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Humic acid complexation of basic and neutral polycyclic aromatic compounds

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Abstract

Complexation by humic acid (HA) of basic (quinoline) and neutral (naphthalene) polycyclic aromatic compounds (PACs) was compared using fluorescence spectroscopy and equilibrium dialysis (ED). These compounds sorb to HA via cation exchange and hydrophobic interactions, respectively. Ionization of quinoline strongly affects its sorption to HA; maximum sorption is observed at pH close to $\log K_b$ (4.92), and competition with H^+ and electrolyte cation (Li^+) is evident. Spectroscopic experiments indicate that quinolinium (QH^+) cation fluorescence is quenched via a static mechanism (i.e., a *dark complex* is formed) when the protonated form is adsorbed via ion exchange to HA. The extent of sorption, calculated from fluorescence data using the Stern–Volmer equation, was compared to independent ED measurements. Although both methods indicated the same trends with solution chemistry, fluorescence quenching data suggested more extensive complexation than that measured using ED. In contrast to ionizable PACs, studied here and previously, interaction of naphthalene with HA is unaffected by changes in solution conditions (pH, ionic strength).

Keywords: Quinoline; Naphthalene; Fluorescence spectroscopy; Dialysis; Complexation

1. Introduction

Polycyclic aromatic compounds (PACs) are widespread and hazardous contaminants produced in large quantities from anthropogenic sources including the combustion of fossil fuels, chemical manufacturing, petroleum refining, metallurgical processes, and some coal, oil shale, and tar sand conversion systems. These compounds exhibit a range in hydrophobicity, polarity and charge that affect the extent and strength of their complexation with humic substances. Since water is a relatively poor solvent for *non-polar* PACs (e.g., naphthalene, anthracene, pyrene), solvation energetics tend to favor humic sorption of such compounds (Gauthier et al., 1987; McBride, 1994). The effect of solution chemistry (e.g., pH, ionic strength (I)), which can influence the extent of sorption by altering compound solubility (Whitehouse, 1984; Brunk et al., 1997) and/or conformation of humic macromolecules (Ghosh and Schnitzer, 1980; Schlautman and Morgan, 1993; Myneni et al., 1999), on PAC–humic interactions is not well documented.

In contrast, humic sorption of *polar* or *ionizable* PACs can involve numerous mechanisms depending on factors such as the functional group chemistry of both PAC and humic substances, and the availability of competing or bridging inorganic ions. For such compounds, PAC–humic complexation may result from ion

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exchange reactions, ion bridging, hydrogen bonds, and charge transfer and covalent bond formation, in addition to hydrophobic interactions. Therefore, changes in solution chemistry can alter the mechanism—as well as the extent—of complex formation. Since many non-polar PACs are degraded to polar and ionizable intermediates, the reactivity and fate of the products are often distinctly different from those of the parent compound. Given the complexity of potential interactions, polar and ionizable PACs clearly merit greater attention.

The major objective of the present study is to directly compare the reactivity towards humic acid (HA) of two contrasting PACs—quinoline (basic) and naphthalene (neutral/non-polar)—under conditions of variable pH and ionic strength. Although several studies pertaining to humic complexation of these PACs exist (Traina et al., 1989; Morra et al., 1990; Chen et al., 1994; Nielsen et al., 1997; Chorover et al., 1999), none of them have employed independent experimental methods as well as consistent solution conditions (sorbate-to-sorbent ratio, *I*) to facilitate direct comparison of results. Our prior studies have shown that quinoline sorbs to HA via cation exchange (Chorover et al., 1999). This paper presents new data with the objective of comparing directly the humic complexation of this compound and its non-polar analog (naphthalene) as affected by changes in solution chemistry.

Our experimental approach employs fluorescence spectroscopy, a simple and non-invasive method for studying PAC sorption to HA (Gauthier et al., 1986; Engebretson and von Wandruska, 1994; Karthikeyan and Chorover, 2000). However, ambiguities exist in resolving the dominant mode of quenching and in quantifying absorption of incident radiation by humic substances (i.e., the "inner-filter" effect (IFE)) (Morra et al., 1990). To avoid misinterpretation of experimental artifacts, we have also measured PAC complexation by HA independently using equilibrium dialysis (ED) (Carter and Suffet, 1982; McCarthy and Jimenez, 1985; Clapp et al., 1997; Chorover et al., 1999; Karthikeyan and Chorover, 2000). Therefore, a secondary objective of this study is to compare these two independent methods, fluorescence quenching (FQ) and ED for measuring PAC–humic complexation.

