was followed by rinsing and soaking in distilled water.

**(D) Ultrafiltration.** Groundwater samples were ultrafiltered by using an Amicon ultrafiltration cell with a 76-mm 500 MWCO YCO5 ultrafiltration membrane. Ultrafilters were prepared for use by soaking overnight in Norganic water, rinsing with 100 mL of Norganic water, and then rinsing with 50 mL of the sample. A new filter was used for samples from each well. Roughly 350 kPa argon back-pressure forced the sample through the membrane.

**(E) Organic Carbon Analysis.** OC levels were established as the difference in total organic carbon (TOC) measured in the unaltered groundwater and the organic carbon measured in the ultrafiltered groundwater. Samples were acidified with phosphoric acid and purged of inorganic carbon by a nitrogen stream. Aliquots (50–200  $\mu$ L) of purged samples were injected directly into the high-temperature combustion chambers of an Ionics total carbon analyzer (Watertown, MA). This instrument utilizes a platinum catalyst to facilitate organic matter oxidation at 900 °C and an infrared detector to measure CO<sub>2</sub> produced. Dilutions of a 1000 mg of C/L potassium bipthalate (Mallinckrodt, reagent grade) solution prepared with Norganic water were used to obtain a calibration curve.

**Typical Procedure. (A) Kinetic Approach.** A 50-mL glass syringe containing either a humic acid solution, ultrafiltered humic acid solution, Norganic water, whole groundwater, or ultrafiltered groundwater and a small Teflon stir bar was spiked with 10  $\mu$ L of a methanol solution containing perylene, phenanthrene, and rhodamine 110 or 10  $\mu$ L of methanol. Immediately after spiking, the syringe was placed on a stir plate. Syringes were covered with foil during experiments to minimize photodegradation of PAH, and samples were spiked with sodium azide to minimize biodegradation. The sample was allowed to mix 3 min; then aliquots were withdrawn sequentially in time for fluorescence and absorbance measurements. At each time point, 5 mL of sample was expelled from the syringe for rinsing and filling the cuvette. Four integrated fluorescence readings were immediately recorded for each of the wavelength pairs listed. Readings were recorded first for the least soluble PAH to minimize the effect of losses to the cuvette walls. After all the fluorescence measurements were obtained, the cuvette was transferred to the spectrophotometer for absorbance measurements. Absorbance measurements were used to calculate inner-filter-effect correction factors for fluorescence measurements (12). This correction was largest for fluorescence wavelengths used to monitor phenanthrene, but never exceeded 1.4. Corrected fluorescence measurements were normalized by rhodamine 110 fluorescence measurements for a given

data set to account for slight variation in spiking volume. These data, along with the difference in measured organic carbon concentration in the whole and ultrafiltered samples, allow determination of  $K_{oc}$  values.

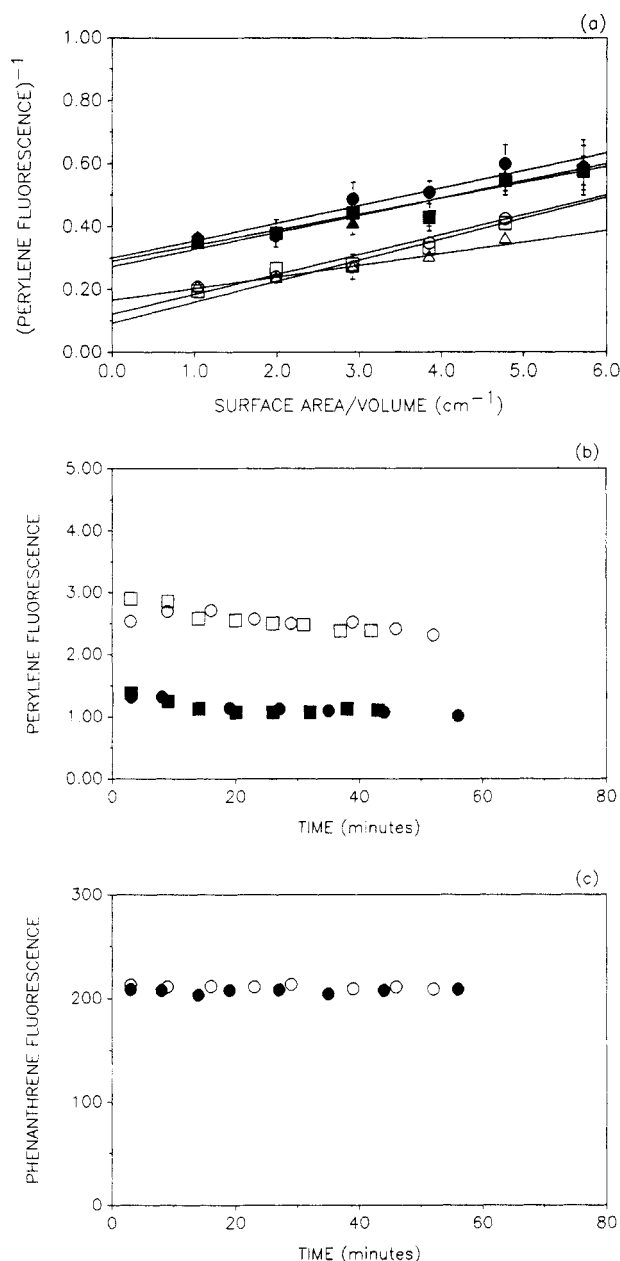
**(B) Equilibrium Approach.** Samples were transferred from BOD bottles to 100-mL flasks and spiked under an argon atmosphere in a glovebox. These flasks were then incubated in the dark at 22 °C for 3 days. Preliminary experiments indicated that PAH sorptive equilibrium between flask walls and sample was reached in less than 3 days (18). Following this equilibration period, an aliquot of the sample was removed from the flask in the glovebox. A portion of this subsample was used to rinse the pipet and cuvette, and then the remainder was transferred to the cuvette for measurement of fluorescence and absorbance. The remainder of the sample in the 100-mL flask was transferred to a clean flask. The sample was then allowed to reequilibrate with this fresh glass surface for 3 days. This sequence was repeated at least four times to obtain fluorescence observations as function of cumulative SA/V.

