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# Polycyclic Aromatic Hydrocarbon (PAH) Binding to Dissolved Humic Substances (HS): Size Exclusion Effect

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## ABSTRACT

Binding mechanisms of polycyclic aromatic hydrocarbons (PAHs) with a purified Aldrich humic acid (PAHA) and its ultrafiltration (UF) size fractions were investigated. Organic carbon normalized binding coefficient ($K_{oc}$) values were estimated by both a conventional Stern-Volmer fluorescence quenching technique and a modified fluorescence quenching method. Pyrene $K_{oc}$ values depended on PAHA concentration as well as freely dissolved pyrene concentration. Such nonlinear sorption-type behaviors suggested the existence of specific interactions. Smaller molecular size PAH (naphthalene) exhibited higher $K_{oc}$ value with medium-size PAHA UF fractions whereas larger size PAH (pyrene) had higher extent of binding with larger PAHA UF fractions. The inconsistent observation for naphthalene versus pyrene was well explained by size exclusion effect, one of the previously suggested specific mechanisms for PAH binding. In general, the extent of pyrene binding increased with lower pH likely due to the neutralization of acidic functional groups in HS and the subsequent increase in hydrophobic HS region. However, pyrene $K_{oc}$ results with a large UF fraction (>100K Da) corroborated the existence of the size exclusion effect as demonstrated by an increase in $K_{oc}$ values at a certain higher pH range. The size exclusion effect appears to be effective only for the specific conditions (HS size or pH) that render HS hole structures to fit a target PAH.

**Key words :** humic substances (HS), polycyclic aromatic hydrocarbons (PAHs), organic carbon normalized binding coefficient ($K_{oc}$), sorption; size exclusion

## 요 약 문

정제된 Aldrich 휴믹산(PAHA)과 한외 여과된 다양한 크기의 PAHA 성분들(PAHA UF fractions)을 이용하여 여러 고리 방향족 탄화수소(PAH)와의 결합 메커니즘을 조사하였다. 유기탄소 결합계수($K_{oc}$)는 전통적인 Stern-Volmer 형광 소광법과 변형 형광 소광법 두 가지를 이용하여 구하였다. 구해진 Pyrene $K_{oc}$ 값은 PAHA 농도와 자유 용존 pyrene 농도에 의존하였다. 이러한 비선형 흡착결합 양상은 두 물질간의 흡착성 고유상호작용이 존재한다는 것을 암시하였다. 상대적으로 분자크기가 작은 naphthalene은 중간 크기의 PAHA UF fractions 과의 결합에서 높은 $K_{oc}$ 값을 보여준 반면 분자 크기의 큰 PAH, 즉 pyrene 의 경우에는 PAHA UF fractions 크기가 크면 클수록 더 결합이 잘 되었다. 이러한 두 PAH 물질간의 불일치한 크기별 결합양상은 현재까지 제시된 고유 결합 메커니즘들 중의 하나인 크기별 배제(size exclusion) 효과로 잘 설명되었다. 다양한 pH 조건하에서의 PAH $K_{oc}$ 실험에서는 일반적으로 pH가 낮아질수록 휴믹산의 산성작용기가 중화되고 그에 따라 휴믹산내의 소수성 영역이 커짐으로 인해 pyrene과 휴믹산과의 결합정도는 커졌다. 그러나 큰 사이즈의 PAHA UF fraction(>100K Da)을 사용한 실험에서는 낮은 pH가 아닌 특정 pH 범위에서 또 하나의 높은 pyrene $K_{oc}$ 값을 보여줌으로서 크기별 배제 효과가 존재함을 뚜렷이 보여주었다. 이러한 크기별 배제 효과는 휴믹산의 홀(hole) 구조가 PAH 크기에 적합하게 구성되어 있는 조건(휴믹성분 크기 혹은 pH)에서만 작용하는 것으로 보인다.

**주제어 :** 휴믹산, 여러 고리 방향족 수소탄소물, 유기탄소 결합계수, 흡착, 크기별 배제 효과

# 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), one groups of hydrophobic organic contaminants (HOCs), belong to a class of hazardous organic chemicals made up of two or more fused benzene rings in linear, angular or cluster arrangements, with unsubstantiated groups possibly attached to one or more rings[1]. A number of PAHs are classified as probable human carcinogens by the U.S. Environmental Protection Agency (US EPA)[2].