2. Materials and methods

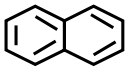
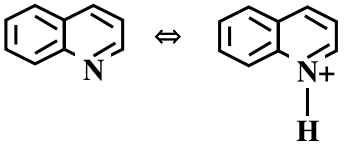
2.1. Humic acid

HA was extracted from the A horizon of a northern hardwood Typic Dystrochrept (forest soil) collected from Nittany Ridge, Centre County, PA. Extraction of the Nittany Ridge HA in NaOH under N_2 (g) and purification in HF/HCl were performed using standard procedures (Swift, 1996). Following acid treatment, HA was dialyzed (SpectraPor 3500 MWCO) against MilliQ H_2O until Cl^- was not detected in the dialysate. Ash content was <2%. The HA was then freeze-dried prior to use. Carbon content of the HA was measured on a Shimadzu TOC 5000A equipped with a solid-sample module and functional group composition of the HA was measured by ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy (CPMAS-NMR) and standard integration methods. These data indicate that the HA carbon is distributed among aliphatic (35%), carbohydrate (12%), aromatic (32%), and carboxylic (21%) functionalities. Carboxylic and phenolic group acidity were 2.84 and 1.36 $mmol_c\,g^{-1}\,C$, respectively, as measured by alkalimetric titration (Bowles et al., 1989). High pressure size exclusion chromatographic analysis of the HA resulted in a bimodal chromatogram. Elution times were calibrated using random coil polystyrene sulfonate standards of known molecular weight (1.4, 4.3, 6.8, 16.8, 32.0, 48.6 and 77.4 kDa, Polymer Standards Service, Inc.) and acetone (Chin et al., 1994). Based on this calibration, the two HA peaks were shown to correspond to weight average molecular weights of 7.1 kDa (86.3% of the integrated chromatogram area) and 61.4 kDa (13.7% of the total area).

2.2. Dialysis experiments

ED was used to quantify sorption of ^{14}C-labeled PACs to HA. Spectra/Por 6 dialysis membrane (1000 MWCO) was washed thoroughly with deionized water and placed in a 250-ml amber glass bottle filled with LiCl/LiOH or LiCl/HCl solution to give equilibrium pH values ranging from pH 2.5 to 11 at 0.001 or 0.01 $mol\,l^{-1}$ ionic strength. The dialysis cell was filled with external solution and a volume of concentrated stock (1.0 $g\,l^{-1}$, pH 7) HA solution was added to provide dissolved organic carbon (DOC) concentrations of 11 or 65 $mg\,l^{-1}$. The cell was then sealed. Stock radiolabeled solution comprising the appropriate PAC, ^{14}C-quinoline (specific activity = 46.5 mCi $mmol^{-1}$, Sigma Chemical) or UL-^{14}C-naphthalene (specific activity = 49.8 $mCi\,mmol^{-1}$, Sigma Chemical) was added to the external solution of each reaction vessel to give a total PAC (quinoline or naphthalene) concentration of 8 $\mu mol\,l^{-1}$ with 13–16% of the total PAC being radiolabeled. Selected physicochemical data of the two PACs are presented in Table 1. Sodium azide (NaN_3, 5 $mg\,l^{-1}$) was added to prevent microbial growth. Replicate experiments without NaN_3 indicated that it had no detectable effect on sorption reactions. Fresh stock solutions were prepared before each experiment. All experimental glassware was autoclaved before use, and solutions were prepared using distilled water that was passed through a MilliQ UV-plus water purification system.

Table 1
Selected physico-chemical properties of quinoline and naphthalene (Zachara et al., 1986; Schwarzenbach et al., 1993)

Compound	Molecular weight ($g\,mol^{-1}$)	Aqueous solubility ($mol\,l^{-1}$)	Octanol–water partition coefficient (K_{ow})	Acid–base equilibrium constant (pK_a)
Naphthalene 	128.2	2.45×10^{-4}	2.29×10^{3}	–
Quinoline 	130.2	4.6×10^{-2}	1×10^{2}	4.92

Humic–PAC suspensions were agitated in a platform orbital shaker in the dark at 65 rpm for 7 d. Concentrations of ^{14}C and respective PAC were determined, respectively, by liquid scintillation counting (LSC, Beckman LS 8100) and high-performance liquid chromatography (HPLC, Waters Inc., with a reverse-phase Beta-basic, Keystone Scientific column followed by photodiode array detection) for both cell interior and exterior solutions within 4 h of terminating an experiment. The HPLC analysis of quinoline employed a mobile phase of 30% (v/v) acetonitrile in a 50 $mmol\,l^{-1}$ triethylamine-PO_4 buffer (pH 3.2) with a run time of 5 min and injection volume of 20 μl. For naphthalene the mobile phase comprised 80% (v/v) methanol. Quinoline and naphthalene were quantified by detection at 306 and 254 nm, respectively. The quantity of ^{14}C label removed from solution after interaction with HA was determined from the difference between inside (free and HA bound) and outside (free) ^{14}C concentrations. A series of controls was established to measure PAC sorption to reaction vessels and membrane surfaces.

2.3. Fluorescence spectroscopy

Variable proportions of LiCl, LiOH, and HCl at the appropriate I (0.001 and 0.01 $mol\,l^{-1}$) were added to 40 ml amber vials to obtain the desired range of final pH values. Sodium azide (NaN_3, 5 $mg\,l^{-1}$) was added to prevent microbial growth. An aliquot of concentrated HA (1.0 $g\,l^{-1}$) was added to yield a final DOC concentration of 11 $mg\,l^{-1}$. Stock solution comprising the appropriate PAC was added to give a total concentration of 2.4 and 8 $\mu mol\,l^{-1}$ of quinoline and naphthalene, respectively. The suspensions were equilibrated in the dark at 25 °C in a platform orbital shaker (65 rpm) for the desired reaction time. To obtain the fluorescence intensity (F_0) of the target compound in the absence of quencher (HA), reaction vessels (PAC blanks) were prepared by following the procedure outlined above except that no HA was added. HA blanks (no PAC) were also prepared. Since the fluorescence intensities of the PACs and HA vary with pH, we ensured that all experimental systems (PAC:HA, PAC blank, HA blank) bracketed the same pH range. Fluorescence measurements were performed using a Photon Technology Inc. spectrometer. Full spectrum experiments were conducted to identify excitation (λ_{ex}) and emission (λ_{em}) wavelengths for each PAC: $\lambda_{ex} = 312$, $\lambda_{em} = 412$ nm for quinoline, and $\lambda_{ex} = 276.2$, $\lambda_{em} = 334$ nm for naphthalene. These values are also consistent with prior fluorescence studies involving these PACs (Traina et al., 1989; Smith et al., 1992).