**(C) Reverse-Phase Separation.** For comparative purposes partition coefficients were determined by the method of Landrum et al. (25). The basis for the method is that dissolved PAH are retained by a C<sub>18</sub> cartridge while OC (humic materials in particular) and associated PAH pass through the cartridge. Glass syringes (20 or 50 mL) were filled with Aldrich humic acid or BSA solutions, distilled water, or groundwater samples. Syringes were spiked with 10–25  $\mu$ L of the PAH spiking solutions or methanol (for determination of background fluorescence) and 10  $\mu$ L of saturated sodium azide solution. Syringes containing distilled water were used to ensure PAH were retained by the cartridge in the absence of OC. Samples in syringes were equilibrated with PAH spikes in the dark with a stir bar on a stir plate for several hours or without a stir bar for at least 18 h. An 8-mL aliquot was then taken to obtain a measure of total PAH (dissolved + OC-associated). The sample was then passed through the C<sub>18</sub> cartridge at a flow rate of 12 mL/min (Maxi Clean cartridge, Alltech Associates, cleaned with 10 mL of methanol and at least 30 mL of distilled water before use) and an 8-mL aliquot of the effluent was taken to determine OC-associated PAH content. These aliquots were extracted with 4 mL of hexane (EM Science, Omnisolve, glass distilled) and analyzed by fluorometry. The dissolved PAH content was determined by difference. The  $K_{oc}$  (mL/g of C) was calculated as  $[PAH-OC]/[PAH_d][OC]$ .

## Results and Discussion

**Method Verification Using Model OC.** Aldrich humic acids and bovine serum albumin were used as model OC to test the fluorescence-quenching method, verifying assumptions inherent in the derivation, as well as to allow comparison of partitioning data obtained by fluorescence quenching with results obtained by more traditional methods.

**(A) Perylene/Aldrich Humic Acids: Equilibrium Approach.** According to eq 3, the reciprocal of observed perylene fluorescence obtained via the equilibrium approach should vary directly with the vessel SA/V ratio. This is observed for perylene fluorescence in the presence of 1.4 mg of C/L Aldrich humic acids as well as for distilled water samples (Figure 2a). The fluorescence measurements obtained in the presence of Aldrich humic acids lie on a distinctly different line than those obtained for the distilled water sample, indicating fluorescence quenching is occurring due to association of perylene with the humic acids. The average  $K_w$  obtained from the slopes of the



**Figure 2.** Partitioning data for Aldrich humic acids. (a) The flask-wall equilibrium approach: perylene. Solid symbols indicate replicate Aldrich humic acid solutions, 1.4 mg of C/L; open symbols indicate replicate distilled water samples. (b) The kinetic approach: perylene. Solid symbols indicate 0.47 mg of C/L clean Aldrich humic acid solutions: (■) spiked with perylene/phenanthrene/rhodamine, (●) spiked with perylene only. (○) Norganic water spiked with perylene, (□) ultrafiltered humic acid solution spiked with perylene/phenanthrene/rhodamine. (c) The kinetic approach: phenanthrene. (●) 0.47 mg of C/L clean and filtered humic acids, (○) ultrafiltered humic acid solution.

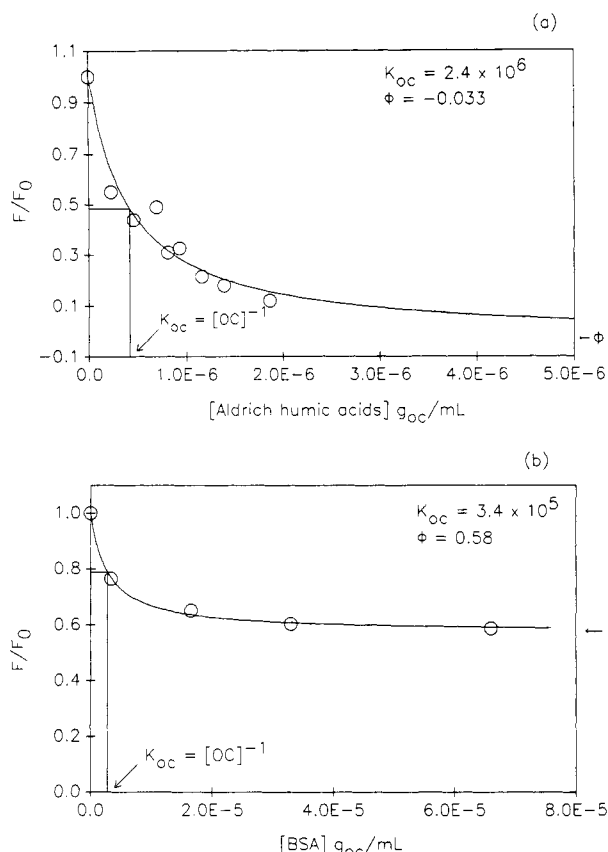
replicate Aldrich humic acid samples ( $K_w = 0.36 \text{ mL/cm}^2$ ) is virtually identical with that obtained for the replicate distilled water samples ( $K_w = 0.39 \text{ mL/cm}^2$ ). This supports the assumption that the PAH probe is partitioning directly to the glass surface. The Aldrich humic acid OC show no tendency to associate with the glass surface. Further substantiation of this assumption is provided by Backhus (18).