Anthropogenic sources of PAHs to the environment often result from activities related to energy production and the transportation, storage, and refining of fuel. The primary sources of PAH contamination in soils and aquifers are leaking underground storage tanks and old manufactured gas plant sites. Often, the contamination by PAHs in subsurface systems is found in the form of nonaqueous phase liquids (NAPLs), such as coal tars, from the uncontrolled disposal of process residuals at former manufactured gas plant sites[2]. The ubiquitous presence of PAHs in soils, sediments and their potential detrimental impact toward human health require a better understanding of their biogeochemistry, and ultimately their fate in the systems.

Humic substances (HS), the largest portion of naturally-occurring organic matter (NOM), are ubiquitous in soils, rivers and groundwater. They originate from the decay of plant and animal materials[3]. HS are mixtures of acidic, polydisperse, randomly polymerized, partly aromatic, refractory, yellow to black in color, high molecular weight organic macromolecules[4]. They are known to strongly bind PAHs and play a role in regulating the fate, transport, bioavailability and toxicity of PAHs in many subsurface environments[5]. The physicochemical characteristics of HS, such as molecular weight and hydrophobicity, are known to affect PAH binding[6-9].

Due to the low aqueous solubility of PAHs, their partitioning from aqueous phase to dissolved HS is a critical factor in controlling their concentrations and transport in aqueous phase. This situation can be commonly found in many subsurface systems because of the ubiquitous presence of HS. Examples of such systems include PAH-contaminated aquifers and sediments. For these systems, binding of PAHs by dissolved HS increases their apparent aqueous solubilities and facilitates their mobilities.

Several mechanisms have been proposed with regard to PAH-HS interaction. Chiou and co-workers proposed a solvent dissolution-like interaction as the mechanism for HOC binding to HS. The change of solute activities in the presence of HS is a driving force for HOC-HS interaction in

the model[10,11]. They utilized Flory-Huggins theory, which is based on solubility parameters and widely used in polymer chemistry, to explain HOC-HS interaction. Wershaw[12] depicted HS as micelle structures with hydrophobic regions in their interior in aggregates, and he suggested that the hydrophobic interiors of the micelle served as a hydrophobic phase into which nonpolar compounds partition. According to a HS model proposed by Schnitzer and Khan,[13] the size of HOC is one of the important parameters to be considered in HOC-HS interaction. They suggested that HS consisted of a broken network of poorly condensed aromatic rings with appreciable numbers of disordered aliphatic or alicyclic structures attached around an aromatic core. HS were assumed to have an open structure into which organic compounds could be trapped. Since the structure of HS is maintained by hydrogen bonds and van der Waals forces in the model, the dimensions and the arrangements are sensitive to solution chemistry such as ionic strength, pH and the presence of cations. Schlautman and Morgan[14] found that their binding coefficient results were not consistent with the Flory-Huggins theory. They suggested, based on the idea of Schnitzer and Khan,[13] that there was a specific interaction (size exclusion effect) between HS and HOC. However, it is still under dispute on whether such a specific interaction is involved in PAH partitioning to HS.

In this paper, binding mechanisms of PAHs with dissolved HS were investigated focusing on the existence of specific interactions. For this purpose, two different sized PAHs (pyrene and naphthalene) and several different size-fractioned HS were utilized. In addition to the use of various materials, different pH conditions were also incorporated to identify the potential binding mechanisms in that HS structure is strongly influenced by solution pH[14].

# 2. Materials and Methods

Concentrated pyrene (Fluka, 99.5%) and naphthalene (Aldrich, 99+%) stock solutions were prepared in methanol (EM Science, HPLC grade) and stored in the dark at 4°C in amber borosilicate bottles. Purified Aldrich humic acid (PAHA) was used for representative HS. Although it was reported that commercial humic acid may not be representative of HS due to the presence of high ash content,[15] Aldrich humic acid used in this study was carefully prepared by repeated pH adjustment, precipitation, and centrifugation to remove ash, humin, and fulvic acid [9]. PAHA were dissolved in distilled, deionized water (DDW) and stored at 4°C at concentrations of~5 g C/L after first adjusting the pH to~7 with 0.1 N NaOH.