For the PAC–HA suspensions, fluorescence of HA alone (F_{HA}, measured on HA blanks) was subtracted from the total prior to corrections for IFEs as described in Gauthier et al. (1986). Absorbance measurements for IFE correction were performed using a Shimadzu PC 3101 UV–VIS–NIR spectrometer at the λ_{ex} and λ_{em} values corresponding to each PAC. The IFE correction factors ranged from 1.75 to 1.96 for quinoline–HA suspensions and 2.6 to 2.98 for naphthalene–HA suspensions, which are below the recommended maximum value of 3.0 (Schlautman and Morgan, 1993). Absorption of UV radiation by HA increased with pH and, therefore, the IFE correction factor exhibited a similar trend.

Detailed studies of quinoline fluorescence lifetime and anisotropy were conducted to assess the quenching mechanism. Time-resolved fluorescence-lifetime experiments for the quinoline/HA system were performed using a time-correlated single-photon counting apparatus at 2.4 $\mu mol\,l^{-1}$ quinoline, 0.01 M LiCl, pH 4.5, and HA concentrations from 0 to 25 $mg\,l^{-1}$ C. The laser

system comprised a cavity-dumped dye laser (modified Coherent Model 599) pumped by a mode-locked Nd:YAG laser (Coherent, Antares Model 70). The laser output was converted to 312 nm, the excitation wavelength for quinoline. Emission from the sample was spectrally resolved by a 0.25-m monochromator (ISA H-10) prior to detection with a microchannel plate photomultiplier (Hamamatsu Model 8447F amplifier). The instrumental time response of this system was typically 48–52 ps. More details regarding the instrumentation used for lifetime measurements is provided in Chapman et al. (1990).

Time-resolved anisotropy decay curves were obtained for 2.4 μmol l^{-1} quinoline in the presence and absence of HA at an aqueous concentration of 11 mg l^{-1} C. The parallel and perpendicular components of the emission were selected using rotating polarization filters. All other experimental methodology and instrumental specifications were similar to those described above for the lifetime measurements.

The effects of temperature (T) on FQ were assessed for $T = 5$–50 °C. Suspensions were placed into quartz cuvettes in the sample chamber of a SPEX F212 spectrofluorometer. The cuvettes were temperature equilibrated in a brass water-jacketed cuvette holder. Temperature measurements were made using a thermocouple placed in the cuvette holder. The samples were allowed to equilibrate for 5 min before recording the fluorescence intensity. For IFE corrections, UV absorbance was obtained as a function of temperature using a Hitachi U-3000 spectrometer. The IFE values for the quinoline–HA suspensions were similar between 17 and 50 °C (1.8–1.83), but increased significantly (≈2.3) at 5 °C. For quinoline blanks, IFE values were close to 1 between 17 and 50 °C and 1.21–1.26 at 5 °C. Methods and results of directly comparable fluorescence and dialysis studies with an acidic PAC (1-naphthol) are reported elsewhere (Karthikeyan and Chorover, 2000).

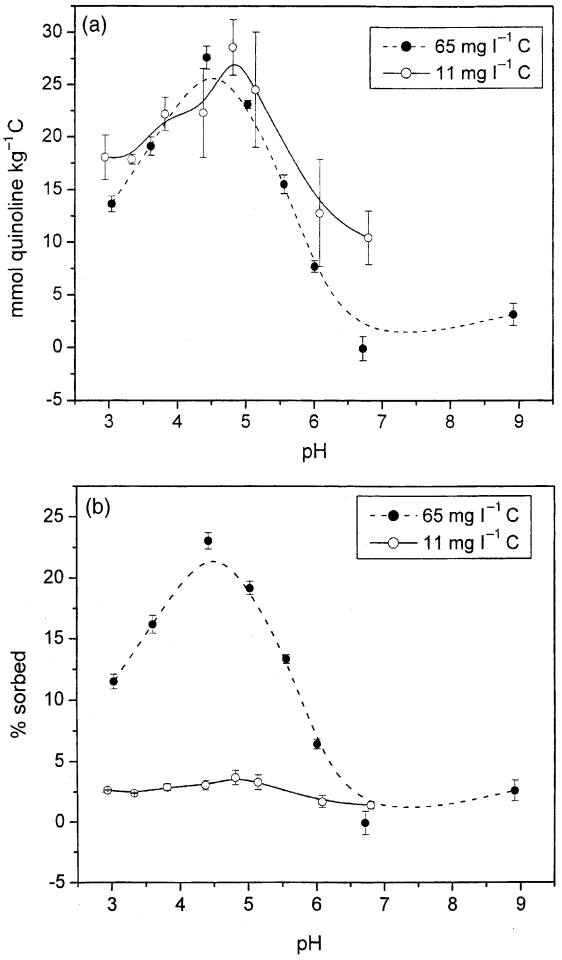


Fig. 1. Sorption of quinoline to HA (measured using ED as the difference between internal and external ^{14}C activities) as a function of pH and HA concentration (quinoline = 8 μmol l^{-1}; $I = 0.01$ mol l^{-1} LiCl). (a) Quinoline sorption per unit mass of organic C. (b) Percent of total quinoline sorbed to HA. The error bars represent standard deviation of duplicate measurements.