**(B) Perylene/Aldrich Humic Acids: Kinetic Approach.** When the kinetic approach is used, perylene fluorescence measurements for both the OC-void and humic acid samples show an exponential decrease in time, asymptotically approaching the solution-glass surface

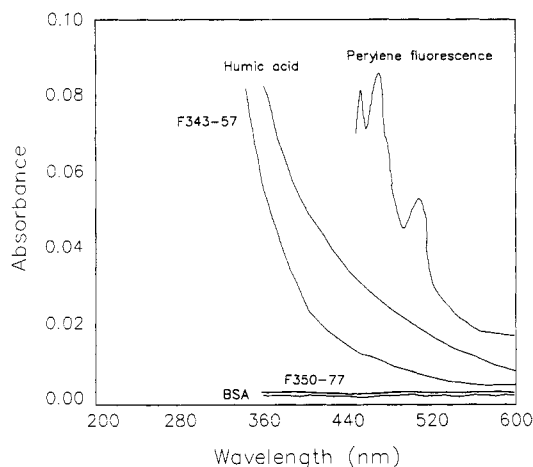
equilibrium value (Figure 2b). The fluorescence measurements obtained for the OC-void and humic acid samples lie on distinct curves, indicating quenching occurs in the presence of OC. The similarity of the average  $K_w$  values for replicates calculated from the ratio of the rate constants obtained from the curve-fitting program for the distilled water samples ( $K_w = 0.35 \text{ mL/cm}^2$ ) and humic acid samples ( $K_w = 0.33 \text{ mL/cm}^2$ ) supports both the assumptions that OC is not partitioning to the glass surface and that PAH-OC partitioning is fast relative to PAH-wall partitioning. Further evidence substantiating these assumptions is provided by Backhus (18).

**(C) Phenanthrene/Aldrich Humic Acids.** Current concepts of hydrophobic organic compound partitioning to OC lead to the prediction that partitioning behavior is proportional to the hydrophobicity of the organic compound, for example, as reflected by  $K_{ow}$ . To test this assumption, phenanthrene was included as a second probe in some samples. At low OC levels (0.47 mg of C/L) the phenanthrene fluorescence measured for the Aldrich humic acid samples is virtually identical with that measured for the OC-void samples (Figure 2c), indicating that phenanthrene, unlike the more hydrophobic perylene, shows little if any association to OC. This result could be predicted from the expected ratio of dissolved to total phenanthrene of 0.996 calculated from the  $K_{oc}$  of  $8.3 \times 10^3 \text{ mL/g of C}$  (25). Not only does phenanthrene show less tendency to associate with OC, but the fluorescence readings in Figure 2c show no significant decrease with time, suggesting no interactions with glass surfaces. This allows the use of phenanthrene to further verify our assumptions regarding the absence of dynamic quenching and losses due to volatilization or biodegradation.

**(D) Fluorescence-Quenching Efficiency.** One final assumption inherent in the derivation of both the equilibrium and kinetic approaches is that the probe fluorescence is totally quenched on association with OC, i.e.,  $\phi \approx 0$ . This assumption was examined by measuring probe fluorescence at various model OC concentrations. The data were analyzed according to eq 7 after accounting for wall losses. The curve should asymptotically approach  $\phi$  as  $[\text{OC}] \Rightarrow \infty$ . The  $K_{oc}$  estimate from these data can be deduced from the curve as the inverse of the  $[\text{OC}]$  where  $F/F_0 = (1 + \phi)/2$ . Results for perylene with clean and filtered Aldrich humic acids and with BSA are shown in Figure 3a and b. For the Aldrich humic acids, the curve asymptotically approaches a  $\phi$  value of  $-0.033$ , which is not significantly different from zero; the fit  $K_{oc}$  is  $2.4 \times 10^6$ . This  $K_{oc}$  is similar to the  $K_{oc}$  value obtained by the kinetic approach for clean unfiltered Aldrich humic acids at a single  $[\text{OC}]$  (Table II). For BSA, however, the curve asymptotically approaches a  $\phi$  value of 0.58, which is significantly different from zero, and  $K_{oc}$  is found to be  $3.4 \times 10^5$ . This nonzero value of  $\phi$  may reflect the diminished overlap of BSA's allowed electronic transitions (e.g., as exhibited by its shorter wavelength absorption spectrum as compared to Aldrich humic acids, Figure 4) with perylene's fluorescent emission energies. Determining a  $K_{oc}$  value for perylene partitioning to BSA by use of the equilibrium or kinetic approach and a single concentration of  $[\text{BSA}]$  would give too small a result if the nonzero value of  $\phi$  were not taken into account. Similarly, since  $\phi$  may not be zero for all types of OC found in groundwaters,  $K_{oc}[\text{OC}]$  estimates obtained by observing diminished probe fluorescence due to quenching by groundwater OC yield only a lower limit on probe-OC association. If concentration and dilution steps do not affect the OC sorbent properties, groundwater OC could be diluted or concen-



**Figure 3.** Determination of  $\phi$  and  $K_{\infty}$  for model OC. (a) Clean and filtered Aldrich humic acids, (b) BSA; each data point obtained by back-extrapolation to SA = 0 using kinetic approach.