A total organic carbon analyzer (Shimadzu model 5050) was used to quantify the dissolved organic carbon (DOC)

concentrations of aqueous samples. External DOC standards were prepared using potassium hydrogen phthalate as necessary. In all cases, sample DOC concentrations were kept well above the detection limit (~0.5 mg C/L). A spectrometer (Beckman model DU640) was used to measure absorbance at the fluorescence excitation and emission wavelengths of pyrene and naphthalene to make inner-filter corrections[16]. Concentrations of freely dissolved PAH were quantified with a luminescence spectrophotometer (Perkin-Elmer, LS-5B) at the excitation/emission wavelengths of 336/373 nm (pyrene) and 278/322 nm (naphthalene) using slits set for bandwidths of 3/5 nm. Relative precisions of 1% and 3% were routinely obtained for absorbance/fluorescence and DOC measurements, respectively.

Fractionation of PAHA was performed by ultrafiltration (UF). Although five different size fractions (<3K, 3-10K, 10-30K, 30-100K and >100K) were obtained from the UF process. The details of the UF fractions were already reported elsewhere[9].

In order to estimate organic carbon normalized PAH binding coefficients ($K_{oc}$) with dissolved HS, freely dissolved PAH and HS-bound PAH should be distinguished quantitatively. In contrast to other techniques requiring laborious work to separate HS from aqueous solution (e.g. dialysis, resin adsorption and ultrafiltration), a fluorescence quenching technique offers many advantages for PAHs. For example, using this technique, it is possible to quantify the freely dissolved HS without any separation step and no disruption of equilibria can be ensured[16]. The fluorescence quenching technique is based on the observation that fluorescence intensity of PAHs proportionately decreases upon the addition of HS.

When PAH is equilibrated with HS, the association of PAH with HS can be represented by the following equation.

$$[PAH]_{free} + [HS] \leftrightarrow [PAH-HS] \tag{1}$$

$$K_b = \frac{[PAH-HS]}{([PAH]_{free} \cdot [HS])} \tag{2}$$

where [PAH]$_{free}$= PAH not associated with HS, [HS] = humic substances, [PAH-HS] = PAH associated with HS, and $K_b$ = the equilibrium constant. $K_{oc}$ is the equilibrium constant when [HS] is quantified by organic carbon. By mass balance, total PAH can be represented by the summation of [PAH]$_{free}$ and [PAH-HS] in the presence of HS.

$$[PAH]_{total} = ([PAH]_{free} + [PAH-HS]) \tag{3}$$

If [PAH]$_{total}$ is divided by [PAH]$_{free}$, the following

equation can be derived from the equation (3).

$$\frac{[PAH]_{total}}{[PAH]_{free}} = 1 + K_b \cdot [HS] \tag{4}$$

If fluorescence intensity is proportional to the concentration of the PAH not associated with HS, the ratio of [PAH]$_{total}$ and [PAH]$_{free}$ is equal to the ratio of the intensity in the presence of HS (F) and in the absence of HS (Fo). This leads to the following Stern-Volmer equation.

$$\frac{F_0}{F} = 1 + K_b \cdot [HS] \tag{5}$$

The slope between $F_0/F$ and the concentration of HS provides a $K_{oc}$ value from equation (5). To generate a Stern-Volmer plot, aqueous HS samples of varying DOC concentration were prepared in 40 mL amber vials by mixing different volume ratios of HS stock solutions with pH adjusted aqueous solutions to obtain a final ionic strength of 0.1 M NaCl. An aliquot of the concentrated PAH stock solution was then spiked into the HS solutions so that the final PAH concentration in a series of samples would be constant (80 μg/L and 1 mg/L for pyrene and naphthalene, respectively). The volume of spiked stock solution was minimal (< 0.1% of total volume) to prevent any adverse effects of methanol. The samples were placed on a shaker at low speed and allowed to equilibrate for 15 min. After equilibration, sample PAH fluorescence intensities were measured, taking into account any inner-filter effects and background HS fluorescence[16]. Corrected PAH fluorescence intensities in the presence and absence of HS were used to construct Stern-Volmer plots[16]. All Stern-Volmer plots were linear with HS concentration, enabling their respective $K_{oc}$ values to be calculated from the slopes.