3. Results and discussion

3.1. Humic complexation of quinoline

3.1.1. Equilibrium dialysis

Figs. 1 and 2 show the effects of solution chemistry (pH, HA concentration, I) on the complexation of quinoline with HA as quantified by ED. The pH-dependent sorption trends are consistent at different HA concentrations and I, with maximum sorption occurring at pH close to $\log K_b$ of quinoline (4.92). Similar pH-dependent trends have been reported previously for quinoline sorption to kaolinite–HA complexes and HA (Nielsen et al., 1997; Chorover et al., 1999). At pH $< \log K_b$, quinoline sorption increases with increasing pH and decreasing competition with H^+ for charged sites. At pH $> \log K_b$, sorption is reduced with decreasing predominance of the ionized (protonated) fraction of quinoline (the *quinolinium* ion, QH^+). This pH-dependent behavior indicates that the cation (QH^+) is the dominant sorbing species and HA has lower affinity for the neutral form. Although higher *percent* sorption is obtained with increasing HA concentration (Fig. 1b), the amount sorbed is similar when the results are normalized on the basis of sorbent mass (Fig. 1a). Competition with background electrolyte is evident from the fact that quinoline sorption is doubled with a 10-fold decrease in Li^+ concentration (Fig. 2). Decreasing sorption at pH $> \log K_b$ and competitive effects of Li^+ and H^+ indicate that sorption of quinoline occurs

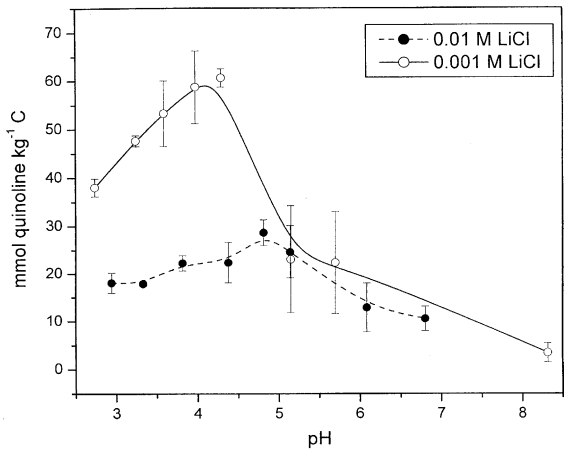


Fig. 2. Sorption of quinoline to HA (measured using ED as the difference between internal and external ^{14}C activities) as a function of pH and ionic strength (quinoline = 8 μmol l^{-1}; HA = 11 mg l^{-1} C). The error bars represent standard deviation of duplicate measurements.

via cation exchange of the QH^+ species (Chorover et al., 1999).

3.1.2. Fluorescence spectroscopy

The fluorescence intensity of quinoline in the absence of HA (F_0) was found to increase in direct proportion to protonation of the heterocyclic *N*. F_0 is plotted as a function of the ionized fraction ($\alpha_0 = [QH^+]/[Q_T]$) in Fig. 3. In the absence of a quencher, quinoline fluorescence is linearly related to the protonated fraction (QH^+) because the neutral form exhibits very weak fluorescence. Conversely, HA fluorescence (F_{HA}) increases with pH and, therefore, F_{HA} dominates the measured values in quinoline–HA suspensions ($F_{quinoline+HA}$) at $\alpha_0 < 0.5$ (pH > log K_b), as shown in Fig. 4. Figs. 3 and 4 indicate that interpretation of fluorescence data for quinoline requires careful background correction for HA and a blank correction to account for the pH-dependence of quinoline fluorescence, especially at pH > log K_b. Such corrections were made systematically in this study by careful use of PAC and HA blanks.

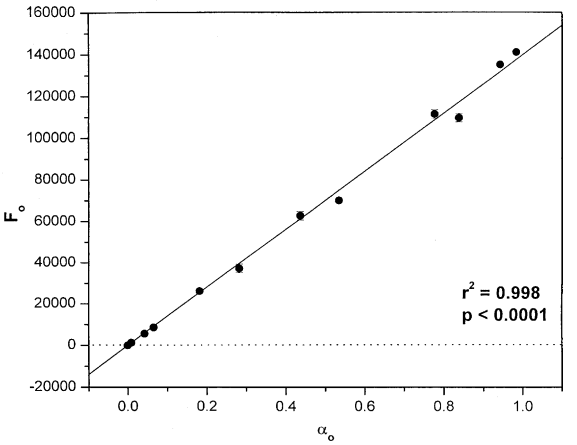


Fig. 3. Fluorescence intensity of quinoline in the absence of HA (F_0) plotted as a function of α_0, ionization fraction of the quinolinium ion, QH^+ (quinoline = 8.0 μmol l^{-1}; $I = 0.01$ M LiCl). The error bars represent standard deviation of quadruplicate measurements.

