Assessing desorption resistance of PAHs in dissolved humic substances by membrane-based passive samplers

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ABSTRACT

Binding of hydrophobic organic compounds (HOCs) to humic substances (HS) has significant influence on their fate and bioavailability in an aquatic environment, yet very little is known on the desorption behavior of HOCs in dissolved HS. In this study, triolein-embedded cellulose acetate membranes (TECAMs) were used as passive samplers in dissolved HS-containing solutions to extract the free polycyclic aromatic hydrocarbons (PAHs). The partitioning coefficients (K_{OC}) of PAHs in dissolved HS from different sources were determined. Then the rapidly desorbable fractions (f_{rd}) of HOCs in dissolved HS were quantified by a quasi-equilibrium model. Results show that the magnitude of K_{OC} of PAHs correlated strongly with the O/C atomic ratio and molecular weight of HS. The PAHs with lower K_{OW} values desorbed almost reversibly from an aquatic fulvic acid, a sediment humic acid (HA), a lignite HA, and Aldrich HA. However, for strongly hydrophobic chrysene binding to the lignite HA, some extent of desorption resistance was found and attributed to sorption hysteresis as well as steric hindrance. The present study illustrated that the quality of HS and the property of solute may play key roles in determining the desorption reversibility.

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1. Introduction

The degree and the pattern of contamination in the aquatic system depends mainly on the availability of the contaminants to be transferred in and by water. The availability is in large extent determined by the binding–desorption behavior of contaminants in dissolved organic matter (DOM), 50–80% of which is humic substances (HS) [1,2]. Many researchers have focused on HS interactions with hydrophobic organic compounds (HOCs) in the past few decades, considering their widespread existence and strong binding capacity.

Generally, it was widely believed that the partitioning of HOCs between water and dissolved HS was nonspecific and the binding of contaminants was rapidly reversible with characteristic equilibration times of hours to a few days at most [3–6]. However, numerous findings were reported recently that our fundamental understanding of sorption and desorption might be in need of reexamination [7]. For instance, a few studies showed that the HS–water system exhibits a complex behavior and a small fraction of the sorbed contaminant remained unexpectedly in HS, just as often observed in sediment–water systems [8]. In Schlebaum et al.'s study, the results showed that part of pentachlorobenzene sorbed to a dissolved humic acid (HA) resisted desorption. They speculated that the nonlabile fraction was a consequence of a change of conformation of

the HA after the binding of pentachlorobenzene [9]. Several different rate models have been used to describe desorption of HOCs from natural sorbents. Intrinsically, desorption of organic compounds from sediment is generally accepted to be at least biphasic, a rapidly desorbing phase followed by a more slowly desorbing fraction [10]. This biphasic model may correspond to desorption in different humic structures that vary in hydrophobicity or rigidity. Therefore, a biphasic desorption model was proposed in the present study, to give a direct description of irreversible desorption.

As in most cases the division between rapid desorption and resistant desorption is rather arbitrary; batch-mode chemical extraction tests appear not able to mimic resistant desorption. Time-integrated techniques have been used as infinite sink for desorbed compounds in the literature for time frames from a few hours to a few days. Examples of these methods include Tenax-aided desorption and equilibrium dialysis [5,9,11–13]. Tenax extraction has advantages of high sorptive capacity and rapid adsorption from water. However, Tenax could not mimic some aquatic complex processes actually, such as reverse adsorption from water and solute effects on HS, since it keeps aqueous sorbate concentrations very low [14]. In terms of an equilibrium dialysis method, one disadvantage reported was the possible leakage of low molecular weight HS through the dialysis membrane. To overcome the above shortcomings, triolein-embedded cellulose acetate membranes (TECAMs) were used in this study as in situ sink under a quasi-balanced state to concentrate free dissolved solute. The overall objective of this research is to investigate the extent of ra-

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pid desorption of contaminants in dissolved HS. Several types of HS from either commercial sources or natural sources were tested. PAHs were selected as model compounds because of their ubiquitous existence and wide range of hydrophobicity. Furthermore, it was also expected that a relation between HS properties and binding affinity for PAHs would be found.