In addition to the conventional fluorescence quenching technique based on Stern-Volmer plots, a modified fluorescence quenching technique was developed and used for this study. This method was based on the assumption that fluorescence intensity is proportional to PAH concentration that is not associated with HS[16]. The newly developed technique was applied only to pyrene because linear relationships between fluorescence intensities and PAH concentrations, which was commonly observed for pyrene, were not found for naphthalene either in the presence or in the absence of HS. First, pyrene standard curves were prepared upon fluorescence measurements using HS-controlled solutions with a range of pyrene concentrations (0 - 80 μg/L). The data were then fitted by a linear regression equation and the obtained equations were used to quantify the concentrations of freely

dissolved pyrene in the presence of HS. The $K_{oc}$ values were calculated using equation (5) and mass balance. A known pyrene concentration (80 µg/L) was spiked into HS samples with a constant concentration of 3.7 mg C/L. The $K_{oc}$ values obtained from this technique matched the results obtained from the Stern-Volmer plot using the constant concentration as a maximum HS concentration.

## 3. Results and Discussion

### 3.1. Stern-Volmer Plots for Pyrene and Naphthalene

Stern-Volmer plots for fluorescence quenching of pyrene and naphthalene with bulk (unfractionated) PAHA are given in Fig. 1. In both cases, strong linear relationships were observed. Although other Stern-Volmer plots with PAHA UF fractions were not presented here, similar good linear Stern-Volmer plots were observed for all other cases ($R^2 > 0.98$). This indicates that only a static fluorescence quenching mechanism is dominant in PAH-HS binding

process. In general, pyrene $K_{oc}$ values were higher than those observed for naphthalene (Table 1). This can be related to the higher hydrophobicity of pyrene versus naphthalene ($K_{ow}$ values of pyrene and naphthalene are 5.13 and 3.36, respectively), as has been reported previously[17]. This hydrophobic effect appears to be more pronounced for larger size of PAHA UF fractions, which are presumably more hydrophobic. For example, pyrene $K_{oc}$ values are almost the same as or slightly higher than naphthalene values for relatively small size of PAHA UF fractions (the <3K and the 3-10K fractions) whereas pyrene $K_{oc}$ value with >100K fraction is a factor of nearly 3 higher than for naphthalene (Table 1). This observation corroborates the findings of previous investigators that partitioning by hydrophobicity is a main mechanism for PAH binding to HS and that binding affinities for PAHs greatly depends both on the hydrophobic properties of PAHs and on the type of HS[7-10].
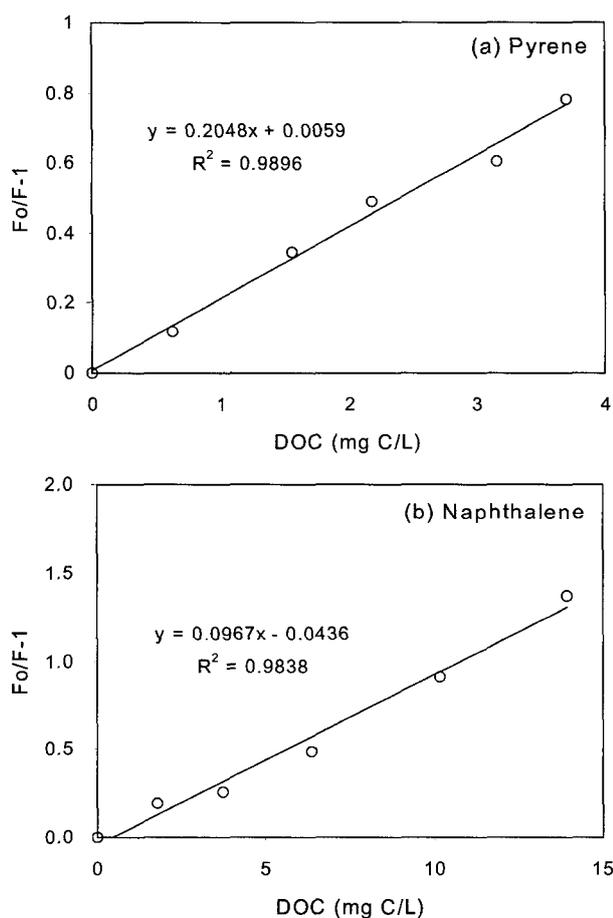


**Fig. 1.** Stern-Volmer plots for quenching pyrene (a) and naphthalene (b) fluorescence with the original bulk PAHA(3.7 mg C/L) in 0.1M NaCl and pH 7 solution.