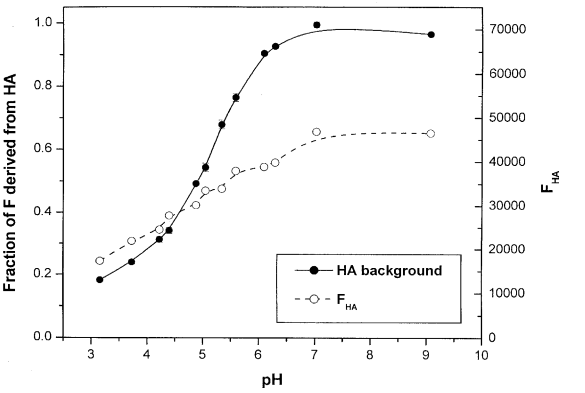


Fig. 4. Fluorescence intensity of HA (F_{HA}) (open symbols) as a function of pH ($I = 0.01$ M LiCl; HA = 11 mg l^{-1} C). Relative contribution of HA to the total fluorescence [$F_{HA}/(F_{quinoline+HA})$] is also provided (solid filled symbols). The error bars represent standard deviation of quadruplicate measurements.

The extent of quinoline complexation by HA can be measured from the ratio of its fluorescence intensity in the absence (F_0) and presence (F) of the quencher (HA). To facilitate direct comparison with macroscopic (ED) results, the Stern–Volmer equation (Lakowicz, 1983) is rearranged to determine the degree of contaminant binding from the quenching ratio (F_0/F). The Stern–Volmer equation is:

$$F_0/F = 1 + K_{SV}[HA] \quad (1)$$

where K_{SV}, the Stern–Volmer constant, is equal to the conditional binding constant for quinoline/HA complexation if a static quenching mechanism is operative. Assuming that the amount of HA consumed in binding quinoline is small ($[HA]_{free} \approx [HA]_{initial}$), then rearrangement of Eq. (1) gives:

$$\text{Humic-bound fraction of quinoline} = [Q_{HA}]/[Q_T] = (1 - F/F_0) \quad (2)$$

where $[Q_{HA}]$ corresponds to humic-bound quinoline and $[Q_T]$ is the total concentration of quinoline.

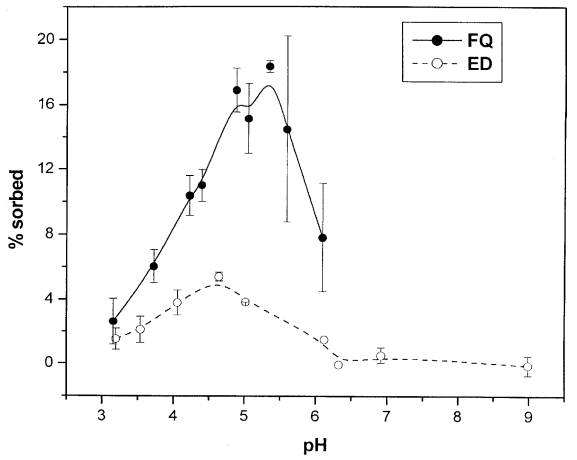


Fig. 5. Percent of total quinoline sorbed to HA as obtained using ED and FQ techniques (quinoline = 2.4 μmol l^{-1}; $I = 0.01$ M LiCl; HA = 11 mg l^{-1} C). The error bars represent standard deviation of duplicate (ED) and quadruplicate (FQ) measurements.

Results from FQ experiments quantified using Eq. (2) are compared with ED data in Fig. 5 (note that the ED data presented in Figs. 1 and 5 correspond to different sorbate-to-sorbent ratios and, therefore, are not directly comparable). Although the results from these independent methods have a similar pH-dependent trend, FQ yields higher sorption values compared to dialysis. In contrast, a similar approach to measuring HA complexation of 1-naphthol resulted in a close agreement between FQ and ED (Karthikeyan and Chorover, 2000). One possible reason for the discrepancy between these two methods in the case of quinoline may relate to the mechanism of fluorescence quenching by HA. The Stern–Volmer equation assumes that static quenching is operative, whereas it does not apply to the case of dynamic quenching where fluorescence is reduced by collisional interactions with the quencher. Static and dynamic quenching mechanisms can be distinguished only with additional measurements of fluorescence lifetime, polarization and the effects of temperature on quenching ratio (von Wandruska and Brantley, 1979; Lakowicz, 1983; Morra et al., 1990; Chen et al., 1994).

Fluorescence lifetime measurements provide the most definitive way of distinguishing between quenching mechanisms. In this method, the lifetime (τ) of a fluorophore is determined from the slope of the fluorescence decay curve measured (at λ_{em}) in the presence and absence of the quencher following a pulse of incident radiation at λ_{ex} (Lakowicz, 1983). With static quenching, the bound PAC does not fluoresce (i.e., a ground state, *dark* complex is formed). Thus, a decrease in fluorescence intensity (F) relative to F_0 is not accompanied by a change in τ because the lifetime of unbound fluorophore is unaffected by the quencher (Lakowicz, 1983). In this case, $\tau_0/\tau = 1$, where τ_0 is lifetime in the absence of quencher. In contrast, dynamic quenching results from collisional interactions with the quencher that occur on the time scale of fluorescence decay and the average value of τ is reduced as a result. Therefore, for purely dynamic quenching, concurrent reduction of both τ and F gives $\tau_0/\tau = F_0/F$. In studies of 1-naphthol binding to HA, Chen et al. (1994) showed that τ was unaffected by quenching and they concluded that a static mechanism was operative. Traina et al. (1989) found that changing the suspension temperature from 1 to 75 °C had no effect on the quenching ratio and, hence, suggested the formation of a ground-state complex between naphthalene and water-soluble organic carbon. However, there have been no prior detailed fluorescence spectroscopy studies on quinoline–HA interactions and so comparable data are lacking.