**Figure 4.** Absorbance vs wavelength scans for model OC and groundwaters at 1.5 mg of C/L. Perylene fluorescence emission wavelength scan excitation  $\lambda = 434$ .

trated to a wide range of  $[OC]$  levels to allow direct assessment of  $\phi$  and  $K_{\infty}$ . The results of Gauthier et al. (13) suggest that  $\phi$  values of zero are found for a wide variety of humic and fulvic materials. Caution must be exercised in interpretation of the linearity of Stern-Volmer plots as an indication that  $\phi = 0$ , as deviations from linearity may not be apparent over limited OC ranges.

Static quenching in general can occur if there is significant overlap of the quencher absorbance spectrum and the fluorescent probe emission spectrum (26). Perhaps there is a correlation between degree of overlap and  $\phi$ . There was a greater degree of overlap for perylene fluorescence energies with the absorption spectra of Aldrich humic acids than with BSA (Figure 4). Further work with a greater variety of OC and probes is required to

**Table II.**  $K_{\infty}$ 's<sup>a</sup> ( $\pm$  rel. std. error) Found Using Model OC in This Work and Reported Previously

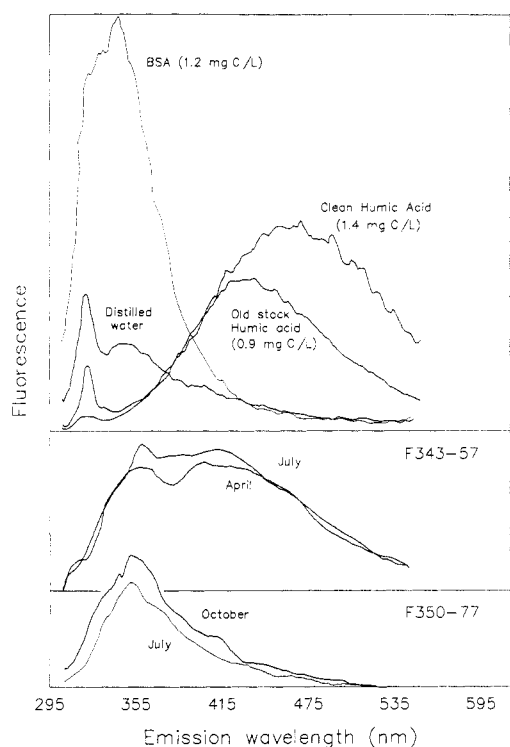
sorbent concn and technique	sorbate		
	phenanthrene	perylene	benzo[a]pyrene
Aldrich humic acid as received			
1.4 mg of C/L by equilibrium approach		$8.8 \times 10^5$ ( $\pm 55\%$ )	
Aldrich humic acid after cleanup			
0.47 mg of C/L by kinetic approach	$<6 \times 10^4$ <sup>b</sup>	$1.7 \times 10^6$ ( $\pm 8.2\%$ )	
0-1.9 mg of C/L by kinetic approach		$2.4 \times 10^6$ ( $\pm 32\%$ )	
1.15 mg of C/L by reverse-phase approach		$8 \times 10^5$ ( $\pm 4.3\%$ )	
11.5 mg of C/L by reverse-phase approach	$9.8 \times 10^3$ ( $\pm 1.4\%$ )		
1-16 mg of C/L by reverse-phase approach (25)	$8.3 \times 10^3$		$8.9 \times 10^5$
0.7-40 mg of C/L by dialysis approach (11)			$1.6 \times 10^6$
bovine serum albumin			
0-66 mg of C/L by kinetic approach		$3.4 \times 10^5$ ( $\pm 11\%$ )	
2.8 mg of C/L by reverse-phase approach		$1.4 \times 10^4$ ( $\pm 50\%$ )	
$K_{ow}$ (27)	$3.7 \times 10^4$	$3.2 \times 10^6$	$9.6 \times 10^5$

<sup>a</sup> Numbers in parentheses,  $\pm$  relative standard error. <sup>b</sup> Indicates no quenching observed, and calculated upper limit  $K_{\infty}$  value based on the  $[OC]$  present and the precision of the fluorescence measurements (generally  $<3\%$  for phenanthrene estimates and perylene reverse-phase estimates) or the standard deviation of the back-extrapolated fluorescence estimates for perylene (generally  $<6\%$ ).

substantiate such a correlation.

**(E) Summary of  $K_{\infty}$  Results and Comparisons with Other Investigators.** After accounting for wall effects and determining that  $\phi = 0$  for Aldrich humic acids,  $K_{\infty}$  values can be calculated from the data presented in Figure 2. These  $K_{\infty}$  estimates for perylene and upper estimates for phenanthrene (calculated from our fluorescence detection precision and observed  $[OC]$  since no quenching was observed) are reported in Table II. The standard deviations reported are based on variability of replicates. Both the kinetic and equilibrium approaches provide reproducible  $K_{\infty}$  estimates (Table II and Figure 2a and b). Concurrent spiking of perylene, phenanthrene, and rhodamine did not affect the estimated perylene-OC partitioning constants (Figure 2b). Since fluorescence measurements for perylene in ultrafiltered humic acid solutions are the same as those obtained in Norganic water, either insignificant quantities of quenchers other than OC are present in the OC-containing samples or ultrafiltration removes only OC from the Aldrich humic acid solutions. We believe the difference in  $K_{\infty}$  found for cleaned versus used-as-received Aldrich humic acids is due to real differences in the mixture of OC in these solutions, as indicated by the shift in maximum emission wavelength (Figure 5). Further discussion of the differences in humic acid solutions is provided by Backhus (18).