**Table 1.** Pyrene and naphthalene $K_{oc}$ values[a] (L/Kg) of PAHA UF fractions and a bulk original PAHA (0.1M NaCl, pH 7)

| PAHA fractions | Pyrene $K_{oc} \times 10^{-5}$[b] | Naphthalene $K_{oc} \times 10^{-5}$ |
|---|---|---|
| Original Bulk | 2.05 (0.10)[c] | 0.91 (0.03) |
| <3K | 1.20 (0.11) | 0.92 (0.04) |
| 3-10K | 0.94 (0.07) | 1.04 (0.02) |
| 10-30K | 1.22 (0.10) | 1.04 (0.04) |
| 30-100K | 1.51 (0.04) | 1.03 (0.02) |
| >100K | 2.54 (0.17) | 0.82 (0.04) |

[a]The $K_{oc}$ values were obtained by Stern-Volmer plots.
[b]The data were adopted from Ref (13).
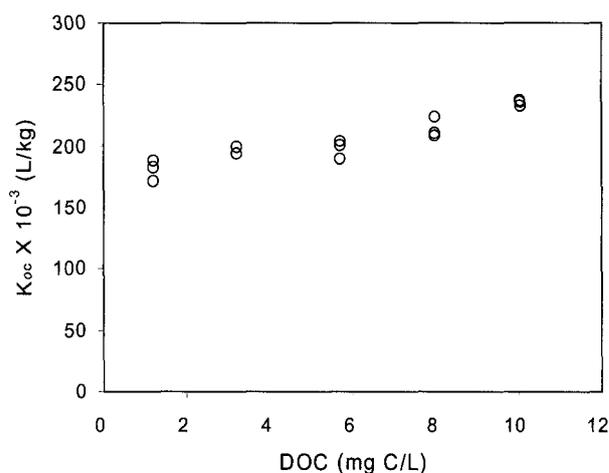[c]Standard error in parentheses.



**Fig. 2.** Pyrene $K_{oc}$ values as a function of PAHA concentrations. The values were estimated by the modified fluorescence technique.

## 3.2. $K_{oc}$ Values with Various PAHA and PAH Concentrations

Fig. 2a shows pyrene $K_{oc}$ values as a function of bulk original PAHA concentration. The modified fluorescence quenching technique was applied to obtain the result. As mentioned earlier, the $K_{oc}$ value estimated from this technique was consistent with that obtained from the Stern-Volmer plots. For example, a similar $K_{oc}$ value was obtained at a HS concentration of 3.7 mg C/L from both techniques (Table 1 and Fig. 2a). However, Fig. 2a clearly demonstrates an increase in pyrene $K_{oc}$ value with HS concentration, indicating that pyrene binding affinity depends on HS concentration.

In fact, such a concentration-dependency was not markedly seen in the Stern-Volmer plots. If there exists partitioning as the sole mechanism for PAH binding to HS, such concentration effects should not be observed because partitioning itself is independent of the solute concentration. This finding suggests that linear partitioning cannot fully describe the interactions of PAHs with HS.

## 3.3. Nonlinear Sorption Behaviors of Pyrene Binding

Pyrene binding with the original dissolved PAHA (~3.7 mg C/L), using varying pyrene concentrations below its water solubility (20 - 80 μg/L), resulted in slightly nonlinear isotherms (Fig. 3 and Table 2). In contrast, many previous studies of PAH binding with dissolved HS did not report such nonlinear binding behavior. The techniques those studies relied on included dialysis[18], reverse-phase separation[19], solubility enhancement[11] and fluorescence quenching method based on Stern-Volmer plots[16].