2. Materials and methods

2.1. Materials

Three deuterated PAH standards, phenanthrene- d_{10} , pyrene- d_{10} , and chrysene- d_{12} , were purchased from Supelco Inc. (Bellefonte, PA). The mixed standard solution was prepared by diluting the original stock solutions with methanol to 20 mg/L. Four kinds of HS obtained from various sources were used in the present study: Aldrich HA (HA sodium salt from Sigma–Aldrich, Steinheim, Germany), Nordic aquatic fulvic acid reference (Nordic FA), and Leonardite HA standard (International Humic Substances Society, Denver, CO, USA), as well as PSHA (a generous gift from professor Pingan Peng, Guangzhou Institute of Geochemistry, isolated from a pond sediment). HS stock solutions were prepared as follows: each HS was dissolved in 0.01 M sodium hydroxide solution (pH 12) separately, and then the solution was stirred for about 24 h and centrifuged at 27,100g average relative centrifugal force (20 °C) for 1 h to remove most of the particles and finally filtered with prerinsed cellulose acetate nitrate membrane filters of 0.45- μ m pore diameter (Whatman, Clifton, NJ) to remove any remaining particulate material. The organic carbon content of the HS stock solution was measured with a total organic carbon analyzer (Phoenix-8000, Tekmar–Dohrmann, Mason, OH). The ultrapure water (OC < 0.1 mg C/L) used in this study was purified by a Milli-Q Gradient system (Millipore, Bedford, MA). Solvent and other chemicals used in the study were of GC or analytical reagent grade. Small pieces of TECAM (4 × 6 cm) were used and the procedure for preparing was the same as described previously [15]. The TECAM consists of two immiscible phases: an outer hydrophilic membrane and inner hydrophobic triolein drops. The image from a scanning electron microscope (SEM) showed that there were many micropores of nanosize on the surface of the outer layer. The porosity of TECAM is $55.3 \pm 3.1\%$. In TECAM, the triolein drops embed in the matrix of cellulose acetate polymers and closely adhere with the polymers. Our previous study showed that the structure of TECAM was stable and the distribution of triolein drops did not change with the HS concentration, pH, or ionic strength of exposure water samples.

HS sample solutions were produced by diluting the HS stock solutions with ultrapure water to reach a content of 5 mg C/L. The solutions were then adjusted to a pH of 7.0 ± 0.3 with 0.1 M HCl and 0.1 M NaOH, transferred to sterilized 1000-mL glass flasks, and spiked with 500 ng/L of each PAH separately. The concentration of carrier did not exceed 0.1 mL/L. The flasks were kept sealed with Teflon-lined caps and shaken gently on a shaker for 48 h in the dark at 20 ± 1 °C.

2.2. Binding experiments

A previous study has shown that the uptake rate of HOCs in TECAM is independent of HA concentrations [16]. The system consists of two round-bottomed flasks connected by a bent tube. One flask was filled up with 1 L of Milli-Q water, the other was filled with 1 L of 15 mg C/L humic acid solution, and both solutions were spiked with 20 OCPs to obtain an aqueous concentration of 200 ng/L for each analyte. Then the two flasks were connected by the vapor-tight air bridge and the flasks were magnetically stirred for

3 weeks to equilibrate the system. After equilibration, one TECAM was put in each flask and sampled for 1 h on a rotatory shaker (90 rpm, 20 °C), and the mean concentration for each OCP in the TECAM samples in the presence and the absence of humic acid was similar ($P > 0.05$). As the free concentrations of OCPs in both flasks of the air-bridge system were equal, the results confirmed our assumption that matrix effects could be neglected in our study. Thereby it can be concluded that the presence of DOM does not influence the uptake kinetics by TECAM, and this method could be used to determine the freely dissolved concentration of analytes in HS-containing samples.

Organic carbon normalized partition coefficients (K_{OC}) of PAHs in HS were determined in batch-mode experiments in three replicates. TECAM exposed for 1 h in solution without HS accumulated less than 5% PAHs of total amount (negligible depletion sampling) [17], suggesting that the aqueous concentration of PAHs remains constant. Therefore, it could be extrapolated that the depletion of TECAM in HS-containing solution also was negligible during this time period. Uptake-time plots of TECAM in 100 ng/L PAHs solutions over 12 h showed that accumulation is in the linear-uptake phase when test solutions were changed per hour. The slopes of fitting lines by linear regression in Fig. 1 were used to calculate K_{OC} . The uptake profiles of PAHs in TECAM were produced in a series of HA-free solutions (50–1000 ng/L) at first for measuring uptake rate constant k_1 according to

$$C_m = k_1 C_w t, \quad (1)$$

where C_m represents the concentration of target compound in the TECAMs; k_1 is the uptake rate constant of compound in TECAMs; C_w is the concentration of compound in aqueous phase; t is the sampling time. Subsequent experiments were carried out in PAH–HS solutions. K_{OC} was determined from the isotherm slopes of solutions with and without HS.