We measured a fluorescence lifetime of 2450 ps for quinoline in the absence of quencher. The fluorescence decay of HA itself was fit to a function dominated by a very short relaxation time of less than 100 ps, in addition to longer times of 950 and 3700 ps. Since there is some overlap in τ_0 values for HA and quinoline, isolating the effects of HA on τ_0 for quinoline is not possible. However, important information may still be extracted from the decay data. Fig. 6 shows the fluorescence decay curves of quinoline in the (a) absence and (b) presence of HA and (c) HA blank. These lifetime data was analyzed using the Marquart–Levenberg non-linear minimization algorithm described in Grinvald and Steinberg (1974). A χ^2 value of less than 1.2 was obtained in all cases indicating excellent fits to decay curves.

The shapes of the fluorescence decay curves provide indirect evidence for the existence of a static quenching mechanism. Fluorescence decay of quinoline is mono-exponential in nature (i.e., linear in the semi-log format), whereas that of HA is non-exponential (non-linear in the semi-log format). The decay curve for quinoline reacted with HA is almost identical to that of quinoline blank (no HA). If quenching was dynamic, then the fluorescence decay curves would be altered in the presence of quencher due to a change in fluorescence lifetime. The fact that the decay curves are unaffected by HA strongly suggests that bound quinoline is non-fluorescent (i.e., static quenching is operative).

The results from fluorescence polarization experiments, in which the time decay of fluorescence intensity is measured in directions both parallel ($I_\parallel$) and perpendicular ($I_\perp$) to the excitation beam, are shown in Fig. 7. Fluorescence anisotropy is a measure of the difference between $I_\parallel$ and $I_\perp$ (Lakowicz, 1983). Equivalency of decay curves for both parallel and perpendicular excitation directions indicates that quinoline depolarizes isotropically. Furthermore, this behavior is unaffected by the absence (Fig. 7a) or presence (Fig. 7b) of HA

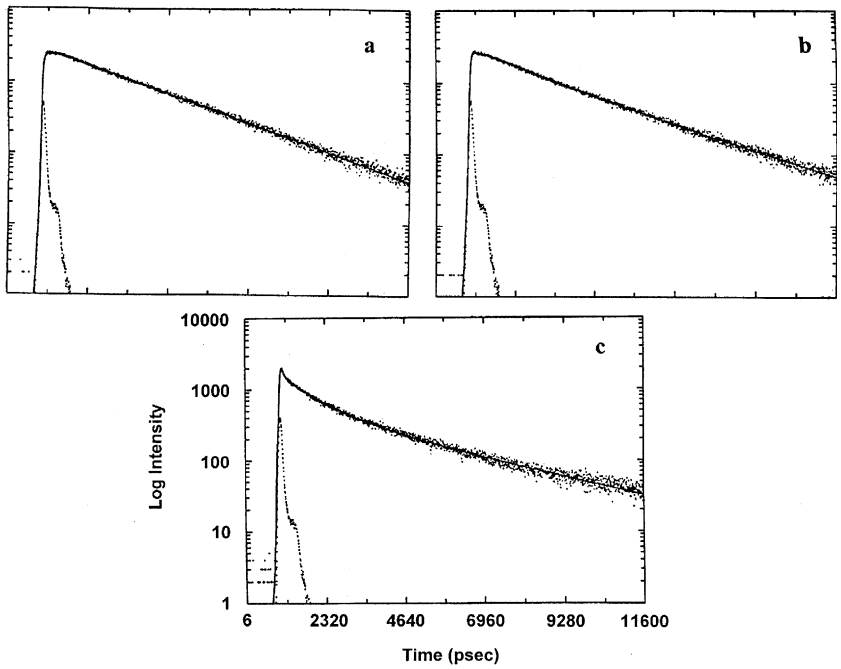


Fig. 6. Time-resolved fluorescence decay curves: (a) quinoline (2.4 μmol l^{-1}; $I = 0.01$ M LiCl; pH 4.5) in the absence of HA. (b) quinoline (2.4 μmol l^{-1}; $I = 0.01$ M LiCl; pH 4.5) in the presence of HA (11 mg l^{-1} C). (c) HA blank (11 mg l^{-1} C; $I = 0.01$ M LiCl; pH 4.5).