In a recent paper, Laor and Rebhun[20] reported nonlinear binding of several PAHs to dissolved humic acids using the complexation-flocculation (CF) and the fluorescence quenching method. They discussed in detail the inherent problems of the prior studies in evaluating binding linearity. In both dialysis and reverse-phase separation analyses, critical experimental artifacts were pointed out such as leaking of HS from the dialysis bag and the formation of large humic aggregates resulting in nonspecific trapping. Evaluation of binding linearity using the solubility enhancement method does not seem to be appropriate either, because the data obtained from the method cannot be converted into a sorption isotherm with various free solute concentrations. Laor and Rebhun[20] also pointed out that using Stern-Volmer plots make interpretation of HOC binding linearity difficult because (1) dynamic quenching may be responsible for the curvature of a Stern-Volmer plot, which is often interpreted as being evidence of nonlinear binding, and (2) when the plot is
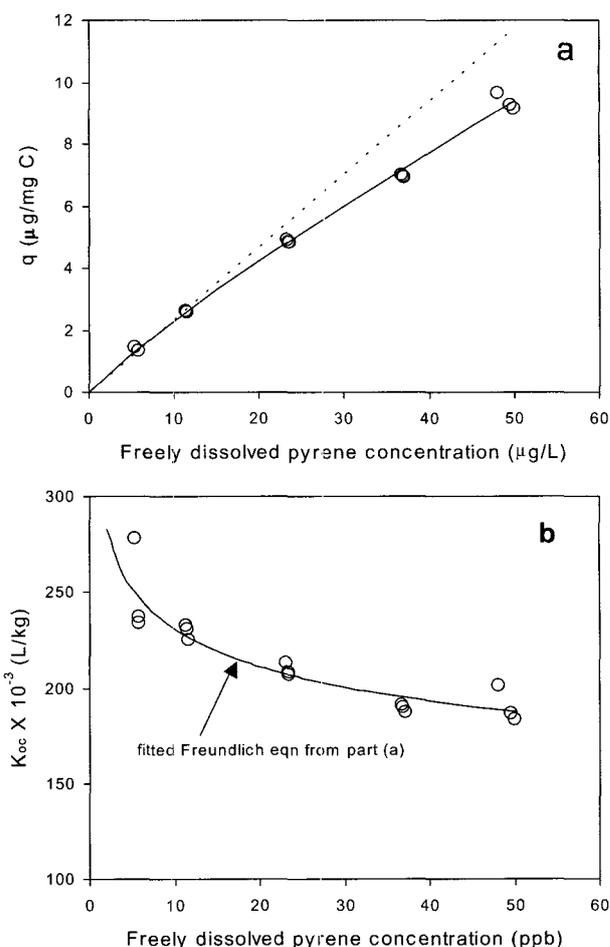
**Fig. 3.** Nonlinear pyrene binding behavior. (a) Pyrene sorption isotherm in original bulk PAHA (3.7 mg C/L). The dotted line is an arbitrary linear line for a comparison. (b) Dependency of $K_{oc}$ on the freely dissolved pyrene concentrations.

**Table 2.** Parameters of pyrene sorption isotherms using dissolved original bulk PAHA[a]

| Freundlich model | | | Linear model | |
|---|---|---|---|---|
| $K_{D,F}$[b] | N | $R^2$ | $K_{D,L}$[c] | $R^2$ |
| 0.3092 ± 0.0267 | 0.8726 ± 0.0237 | 0.9951 | 0.1942 ± 0.0031 | 0.9855 |

[a]Number of data points is 15.
[b]Units in $(\mu g/mg\ OC)/(\mu g/L)^N$.
[c]Unit in (L/mg OC).

constructed over a narrow range of y-axis, nonlinear binding is often not apparent and the plot looks linear. In this study, therefore, the linearity of pyrene binding with the original dissolved PAHA was evaluated based on a sorption isotherm constructed by the binding of various pyrene concentrations to PAHA with a constant concentration instead of Stern-Volmer plot.

Nonlinear HOC sorption isotherms in soils and sediments have recently been reported, and several conceptual models have been proposed to account for nonlinear sorption behavior[21-23]. Although the models may not be directly applied to dissolved HS, the concept of the site-specific interaction such as size exclusion or hole-filling mechanism may be useful to explain this particular situation. Schlautman and Morgan[14] found that a Flory-Huggins partitioning model failed to predict PAH binding by Suwannee River humic and fulvic acids at varying solution chemistry. They hypothesized that hydrophobic cavities might play a role in binding PAH as secondary structures of HS. Xing and Pignatello[22] incorporated the size exclusion effect into the dual-mode model (glassy/rubbery model), and suggested that two mechanisms, partitioning and hole-filling mechanism, control HOC binding by HS. According to Xing and Pignatello[22], HS can be described as having expanded regions and condensed regions, which are analogous to rubbery polymers and glassy polymers, respectively, and HOC sorption to the expanded regions occurs by partitioning whereas its sorption to the condensed regions is governed by concurrent partitioning and hole-filling mechanisms. The concept of "sorption to voids of HS" is similar to the specific interaction proposed by Schlautman and Morgan[14]. Xing and Pignatello[22] also reported such nonlinear sorption behaviors in extracted humic acids as well as in soils. Laor and Rebhun[20] considered site-specific hydrophobic interaction at limited interior or external molecular surfaces as a possible mechanism. On the other hand, the presence of a small quantity of high-surface area carbonaceous material (HSACM) such as charcoal in HS has also been attributed to the nonlinearity of HOC sorption[23]. However, it is still in debate that such a material can also exist in dissolved extractable HS[20]. More extensive studies are required to evaluate underlying mechanisms for nonlinear sorption behavior in dissolved HS.