$$K_{OC} = \frac{k_1 - k'_1}{k'_1 [OC]}, \quad (2)$$

where k'_1 represents the apparent uptake rate constant of TECAM in HS solutions. All regressions were done with relative coefficients above 0.9 and $P < 0.01$ (significant difference value).

Then a dynamic experiment for measurement of K_{TECAM} was performed with a laboratory static aqueous method developed by Banerjee et al. [18]. Distilled water (1000 mL) in conical flasks was spiked with 500 ng/L mixed PAHs and deployed with two pieces of TECAM. To prevent losses of PAHs due to volatilization, the flasks were sealed and shaken on a constant temperature-reciprocating shaker throughout the whole accumulation course. Temperature was kept at 20 ± 1 °C in all experiments. Preliminary

Fig. 1. Determination of the pyrene partition coefficient K_{OC} for HS of different origin from binding experiments. Bars correspond to standard error at $n = 3$.

tests were conducted to assess the experiment time for attaining apparent equilibrium in the tested system. The results showed that 72 h was sufficient for attainment of equilibrium in pure water. Then accumulation experiments spanning 72 h were conducted to determine K_{TECAM} in four replicates. Water concentrations after accumulation were measured by liquid–liquid extraction. Sample processing and chemical analysis were performed as previously described [19]. QA/QC is detailed in the [Supplementary material](#).

2.3. Equilibrium experiments

Acute and subchronic toxicity tests for aqueous animals usually last for several days. To mimic the process, the equilibrium experiments were run for 6 days with four pieces of TECAM per flask. According to the calculation detailed in the [Supplementary material](#), equilibrium between membranes and water was reached over the duration for those HS samples.

A quasi-equilibrium model based on first-order mass transfer was used to analyze our experimental results. Quasi-equilibrium is the quasi-balanced state of a thermodynamic system near thermodynamic equilibrium in some sense or degree. Hereafter, the term was characterized by the slow change of sorption and desorption processes in the static system. In a two-compartment system, concentration of target compound in the TECAMs (C_m) can be expressed by a first-order kinetics equation as

$$\frac{C_m}{C_t} = \frac{k_1}{k_1 M + k_2} [1 - e^{-(k_1 M + k_2)t}], \quad (3)$$

where k_2 represents the rate constants of mass transfer from TECAMs to water phase, C_t is the total concentration of target compound in the system, and M is the mass of membrane per unit volume of water. When equilibrium is reached between membrane phase and aqueous phase in the definite system

$$\frac{C_m}{C_w} = \frac{k_1}{k_2} = K_{\text{TECAM}}, \quad (4)$$

where K_{TECAM} is the TECAM–water partition coefficient.

When dissolved HS is present, the partition of compounds in the system obeys the Stern–Volmer equation

$$C_{\text{OC}} = K_{\text{OC}} C_w, \quad (5)$$

where C_{OC} is the bound compound concentration in HS, and K_{OC} is the organic carbon normalized partition coefficient in HS. After TECAMs are introduced into the system, desorption occurred from HS to water. On the condition that part of the bound compound does not take part in partition between water phase and HS phase, or this fraction is desorption resistant during the exposure period, a mass balance of solute at equilibrium can be expressed by the following equation:

$$\frac{C_t(1 + K_{\text{OC}}[\text{OC}]f_{\text{rd}})}{1 + K_{\text{OC}}[\text{OC}]} = MC_m + C_w(1 + K_{\text{OC}}[\text{OC}]f_{\text{rd}}). \quad (6)$$

Herein f_{rd} is the rapidly desorbable fraction of HOCs in dissolved HS ranging from 0 to 1, and $[\text{OC}]$ represents the content of organic carbon. Substituting Eq. (3) into Eq. (5) yields

$$C_m = \frac{C_t(1 + K_{\text{OC}}[\text{OC}]f_{\text{rd}})}{\left[M + \frac{(1 + K_{\text{OC}}[\text{OC}]f_{\text{rd}})}{K_{\text{TECAM}}}\right](1 + K_{\text{OC}}[\text{OC}])}. \quad (7)$$

Therefore, f_{rd} can be calculated by simply measuring C_m while K_{TECAM} is known. The main advantage of our model is that it has few independent variables as well as a direct physical meaning of the reversible descriptor.