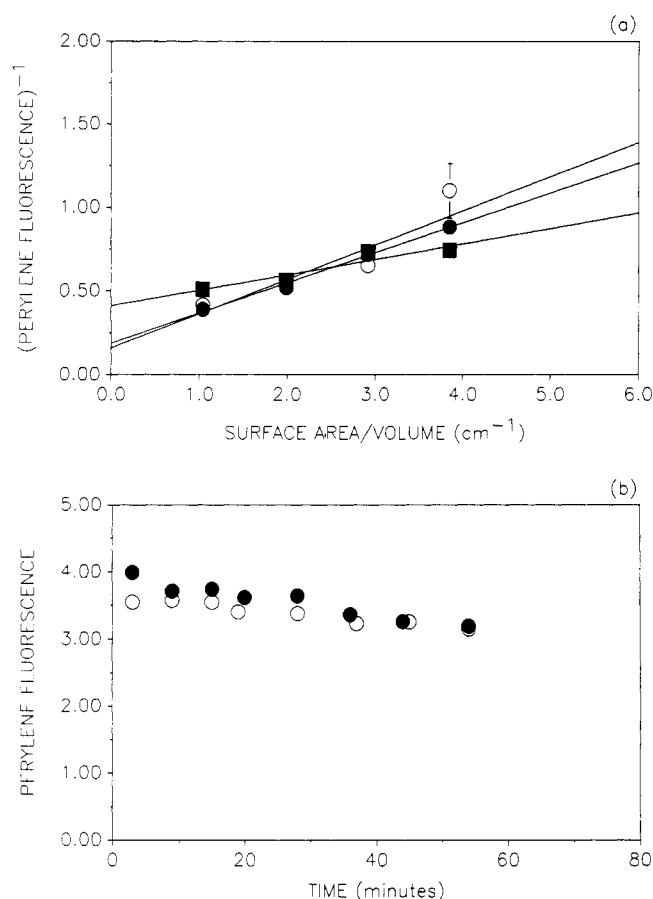
A comparison of these  $K_{\infty}$  values obtained by fluorescence quenching with  $K_{\infty}$  obtained by other techniques provides further verification of our method.  $K_{\infty}$  values for perylene and phenanthrene with Aldrich humic acids were measured by a reverse-phase separation technique (Table II). The  $K_{\infty}$  value for perylene with clean Aldrich humic acids measured by the reverse-phase technique is only  $1/2$  to  $1/3$  that obtained by the fluorescence-quenching method. Invalid assumptions in the fluorescence-quenching method should lead to underestimation rather than overestimation of the  $K_{\infty}$  value; problems involved in the separation of equilibrated phases using reverse-phase cartridges may



**Figure 5.** Fluorescence emission wavelength scans (excitation wavelength  $\lambda = 290$ ) for model OC and groundwater OC. Note groundwater scans represent the difference between the whole and ultrafiltered scans.

account for the observed discrepancy in  $K_{oc}$  values. Though no data exist in the literature regarding perylene partitioning to Aldrich humic acids, comparison of our results with those obtained by others for benzo[a]pyrene (B[a]P), a five-ring PAH of similar hydrophobicity (but markedly greater carcinogenicity), is instructive. The  $K_{oc}$  values obtained for perylene by the fluorescence-quenching method are in the same range as those obtained by others for B[a]P (Table II). The  $K_{oc}$  values obtained by the reverse-phase method for phenanthrene are consistent with the upper limit  $K_{oc}$  estimated by considering the precision of the fluorescence-quenching method and match the  $K_{oc}$  value obtained by Landrum et al. (25) using a reverse-phase method. Thus, the fluorescence-quenching method appears consistent with other approaches.

**Fluorescence Quenching To Quantify the Impact of OC in Groundwater.** The fluorescence-quenching method was applied to several groundwater samples to investigate the potential importance of OC-sorbed species in such waters. Three wells were tested on two separate occasions. The groundwater properties vary substantially between these wells (Table I); most notably, wells F343-57 and F350-77 contain water with  $\sim 10$  times more OC than the background well F242-77. The TOC does not vary so strongly. The groundwater nearest the infiltration beds had the highest pH, the lowest  $E_h$ , and the highest conductivity. Oxygen was present at or below detection limits in groundwater from both wells in the contamination plume, and laser light scattering intensity was greatest near the sewage beds. This level of light scattering, a few times above our background, suggests less than 1 mg/L inorganic colloids, possibly organic coated, was present (based on a polystyrene bead standard). Previous samples recovered from this particular site contained much greater inorganic colloid concentrations (2). Both wells F343-57 and F350-77 showed significant temporal changes in conductivity, further indicating the variable nature of the groundwater plume.