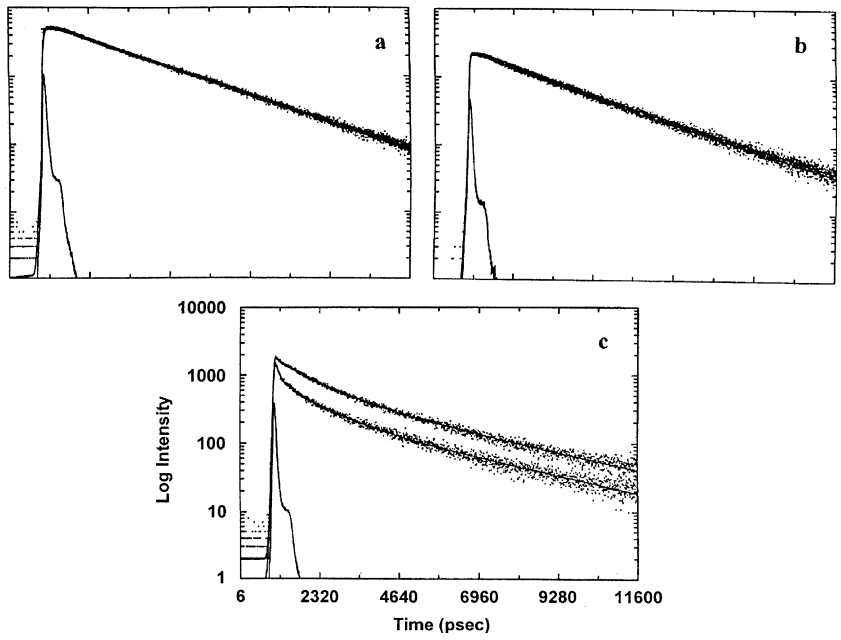


Fig. 7. Time-resolved fluorescence anisotropy curves: (a) quinoline (2.4 μmol l^{-1}; $I = 0.01$ M LiCl; pH 4.5) in the absence of HA. (b) quinoline (2.4 μmol l^{-1}; $I = 0.01$ M LiCl; pH 4.5) in the presence of HA (11 mg l^{-1} C). (c) HA blank (11 mg l^{-1} C; $I = 0.01$ M LiCl; pH 4.5).

quencher. In contrast, HA itself exhibits a measurable anisotropy (Fig. 7c), as indicated by two different decay curves. Given that the fluorescence polarization of quinoline is unaltered by the presence of HA (i.e., Fig. 7a

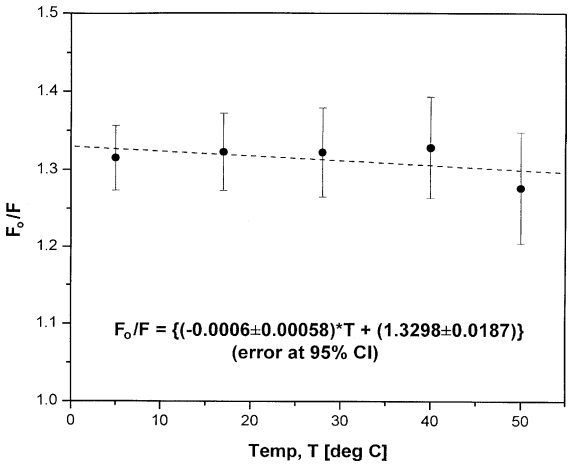


Fig. 8. Effect of temperature (5–50 °C) on the quenching ratio (F_0/F) of quinoline by HA (quinoline = 2.4 μmol l^{-1}; $I = 0.01$ M LiCl; HA = 11 mg l^{-1} C; pH 4.5). The error bars represent standard deviation of triplicate measurements.

and b are identical), these data provide additional evidence that bound quinoline is non-fluorescent.

Further support for a static quenching mechanism derives from the study of temperature (T) effects on the quenching ratio of quinoline. Results show that changing the suspension T from 5 to 50 °C had no significant effect on the efficacy of quenching (Fig. 8). Since dynamic quenching results from collisional interactions between the fluorophore and quencher (Lakowicz, 1983), an increase in T would result in an increase in F_0/F, concurrent with an increased frequency of molecular collisions, if this mode were operative. According to the data in Fig. 8, there is no effect of T on F_0/F. The slope of the regression line is slightly negative and not significantly different from zero.

Multiple lines of evidence from fluorescence spectroscopy experiments (lifetime, polarization and T effects) indicate that quinoline forms a ground-state complex with HA during cation exchange reactions. Therefore, the lack of agreement between FQ and ED results (Fig. 5) cannot be attributed to dynamic quenching. It is more likely to be due to high background contributions from HA, especially at pH > $\log K_b$ (Fig. 4). The IFE correction factors are affected by background humic contributions, which, in turn, will significantly influence the quenching ratio. As the fraction of fluorescence intensity derived from HA $[F_{HA}/F_{quinoline+HA}]$ increases, the difference between FQ and ED results increases (Figs. 4 and 5). For example, at pH values of 4, 4.92 ($\log K_b$) and 6, this fraction (Fig. 4) is 0.28, 0.52, and 0.79, respectively, and the corresponding difference between FQ and ED results (%sorbed$_{FQ}$/%sorbed$_{ED}$ in Fig. 5) is 2.43, 3.6, and 6.17, respectively.