HgCl₂ (50 mg/L) was added to prevent microbial degradation of PAHs [12]. Control experiments were run simultaneously to test the loss of chemicals in 6 days. Typically, less than 10% reduction

of the solution-phase concentration was observed in the controls over the whole experiment period and the losses were neglected. Two replicates were made for each sample.

2.4. Fluorescence experiments

For the fluorescence experiments, pyrene-*d*₁₀/HS solutions were prepared as described above. But in this case, concentrations of 10 µg/L pyrene and 10 mg/L HS were used. Fluorescence spectra were recorded, using a quartz cuvette (3 mL), on a Hitachi F3000 fluorospectrometer at room temperature. These solutions were left to equilibrate in the dark for at least 24 h before measurements were carried out. Slits were set at 5–3 nm bandpass. Excitation wavelengths of 240 nm were employed to minimize the influence of HS emission. The emission range was from 350 to 400 nm. The ratios of fluorescence emission intensities corresponding to the first and third vibrational peaks at 372 and 392 nm (I_1/I_3) were used to study micellar systems [20]. Three replicates were made for each sample.

3. Results and discussion

3.1. Relationship between HS properties and binding affinity for PAHs

Typical plots of the relationship of C_m versus C_w are given in [Fig. S1 in supporting information](#) for the HS samples of different origin. A linear regression procedure using a statistics software Origin-8.0 (Microcal Software, Northampton, MA) was utilized to fit

Fig. 2. Correlation between partition coefficients of PAHs and HS structural properties for: (A) relationship of the $\log K_{\text{OC}}$ values versus O/C atomic ratios; and (B) relationship of the $\log K_{\text{OC}}$ values versus HS molecular weights. The error bars represent the standard deviations of regression and the values of R^2 represent the adjusted coefficient of determination.

the data. All the plots yielded high correlation coefficients with R^2 ranging from 0.95 to 1. The slopes were then used for determination of the K_{OC} values. The corresponding results for all the HS are summarized in Table 1. The obtained K_{OC} values for different HS lay in the range of 2.34×10^4 – 1.53×10^6 . These values agreed well with the reported K_{OC} for PAHs binding to commercial HS [22]. The observed trend that K_{OC} values for individual HS increased with increasing K_{OW} of PAHs is consistent with the hypothesis of solute distribution [23]. On the basis of the measured K_{OC} values, the target HS can be arranged in the following order of affinity for phenanthrene and pyrene: reservoir FA < Aldrich HA < PSHA < Leonardite HA. The lowest factors occurred in interactions with fulvic acid from a drinking water reservoir. Nevertheless, the difference was not very significant, with the K_{OC} values for FA less than one order of magnitude lower than those for HA. The binding affinity of Leonardite HA extracted from lignite was higher than that of the PSHA or Aldrich HA extracted from peat [24]. These results are in good accordance with the degree of diagenesis, as the geological evolution theory illustrates that HS and source materials initially form peat during diagenesis, and then peat undergoes condensation reactions to form lignite as diagenesis continues [25].

Since the affinity of natural organic materials for nonpolar organic compounds depends on its origin and geologic history, variability in the composition and structural property of HS was proposed as an approximation to describe the binding affinity. Several selected physicochemical parameters including ^{13}C NMR spectral data, elemental analyses, and molecular weight measurement results are listed in Table 2. The main differences between HA and FA used in this study were their overall O/C content and molecular weight. The three humic acid samples have lower O/C atomic ratios of 0.37–0.58 than that (0.65) of the fulvic acid, whereas higher weight-averaged molecular weight (M_w) of 4006–5867 than M_w (1690) of the FA.