The slightly nonlinear binding results in a dependency of $K_{oc}$ on the freely dissolved pyrene concentrations (Fig. 3b), meaning that the $K_{oc}$ values for dissolved PAHA decrease with pyrene concentration. This trend clearly demonstrates that there may be specific limited sites on PAHA for pyrene binding. In these case, low concentration of pyrene molecules will have more accessible site for HS with higher $K_{oc}$ value whereas the sites is expected to be limited for a high concentration of pyrene molecules.

### 3.4. Variation of $K_{oc}$ Values with Different PAHA UF FRACTIONS

To investigate the existence of specific interactions

between PAHs and HS, the $K_{oc}$ values obtained from PAHA UF fractions were compared using two PAHs, pyrene and naphthalene (Fig. 4). This approach was based on an assumption that the number of specific binding sites (e.g., hole structures) varies with each UF size fractions. The heterogeneous structural characteristics among PAHA UF fractions were already well discussed elsewhere[9]. As seen in Fig. 4, larger size UF fractions exhibit higher binding affinity for pyrene. In contrast, higher $K_{oc}$ values of naphthalene were found in the medium size UF fractions for naphthalene although the values did not vary as much as that for pyrene $K_{oc}$. The inconsistent trend of naphthalene $K_{oc}$ versus pyrene $K_{oc}$ cannot be explained with their different hydrophobic properties because if hydrophobicity is important for the binding, larger size UF fractions should have exhibited higher $K_{oc}$ value for naphthalene as well as pyrene. In this context, size exclusion effect[14] or hole-filling mechanism[22] may emerge as a plausible explanation for the inconsistent trends for pyrene versus naphthalene if one assumes that larger size PAHA UF fractions contain more number of larger hole structures within them than do smaller size fractions. In
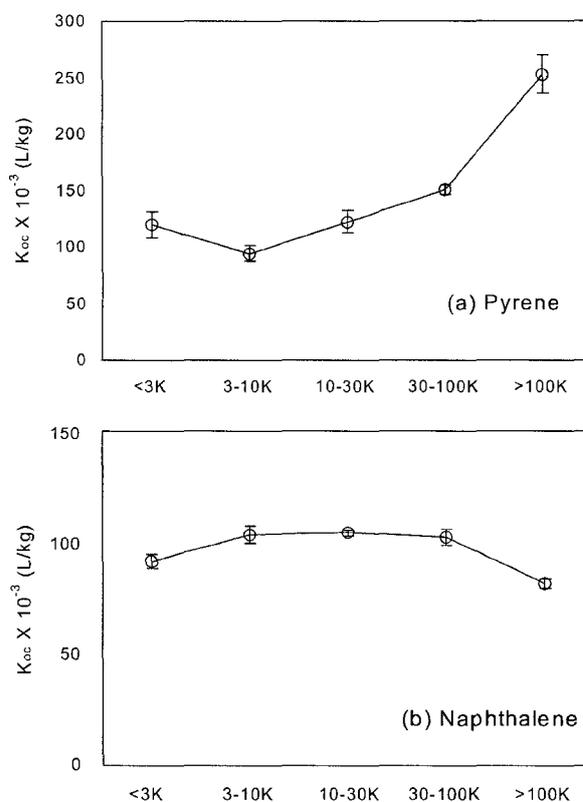


Fig. 4. Pyrene (a) and naphthalene (b) $K_{oc}$ values using different PAHA UF fractions. The HS concentration was 3.7 mg C/L. The values were estimated by Stern-Volmer fluorescence quenching technique.

other words, since the molecular size of naphthalene is smaller than pyrene, the HS hole structures to fit naphthalene are likely to be more distributed in the medium size UF fractions rather than in the large size fractions.