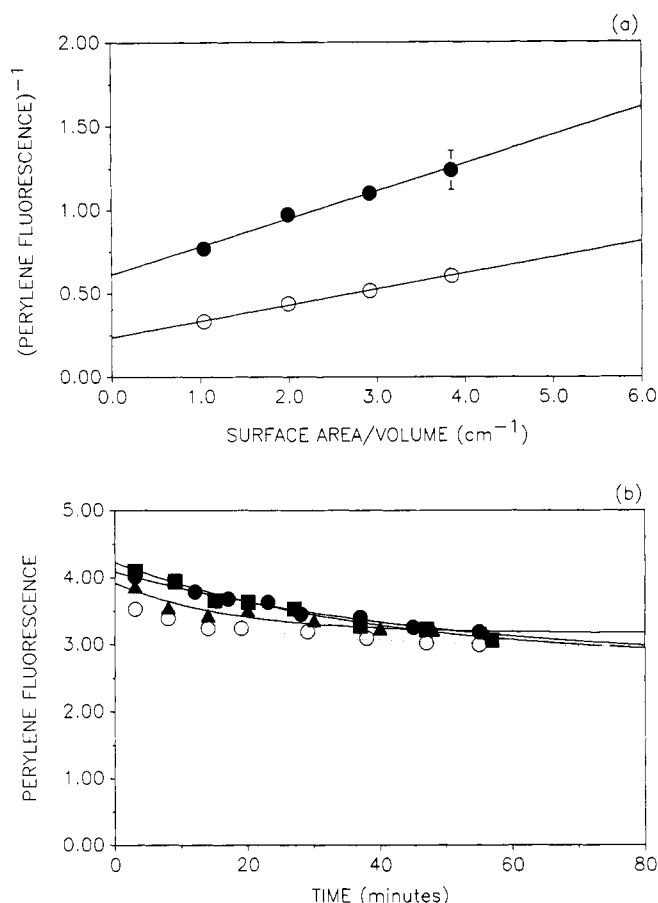


**Figure 6.** Perylene partitioning data for groundwater samples from well F242-77. Solid symbols indicate whole groundwater samples; open symbols indicate ultrafiltered groundwater samples. (a) Equilibrium approach: samples collected in October 1987. (b) Kinetic approach: samples collected in April 1988.

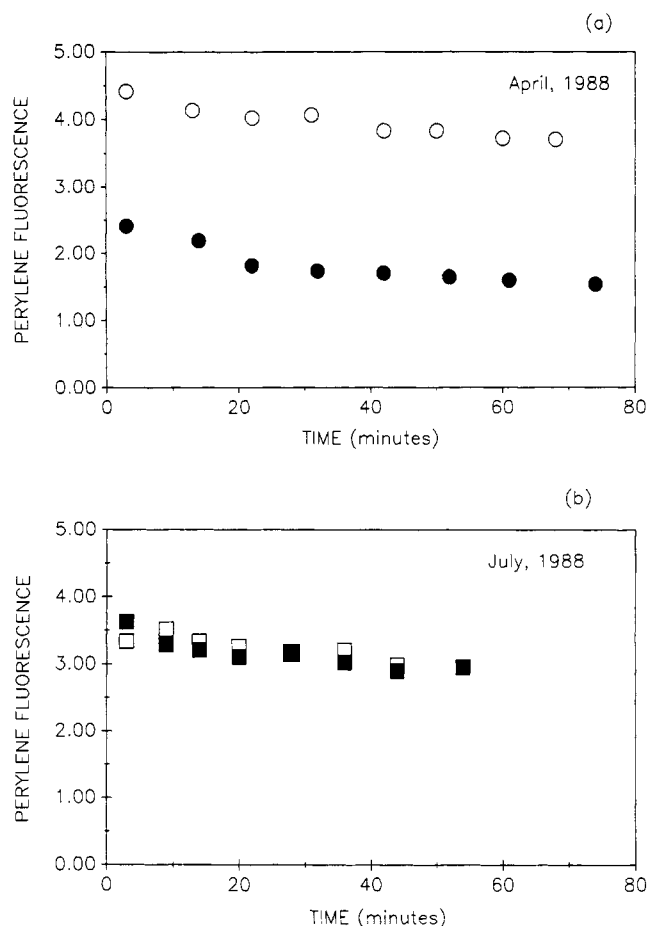
Maintenance of in situ conditions, especially anoxia, was especially challenging for samples from the groundwater contamination plume. Introduction of even slight amounts of oxygen into these samples quickly led to precipitation of iron oxides. By filling 50-mL glass syringes in the field, and using the kinetic approach for determining partitioning, we were able to observe fluorescence quenching while avoiding such water chemistry changes. With the most sensitive samples from well F343-57, the transfer steps involved in the equilibrium approach resulted in precipitation of ferric oxides. Hence, for well F343-57 only data obtained with the kinetic approach are reported here.

Fluorescence-quenching results for perylene, added to the whole and ultrafiltered groundwater from these three wells, are shown in Figures 6–8. We note that perylene fluorescence in the ultrafiltered water (containing between 0.7 and 1.8 mg of C/L residual DOC) never differed from that observed in Norganic water, indicating that this ultrafilterable organic matter did not participate in binding. Thus, calculations using TOC values to deduce  $K_{oc}$  will underestimate the true result. Using the measured OC concentration and assuming  $\phi = 0$ ,  $K_{oc}$  values for these samples can be calculated. No evidence for any association of either perylene or phenanthrene with OC was ever observed for samples obtained from the background well F242-77, either via fluorescence quenching (Figure 6) or via reverse-phase separation (Table III). Similarly, no association of the more water soluble probe, phenanthrene, was observed for any sample obtained from well F343-57 or F350-77 regardless of the method used to quantify partitioning (Table III). The results for the more hydrophobic probe, perylene, for the wells within the contam-





**Figure 7.** Perylene partitioning data for groundwater from well F350-77. Solid symbols indicate whole groundwater samples; open symbols indicate ultrafiltered groundwater samples. (a) Equilibrium approach: sample collected in October 1987. (b) Kinetic approach: sample collected in July 1988.