The difference in the relative amount of oxygen-containing functional groups among HS samples was in agreement with previous studies that the oxygen/carbon (O/C) atomic ratios of natural organic matter decrease with increased age and, thus, apparently with diagenetic alteration [30]. The oxidation degree of HS appears to be significant in controlling reactivity with HOCs since a negative correlation was found between the log K_{OC} values and the atomic O/C ratios of HS (Fig. 2A). However, PSHA and Aldrich HA seemed to have lower degrees of unsaturation than Nordic FA, and there was a lack of strong correlation between log K_{OC} value and H/C atomic ratio ($P > 0.5$). This misleading role of the atomic H/C ratio could be interpreted by different influences of functional groups with the same H/C ratio on binding. As Haitzer et al. pointed out, the presence of a high concentration of COOH groups can adversely affect the use of atomic H/C as a measure of $-\text{HC}=\text{CH}-$ groups because the sorption of hydrophobic organic compounds is assumed to be promoted by (hydrophobic) $-\text{HC}=\text{CH}-$ groups and impeded by (hydrophilic) carboxylic groups [2]. For Nordic FA, there is sufficient evidence for large existence of O- and N-substituted aliphatic groups (50–100 ppm) and anomeric carbons (100–110 ppm) considering the large area of ^{13}C NMR spectrum 50–110 ppm.

Statistical analyses also show that partition coefficients correlated well with HS M_w (Fig. 2B), while HS aromatic carbon content (percentage of peak area of ^{13}C NMR spectrum 110–165 ppm) has no linear relationship with log K_{OC} values, with $P > 0.1$ for all the three PAHs. Although Perminova et al. found that molecular weight was a poorer predictor than aromaticity and other ^{13}C descriptors [31], the prevalence of molecular weight over aromaticity in our study can be explained by several factors. First, discrepancy exists because almost all of their HS samples are from a terrestrial source and most are a combination of FA and HA. Second, the peak area of 110–165 ppm was poor in group specificity. The signal spreads over a wide range and can be assigned to alkenyl structure, ester, and other chemical groups. Moreover, although the application of

Table 1
Partition coefficients of the PAHs for hs used in this study.^a

Chemical	Log K_{OW}^b	K_{OC} (L/kg C)			
		Leonardite HA	PSHA	Aldrich HA	Nordic FA
Phenanthrene	4.46	7.47×10^4 (5.10×10^3)	5.24×10^4 (3.66×10^3)	4.75×10^4 (3.08×10^3)	2.34×10^4 (1.37×10^3)
Pyrene	5.30	3.75×10^5 (1.96×10^4)	2.89×10^5 (9.03×10^3)	1.23×10^5 (4.81×10^3)	1.02×10^5 (4.34×10^3)
Chrysene	5.61	9.11×10^5 (8.91×10^4)	1.53×10^6 (1.33×10^5)	6.26×10^5 (4.09×10^4)	5.40×10^5 (4.96×10^4)

^a Values in parentheses represent standard deviations.

^b Preferred or selected values from Mackay et al. [21].

Table 2
Selected physicochemical parameters of hs used in this work.

Sample	Elemental compositions (wt.%) ^a				Atomic ratio		M_w (Da)	Integrated areas of solid-state ^{13}C NMR spectrum (%)				
	C	H	O	N	O/C	H/C		Aliphatic 0–50 ppm	Carbohydrate 50–110 ppm	Aromatic 110–165 ppm	Carboxyl 165–190 ppm	Carbonyl 190–230 ppm
Leonardite HA ^b	68.8	4.0	33.7	1.3	0.37	0.70	5867 ^c	21 ^d	9	45	16	9
PSHA ^e	54.8	5.0	30.6	3.4	0.42	1.1	4940 ^f	24	16	33	23	4
Aldrich HA ^g	53.3	4.7	41.1	0.81	0.58	1.0	4006 ^c	44 ^d	15	26	9	6
Nordic FA ^h	57.6	4.4	49.7	0.80	0.65	0.92	1690 ^h	27	28	29	14	2

Data were from the references labeled in the superscript of the sample unless otherwise denoted.

^a Dry, ash-free basis.

^b Data from International Humic Substance Society.

^c Ref. [26].

^d Ref. [27].

^e Ref. [28].

^f Measured by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

^g Ref. [24].

^h Ref. [29].