3.2. Humic complexation of naphthalene

ED experiments did not yield reproducible results for naphthalene sorption to HA. With wide mouth amber bottles (250 ml) significant loss of naphthalene by volatilization resulted in ^{14}C recoveries (defined as the ratio of the total (inside + outside) ^{14}C activities after and before the reaction) less than 80%. Recovery was improved by the use of narrow-mouth serum bottles with Teflon-lined rubber septa, but variability in the sorption data persisted. We attribute this variability to (i) small differences between internal and external ^{14}C activities because of low overall naphthalene sorption to HA and (ii) the difficulty in maintaining identical headspace volumes in the dialysis cell and external solutions.

The results of naphthalene binding to HA as obtained using FQ are shown in Fig. 9. The binding constant $[K_{oc} = \{(\text{naphthalene:HA})\}/\{(\text{naphthalene})(\text{HA})\}]$ obtained in this work (Table 2) is intermediate relative to other studies. For quinoline, K_{oc} varies significantly with pH and I ($10^{2.64}$ and $10^{3.92}$ at pH 8.31 and pH 4.3, respectively, at 0.001 M I; $10^{3.11}$ and $10^{3.54}$ at pH 6.8 and pH 4.81, respectively, at 0.01 M I). At the lower ionic strength, the maximum K_{oc} value is almost identical for naphthalene ($10^{3.96}$) and quinoline ($10^{3.92}$), whereas HA exhibits a higher affinity for naphthalene at 0.01 M I ($K_{oc(naphthalene)} = 10^{4.02}$; $K_{oc(quinoline)} = 10^{3.54}$). Unlike the ionizable quinoline, changes in pH and I had a negligible effect on sorption of naphthalene to HA (Fig. 9). Hydrophobicity is considered to be the dominant factor controlling the binding of non-polar PACs, e.g., naphthalene, to HA (Gauthier et al., 1987; Traina et al., 1989). The force driving this interaction is the poor relative compatibility of the PAC for aqueous solvent as compared to HA (Traina et al., 1989). Change in I and

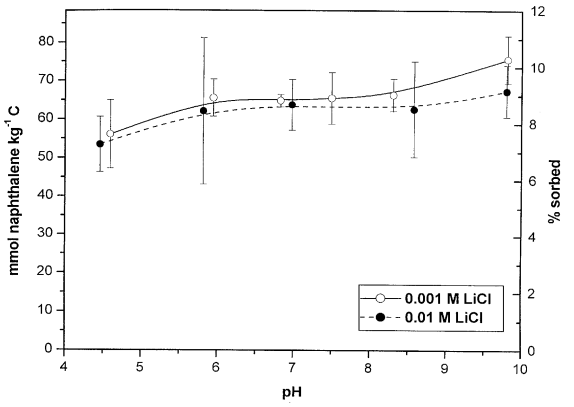


Fig. 9. Fluorescence quenching of naphthalene in the presence of HA as a function of pH and ionic strength (naphthalene = 8 μmol l^{-1}; HA = 11 ppm C). The humic-bound fraction (%) is provided in the right Y-axis as obtained using Eq. (2). The error bars represent standard deviation of triplicate measurements.

Table 2
Binding constants (K_{oc}) for naphthalene sorption to humic substances

Sorbent	Background cation	Method	$\log K_{oc}$	Reference
Soil HA	Li^+	FQ	3.85–4.02	This study
Soil HA	Na^+	FQ	4.92	Morra et al. (1990)
Aldrich HA	Na^+	Dialysis	3.00	McCarthy and Jimenez (1985)
Aldrich HA	Na^+	HPLC	3.74	Nielsen et al. (1997)
WSOC	Na^+	FQ	4.89	Traina et al. (1989)

pH affects the conformation of humic substances, with increasing electrolyte concentration and decreasing pH tending to favor coiling and co-association of macromolecules (Ghosh and Schnitzer, 1980; Myneni et al., 1999). However, a quantifiable effect may require relatively large shifts in solution chemistry (Lead et al., 2000). It appears that the range of solution conditions used in this study (pH: 4.5–10; I: 0.001–0.01 mol l^{-1} LiCl) does not alter HA conformation sufficiently to change the availability of complexation sites or otherwise affect its suitability as a sorbent for naphthalene. Traina et al. (1989) obtained similar results for naphthalene sorption to water-soluble organic carbon; they reported no influence of pH (1.5–7.3) or I (Ca^{2+} 0.05–0.5 M). In the presence of Al^{3+} (0.05 M), however, there was a significant decrease in sorption, caused possibly by conformational changes in humic structure (Traina et al., 1989).

4. Conclusions

Both fluorescence spectroscopy and ED are applicable to study PAC–HA systems, but limitations exist for both methods. The fluorescence characteristics of quinoline impose an ''apparent'' limitation to the solution conditions for which FQ can provide reliable results. Due to the difficulties involved in performing ED experiments with naphthalene, the two methods could not be compared.

Contrasting solution chemistry effects, related to the mechanism of interaction, are observed during the complexation of quinoline and naphthalene to HA. With quinoline, greater retention is obtained under acidic conditions and at lower I, and HA concentration (when results are normalized on ''C'' basis) has no effect on the sorption extent. These results indicate that solution chemistry may bear heavily on the sorption of ionizable PACs through its impact on humic dissociation, sorbate charge and sorptive competition with inorganic ions. In contrast, under the range of experimental conditions used, pH and I had no influence on the retention of naphthalene to HA, which indicates that the factors governing sorption of this neutral PAC were unaffected by dissociation and resulting conformational changes in HA that accompany such changes in solution chemistry.

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