**Figure 8.** Perylene partitioning data for groundwater from well F343-57: kinetic approach. Solid symbols indicate whole groundwater samples; open symbols indicate ultrafiltered groundwater samples. (a) Sample collected in April 1988; (b) sample collected in July 1988.

**Table III. PAH Partitioning Behavior to Groundwater OC<sup>a</sup>**

sample and method	[OC], mg of C/L	$K_{oc}$ , mL/g of C	
		perylene	phenanthrene
F343-57, Apr 88 kinetic	1.1	$6.7 \times 10^5 \pm 3.6 \times 10^5$	$<1.8 \times 10^5$ *
reverse-phase	1.1	$2.6 \times 10^5 \pm 2 \times 10^4$	$<2 \times 10^3$ *
F343-57, July 88 kinetic	1.5	$<4.1 \times 10^4$ *	$<6.5 \times 10^4$ *
reverse-phase	1.5	$<1.7 \times 10^4$ *	$<1.2 \times 10^4$ *
F350-77, Oct 87 equilibrium	1.4	$1.1 \times 10^6$	
F350-77, July 88 kinetic	1.3	$<4 \times 10^4$ *	$<4 \times 10^4$ *
reverse-phase	1.3	$<1.6 \times 10^4$ *	$<7 \times 10^3$ *
F242-77, Oct 87 equilibrium	0.1	$<5.5 \times 10^6$ *	
F242-77, Apr 88 kinetic	0.2	$<3 \times 10^5$ *	$<8.4 \times 10^4$ *
reverse-phase	0.2	$<1.4 \times 10^5$ *	$<8.1 \times 10^4$ *

<sup>a</sup> Asterisk indicates no quenching observed, and calculated upper limit  $K_{oc}$  value based on the [OC] present and the precision of the fluorescence measurements (generally <3% for phenanthrene estimates and perylene reverse-phase estimates) or the standard deviation of the back-extrapolated fluorescence estimates for perylene (generally ≤6%).

inant plume demonstrate temporal variability. Fluorescence measurements for the October 1987 samples from well F350-77 exhibited perylene quenching by OC (Figure 7a). The April 1988 samples from well F343-57 also demonstrated perylene association with OC both via fluorescence quenching (Figure 8a) and via reverse-phase methods (Table III); the two  $K_{oc}$  estimates agree reasonably well. In contrast, perylene showed no tendency to associate with

OC in the July 1988 samples obtained from either of these wells, when measured by fluorescence quenching (Figures 7b and 8b) or by reverse-phase methods (Table III).

The magnitudes of the computed  $K_{oc}$  values for most cases seem reasonable. The lack of association of phenanthrene or perylene with OC in samples from the background well could have been predicted given the low OC concentrations. Calculations suggest only 5–9% of the perylene and less than 0.2% of the phenanthrene would be associated with OC in these samples (assuming  $K_{oc}$  values of  $5 \times 10^5$  and  $8 \times 10^3$ , respectively). Similarly, phenanthrene is insufficiently hydrophobic to be significantly bound even at the higher OC concentrations found in wells F343-57 and F350-77 (less than 1% association predicted).

For the first set of samples collected from wells F343-57 (April 1988) and F350-77 (October 1987), the perylene  $K_{oc}$  estimates agree with values that might have been predicted. Both proteins and humic materials can be important components of secondary sewage effluent (28, 29), leading us to expect  $K_{oc}$  values somewhere between those measured for the two model OC; the measured groundwater  $K_{oc}$  values are comparable to those measured for BSA and about half that measured for the clean Aldrich humic acids. If, however, the  $\phi$  values for the groundwater OC were greater than zero, the true  $K_{oc}$  values would be greater than those reported in Table III. Using the  $K_{ow}$  value for perylene as an upper estimate of the true  $K_{oc}$  for OC in these groundwaters indicates that the  $\phi = 0$  assumption leads to at most a factor of 3–5 underestimation of the true  $K_{oc}$  value.



It is difficult to interpret the temporal variability in the  $K_{oc}$  values measured for samples from wells F343-57 and F350-77. Conceivably, the OC in the July 1988 samples differed in composition and  $\phi$  values from that previously collected from these wells; for example, they could have been rich in hydrophilic polysaccharides or consisted of quite small macromolecules. Other workers have previously shown how differences in the character of OC, for example C/O ratios or aliphatic/aromatic proportions can significantly affect  $K_{oc}$  (13, 30, 31). We briefly examined this possibility by comparing the fluorescence emission spectra of the July samples with the earlier samples (Figure 5). Both the fluorescent intensities and the overall spectral response did not appear significantly different for the samples collected at any one well, tending to counter the hypothesis that the OC was compositionally different. Another possibility is that the groundwater composition was varying so as to effect differential OC-*perylene* interactions. For both July 1988 samples, the electrical conductivity was lower than previously seen. Although this decrease in salt content would increase the solubility of *perylene* in the water, the Setchenow relationship (e.g., refs 32 and 33) suggests this effect would be quite small. Using a value of the Setchenow constant,  $K_s$ , similar to that reported for other PAH ( $0.4 \text{ M}^{-1}$ ) and converting conductivity measures to ionic strengths ( $10^{-2} \text{ M}$ ) by using Langmuir's equation (34), we find

$$\frac{\text{solubility}_{\text{saltwater 2}}}{\text{solubility}_{\text{saltwater 1}}} = 10^{-(K_s)(\Delta[\text{salt}])} = 10^{-0.004} \approx 0.99 \quad (8)$$

The corresponding change in *perylene*'s aqueous activity coefficient due to these changing concentrations is insufficient to explain the observed lack of OC association. The observed differences in conductivities or specific ion concentrations (14) could also signal a shift in the macromolecular configuration of the OC. Increasing ionic strength has been shown to cause organic polyelectrolytes like humic acids and proteins to take on a more globular configuration (35). Chiou et al. (30) demonstrated that organic macromolecules occurring in a stretched-out configuration (e.g., polyacrylic acid) do not act as sorbents for hydrophobic chemicals. Conceivably, the OC present in the July 1988 groundwaters, although present at roughly the same concentrations and composed of similar structural elements as in the early samples, were simply "denatured" and unable to bind *perylene*. Further work is necessary to substantiate this hypothesis or other explanations.