NMR has facilitated conclusions in several investigations that contaminant K_{OC} values are proportional to the aromatic carbon, Chetetz and co-workers indicated that aromaticity is not a suitable variable, especially when used to predict or explain K_{OC} values, as their study demonstrates that aliphatic structural components of HAs can contribute appreciably to phenanthrene and pyrene in aqueous solution sorption [32,33]. Also as shown in the NMR results, large amounts of aliphatic groups exist in Aldrich HA while FA is composed of relatively small oligomeric molecules containing predominantly polar and ionizable groups. It has been reported that larger macromolecules of HA that are held together by weak interactions can form a more compact conformation assembly after the sorption of PAHs and may form aggregated hydrophobic regions that are more effective in binding [13,34,35]. As stated above, we can draw the conclusion that the M_w of HS might play an important role in binding of PAHs to dissolved humic and fulvic acids.

3.2. Desorption of the bound fraction

In Fig. 3 results of equilibrium experiments are shown. f_{rd} for the PAHs in HS ranged from 0.46 to 1.19. The estimated standard deviation in f_{rd} is calculated from the error propagation relationship, and the magnitude of deviation generally is below 40% due to the complexity of the data and the potential for error propagation. The f_{rd} value essentially approaches to one for almost all HS-bound PAHs and one-way ANOVA showed that there was no significant difference between f_{rd} of different HS ($P > 0.05$), which indicates that the chemicals can desorb almost reversibly. However, a t test showed that at the one-tailed probability level of 0.05, the minimum f_{rd} value of 0.46 for Leonardite HA-bound chrysene, was an outlier (with the statistic of 2.08), and that may reflect some extent of desorption irreversibility.

There are several possible causes of the observed resistant desorption. As pointed out by Wang et al. “pseudo-hysteresis” could be a manifestation resulting from nonattainment of equilibrium due to rate-limited diffusion before desorption was started [36]. Although most previous studies reported that the sorption of PAHs to various types of dissolved HS was completed within minutes and the extent of reversibility was unrelated to the sorption time [5,31,37], Haitzer et al. have shown decreasing bioavailability with increasing contact times up to 12 days for one HS sample out of three tested [38]. However, our further study showed that for HS, a contact time of 6 days has no influence on partition coefficients ($P < 0.05$) (data unpublished); therefore pseudo-resistance associated with nonequilibrium of partition can be eliminated. Another explanation for the decreased f_{rd} is related to

physical entrapment. Cornelissen et al. have proposed the entrapment of organic chemicals in “voids” in the organic matter (similar to those in glassy polymers) to account for (de)sorptional retardations [14]. Humic acid is a relatively soft, yet still glassy form [39]. Sander et al. also provide compelling evidence of the glassy character of a soil HA. These voids have recently been hypothesized to undergo a physical rearrangement on sorption of a sorbate molecule through which this sorbate becomes more strongly bound [40]. Therefore it was suggested that complexation may be activated due to the self-coiling and aggregation of dissolved HS molecules. The conformation changes could result in slower diffusion to specific sorption sites, and a fraction of solute may be trapped [8,12,13].

However, the significant low value of f_{rd} was observed for chrysene in only one of four HS. The quality of HS and the properties of a particular solute thus seem to be key factors determining the extent of HS–pollutant interactions. Previously, it was concluded that the sorption–desorption properties of natural organic matter are related to their degree of aromatization and condensation [41]. As known, PAHs tend to absorb onto other nonaqueous phases either through hydrophobic interaction with nonpolar moieties or through conjugate π bonding with polar moieties [6]. Therefore an increase in aromaticity of the dissolved HS may serve to increase the polarizability of the polymer and increase the strength of binding [20]. Also, long diagenesis history determining the degree of condensation may lead to more steric hindrance. In general, more aromatic and more highly condensed structure of Leonardite HA relative to aquatic material, as well as the largest molecular volume of chrysene among the three PAHs, may lead to larger extent of steric hindrance in the same HS sample and explain the discrepancy.

3.3. Aggregation and conformational behavior of HS

HS was composed of long-chain amphiphatic molecules; therefore HS molecular conformation could be affected by HS origin and its concentration as well as water chemistry [42]. I_1/I_3 band ratio has been used to monitor the microenvironment of pyrene, which decreases when micelle-like agglomerates are produced. Results of fluorescence experiments showed that Leonardite HA had the lowest I_1/I_3 (Fig. 4), indicating the higher tendency of Leonardite HA to form structured pseudomicellar domains that can sequester non-polar species. Similar results were drawn by Kučerík et al. They found that sodium salts of humic acids from lignite showed aggregates from very low concentration at neutral pH [43]. For all HS samples, the I_1/I_3 seemed to decrease with the increasing M_w and increase with the O/C ratio. These observations were consis-

Fig. 3. f_{rd} for HS-bound PAHs used in this study. The error bars represent the standard deviations calculated according to error propagation.