### 3.5. Solution pH Effect on Pyrene $K_{oc}$

Pyrene $K_{oc}$ binding to bulk original PAHA and two UF fractions was investigated at varying pH conditions (Fig. 5). In general, PAH binding affinity is influenced by solution pH because of possible structural arrangement of HS. A decrease in pH at a constant ionic strength protonates acidic functional groups of HS, which renders HS more hydrophobic and increases binding affinity for PAHs. In this study, contrasting trends of $K_{oc}$ with pH were observed for the <3K fraction versus the >100K fraction, which is the smallest size fraction and the largest size fraction from UF process, respectively (Fig. 5). Pyrene $K_{oc}$ monotonously increases with lower pH, which is easily explained with partitioning. In contrast, a similar trend of increasing $K_{oc}$ value with lower pH was observed only in the pH range from 3 to 6 for the >100K UF fractions. The bulk original PAHA shows a mixed trend. The sharp increase in $K_{oc}$ values at pH~8 may be attributed to the size exclusion effect, which was not observed for the <3K UF fractions. If solution pH changed the hole environments by contracting or expanding HS structure, the relative contributions of both partitioning and specific interactions could influence the extent of binding. At lower pH range[3-6], HS may adopt more a constricted conformation because of reduced repulsive forces between negatively charged functional groups in HS and, possibly, more H-bonding. In this case, the more compact conformation of HS at lower pH may restrict pyrene accessible to HS attachment sites, and there may not be a sufficient number of holes to fit pyrene. For this particular situation, the extent of binding would be determined by the hydrophobicity of HS rather than specific interactions. As pH increases, however, a greater number of acidic functional groups dissociate and specific interactions (or hole-filling mechanism) participate more effectively in the overall binding. The results of the >100 K fraction shown in Fig. 5 suggest that pH 8 is an optimum pH condition to allow PAHA hole structures best to fit pyrene molecules. However, this enhancement was not observed for the <3K fraction likely due to the size limitation of the enlargement of hole structures. It should be kept in mind that even for the >100 K fraction, pyrene $K_{oc}$ value at pH 3 is still higher than the value in pH 8, suggesting that partitioning is typically more important in PAH binding to HS for the wide range of pH conditions and also that the size exclusion effect plays only a
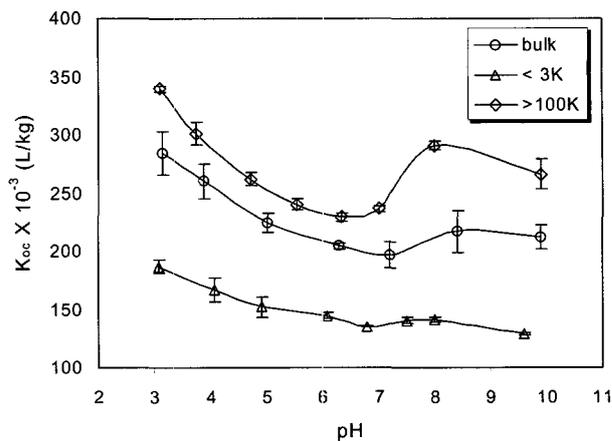


**Fig. 5.** Variation in pyrene $K_{oc}$ values with pH. The values were estimated by the modified fluorescence quenching technique.

secondary role in the binding.

## 4. Conclusions

A newly developed fluorescence quenching method enabled one to discern subtle differences in $K_{oc}$ values changing from different conditions (PAHs or HS concentrations and solution chemistry), which are hardly distinguished with the typical Stern-Volmer quenching method. Pyrene $K_{oc}$ values depended on PAHA concentration as well as freely dissolved pyrene concentration, suggesting that specific interactions related to nonlinear sorption sites may exist. Smaller molecular size PAH (naphthalene) exhibited higher $K_{oc}$ value with medium-sized PAHA UF fractions whereas larger size PAH (pyrene) had higher extent of binding with larger PAHA UF fractions. The inconsistent observation for naphthalene versus pyrene was well explained by size exclusion effect, one of the previously suggested sorption mechanisms for PAH binding. The results of pyrene $K_{oc}$ values at varying pH condition corroborated that size exclusion effect might be involved in PAH binding to HS. The size exclusion effect was pronounced only for the specific pH range, which renders HS hole structures to fit a target PAH.

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