**Implication to Subsurface Transport.** The quenching results for groundwater obtained within the plume at Otis Air Base suggest that sufficient OC is, at least sometimes, present to augment the mobile load of very hydrophobic chemicals. Assuming that OC moves at the groundwater seepage velocity, and that immobile organic matter has a  $K_{oc}$  value similar to mobile OC, an adjusted retardation coefficient can be calculated for such OC-interactive species:

$$\begin{aligned} \text{retardation factor} &= 1 + \frac{\text{fraction immobilized in soil}}{\text{fraction dissolved} + \text{fraction OC-bound}} \\ &= 1 + \frac{\rho_b f_{oc} K_{oc}}{n + n[\text{OC}]K_{oc}} \quad (9) \end{aligned}$$

where  $\rho_b$  is the soil bulk density,  $f_{oc}$  is the organic carbon content of the soil, and  $n$  is the soil porosity. Obviously, for cases where the product  $[\text{OC}]K_{oc}$  is small compared to 1, that is, when the mobile OC concentration is low or pollutants of concern exhibit low  $K_{oc}$  values, the retarda-

tion expression simplifies to that derived for the two-phase transport case (e.g., ref 36).

The significance of OC association to transport of compounds like *perylene* in the sewage plume at Otis Air Base can be illustrated as follows. Barber et al. (37) found for these aquifer solids,  $n \approx 0.3$ ,  $\rho_b = 1.8 \text{ g/cm}^3$ , and  $f_{oc} \approx 5\%$  fines at 0.1% organic content  $= 5 \times 10^{-5}$ . For *perylene* with  $K_{oc} \approx 7 \times 10^5$  and groundwater  $[\text{OC}] \sim 1 \text{ mg C/L}$ , we find

$$\begin{aligned} \text{retardation factor} &= 1 + \frac{63}{0.3 + 0.2} \\ &\approx 130 \quad (10) \end{aligned}$$

In other words, the presence of OC in the flowing groundwater approximately doubles the mobile load (halves the retardation factor calculated in the absence of OC) of a contaminant with hydrophobicity similar to *perylene*. The presence of OC could be even more important than the retardation coefficient predicts if size exclusion effects allow colloids to travel faster than the seepage velocity (38).

### Conclusion

The fluorescence-quenching method described in this paper provides an alternative method for determination of partition coefficients between OC and fluorescent sorbates in environmental samples, allowing direct assessment of the possibility of OC-enhanced pollutant transport. The advantages of this method are that it requires no separation of equilibrated phases, and partition coefficients can be determined under in situ conditions. The method allows determination of partition coefficients for all OC that quench the fluorescence of PAH probes on association. Partition coefficients and the possibility of enhanced transport for nonfluorescent pollutants can be determined by inference. The main limitation of this method is the assumption that probe fluorescence is completely quenched on association with OC ( $\phi = 0$ ). Bounds can be placed on the true  $K_{oc}$  value by using the probe  $K_{ow}$ , as an upper limit, and the  $K_{oc}$  calculated by assuming  $\phi = 0$ , as the lower bound. Determination of the actual groundwater  $[\text{OC}]K_{oc}$  requires further sample manipulation by dilution or OC concentration to determine  $\phi$ . The limitation of this method imposed by this assumption (factor of 3-5 underestimation of  $K_{oc}$ ) must be weighed against the possible errors in other methods due to OC concentration and separation schemes that may change the nature of the OC or collect only a fraction of the OC present. The fluorescence-quenching method described is useful for screening/determining the  $K_{ow}$  range of contaminants for which OC in the sample will significantly increase the mobile load. The method was used successfully on samples from an aquifer contaminated by recharge of secondarily treated sewage. The data indicate the presence of OC in this case will approximately double the mobile load of hydrophobic pollutants such as *perylene* or B[a]P, but will have little effect on the mobility of less hydrophobic pollutants. The temporal variability observed in PAH-OC association in these groundwaters indicates that, unlike organic contaminant partitioning to soil organic matter, partition coefficients for OC may not be a simple function of OC organic carbon content. Understanding this variability will be necessary to estimate accurately the transport of OC-associated hydrophobic organic pollutants through the subsurface. Additionally, groundwater samples from this site indicate that OC may represent a significant fraction of the TOC in environmental samples (e.g., 40-60% for groundwater within the plume) or a minor fraction (e.g., 10-20% at well F242-77). This indicates that use of TOC in calculation of  $K_{oc}$  values will underestimate

the true  $K_{oc}$ , as ultrafilterable DOC (for the samples in this study) does not quench PAH fluorescence and therefore does not appear to bind.

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**Registry No.** C, 7440-44-0; perylene, 198-55-0; phenanthrene, 85-01-8; benzo[a]pyrene, 50-32-8.

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