Fig. 4. I_1/I_3 ratios of the pyrene- d_{10} (10 $\mu\text{g/L}$) emission in 10 mg/L HS solutions. The error bars represent the standard deviations.

tent with the pseudomicellar model that smaller, highly solvated HS and more polar aliphatic fractions aggregated poorly [44,45].

The higher aggregation of Leonardite HA from a lignite, on the other hand, explained the lowest value of f_{rd} observed for chrysene. The colloidal state of humic acids in aqueous solution is very sensitive to the presence of other molecules, both charged and neutral [43]. Sierra et al. observed at neutral pH the partial neutralization of negative charges, associated with the hydrophobic–hydrophobic interactions between HA. The addition of pyrene molecules promoted the formation of relatively stable water-soluble agglomerates similar to micelle arrangements [46]. In our study, PAHs added to HS solution can also act as a nucleus for such arrangements. Such behavior reflects mechanisms which occur during the sequestration of hydrophobic organic compounds and has several implications for the unlabile contaminants which are hypothesized to be encapsulated within hydrophobic pockets of soluble humic aggregates.

It was reported that decreasing pH and increasing ionic strength could promote DOM aggregation and thus enhance K_{OC} , while molecular aggregation promoted by increased DOM concentration led to reducing K_{OC} [47]. Metal ions can neutralize the charge on a number of the phenolic and carboxylic groups of the humic acid macromolecule, and also engage in bridging interactions, causing the larger humic acid polymers to coil up and form relatively structured domains of increased microviscosity [22]. Since the conformation of HS molecules played an important role in its interactions with HOCs, further research is needed to explore the influence of the origin and aquatic chemical parameters on HS conformation and binding/desorption behaviors.

3.4. Environmental implication

There is growing awareness that dissolved HS significantly decreases bioaccumulation of HOCs. However, discussions on the influence of HS on HOC bioavailability were mainly focused on binding of HOCs. In fact it is the irreversible desorption of bound HOCs that affects uptake and bioaccumulation, as illustrated by Pan et al. [13]. The present study showed that true bioavailability of HS-bound HOCs could be lower than that predicted from reversible desorption and higher than that assumed nonbioavailable for HS-bound chemicals. Therefore, reevaluation on binding mechanisms between HOCs and HS as well as their impact on fate and bioavailability of HOCs must be emphasized, although there is still no clear evidence of the environmental significance of sequestered contaminants in HS.

Numerous studies have reported the dependence of binding on the composition and structure of the HS, which also has implications with respect to the formation of predictive models for determining the environmental fate of pollutants. To establish reliable risk assessment models, it appears necessary to use a more complete model considering the nature of the structural phases and the non-ideal HOC–HS interactions, especially the complicated irreversible desorption instead of simple reversible ones. Another notable unknown problem is how prolonged contact time, that is, “aging,” would mean for the binding, and desorption, as well as bioavailability of HOCs in natural waters [12]. In a study by Palomo and Bhandari, it was revealed that the extractability of sorbed solutes in HS solution during organic solvent extraction was lowered with aging [34]. Therefore systematic studies with longer contact times are required in our immediate goal in order to understand the influence of aging on the amount of irreversible compartment.

4. Conclusions

The use of M_w and O/C atomic ratio of HS gave us information on the magnitude of their binding strength for PAHs. With the

application of TECAMs combined with a two-sites quasi-equilibrium desorption model resistant desorption, it was shown that the sorption hysteresis related to some of the features of both humic substance and PAH. The PAHs with lower K_{OW} values desorbed almost reversibly from all HS selected in the study. However, chrysene- d_{12} with the highest K_{OW} in the three PAHs showed some extent of desorption irreversibility in the most diagenetic HA, Leonardite HA. Morphological research on HS also showed evidence for the desorption resistance. Such phenomena illustrated that more information is needed to deepen the understanding of desorption processes of HOCs in dissolved HS. This knowledge is also critical to ultimately link the behavior of HOC in passive samplers such as TECAM to bioaccumulation, for the purpose of correctly quantifying the bioavailability of HOCs in a natural environment.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcis.2010.06.022.

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