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Measuring Binding and Speciation of Hydrophobic Organic Chemicals at Controlled Freely Dissolved Concentrations and without Phase Separation

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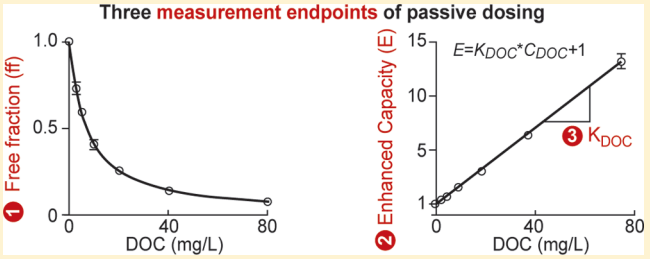
Supporting Information

ABSTRACT: The binding and speciation of hydrophobic organic chemicals (HOCs) in aqueous solutions were determined by controlling chemical activity and measuring total concentrations. Passive dosing was applied to control chemical activities of HOCs in aqueous solutions by equilibrium partitioning from a poly(dimethylsiloxane) polymer preloaded with the chemicals. The HOC concentrations in the equilibrated solutions [$C_{solution(eq)}$] and water [$C_{water(eq)}$] were then measured. Free fractions of the HOCs were determined as $C_{water(eq)}/C_{solution(eq)}$, whereas enhanced capacities (E) of the solutions for HOCs were determined as $C_{solution(eq)}/C_{water(eq)}$. A mixture of polycyclic aromatic hydrocarbons served as model analytes, while humic acid, sodium dodecyl sulfate, hydroxypropyl-β-cyclodextrin, and NaCl served as model medium constituents. The enhanced capacities were plotted versus the concentrations of medium constituents, and simple linear regression provided precise partition ratios, salting out constants, and critical micelle concentrations. These parameters were generally in good agreement with published values obtained by solid phase microextraction and fluorescence quenching. The very good precision was indicated by the low relative standard errors for the partition ratios of 0.5−8%, equivalent to 0.002−0.03 log unit. This passive dosing approach allows binding and speciation of HOCs to be studied without any phase separation steps or mass balance assumptions.



Solid phase microextraction (SPME) and other diffusive sampling techniques can be used to measure freely dissolved concentrations and chemical activities for nonpolar and polar analytes in a wide range of sample types.[1−4] This has created completely new possibilities for the measurement of speciation and binding phenomena such as binding of toxicants and drugs to proteins[5−7] and algae,[8] binding of environmental pollutants to dissolved organic carbon (DOC),[9,10] tissue to blood partitioning of semivolatile and nonvolatile organic chemicals,[11] and sorption of polycyclic aromatic hydrocarbons (PAHs) to engineered carbon nanomaterials.[12] SPME features analytical performance and flexibility, while it avoids phase separation steps such as filtration and centrifugation. However, its correct application can be challenging in some cases. For hydrophobic analytes, it can be difficult to achieve equilibrium sampling within a practical time period and to satisfy the negligible depletion criterion in small samples.[13] For very complex media such as biological fluids, it can be difficult or just tedious to confirm that surface fouling of the thin sampling phase does not compromise the analytical results.[14,15] Most SPME methods that deduce speciation in the sample from the measured concentration in the polymer also rely on some kind of complete mass balance assumption, which can lead to biased partition ratios in the case of binding of the analyte to the test vessel.[16,17]

Passive dosing is the "inverse" of passive sampling: the polymer is preloaded with the chemicals and then applied as a dominating partitioning source to control the freely dissolved concentration and chemical activity in an aqueous sample. Passive dosing was initially developed to establish and control freely dissolved concentrations of hydrophobic organic chemicals (HOCs) in aquatic toxicity tests and in vitro assays.[18,19] The approach has recently been improved by using thicker polymer donor phases and new loading procedures based on partitioning from methanol standard solutions.[20,21] In a recent technical note, we have shown that the technical quality and performance of passive dosing have reached levels that allow its utilization within analytical chemistry.[22] The preloaded polymer was used to control the freely dissolved concentration of one radiolabeled analyte in aqueous environmental solutions and pure water. The total analyte concentrations were then measured by liquid scintillation counting and free fractions in the environmental solutions determined as the concentration ratio between equilibrated pure water and the environmental solution.

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This study aims (1) to significantly improve this new analytical passive dosing approach and extend its applicability domain, (2) to provide the first comprehensive presentation of this analytical principle, and (3) to apply it to mixtures of nonlabeled analytes and several types of medium constituents. The approach was extended to mixtures of nonlabeled PAHs by the use of direct injection high-performance liquid chromatography (HPLC) with multiband fluorescence detection to allow comparisons of binding and speciation phenomena across a group of chemicals. A sequential incubation and calibration approach was developed and applied to further improve the analytical performance and robustness. In addition, the kinetics were markedly improved even for the more hydrophobic analytes by more efficient agitation. Several medium constituents (humic acid, cyclodextrin, and surfactant) were included to cover different types of binding such as partitioning, complexation, and encapsulation into micelles. Furthermore, sodium chloride, a strongly ionizable electrolyte, was included to represent an environmentally relevant medium constituent that does not bind the analytes but rather alters the solvent properties by what is known as the salting out effect.[23] Passive dosing was applied to characterize aqueous solutions using the new measurement end point of "enhanced capacity", which quantifies the capacity of a solution for HOCs relative to pure water. These enhanced capacity values were then fitted to simple linear regressions to determine partition ratios and salting out constants.

WORKING PRINCIPLE

A polymer is loaded with the analytes and then applied as a dominating partitioning source to control freely dissolved concentrations, or more precisely chemical activities, in aqueous solutions of medium constituents and pure water (see Figure 1). The total analyte concentration measured in the equilibrated solution [$C_{solution(eq)}$] is the dependent variable, and the concentration measured in the equilibrated pure water [$C_{water(eq)}$] serves as a surrogate for the freely dissolved concentration in the solution. The free fraction is then determined as the concentration ratio of equilibrated pure water to solution [ff = $C_{water(eq)}/C_{solution(eq)}$]. The enhanced capacity is quantified as the concentration ratio between the solution and pure water [$E = C_{solution(eq)}/C_{water(eq)}$] for the case in which pure water serves as a reference medium and $E = 1/ff$. Finally, E values are plotted versus the concentrations of the medium constituents, and speciation and binding parameters are obtained by simple linear regression.

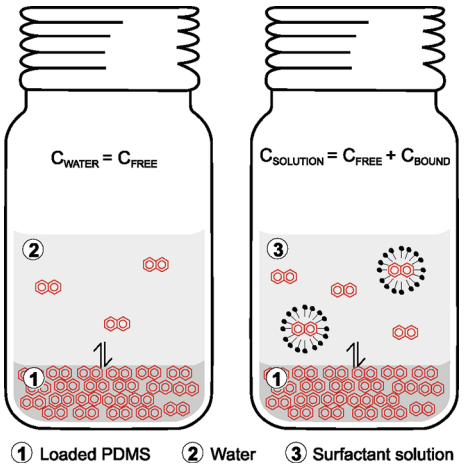

Figure 1. Analytical passive dosing setup based on a 10 mL glass vial with 500 mg of PDMS at the bottom, which is loaded with the analytes. The freely dissolved concentration in 5 mL of water and solution is controlled by equilibrium partitioning from the PDMS. Analyte concentrations in equilibrated pure water [$C_{water(eq)}$] and an equilibrated solution [$C_{solution(eq)}$] are measured to determine free fractions [ff = $C_{water(eq)}/C_{solution(eq)}$] and enhanced capacities [$E = C_{solution(eq)}/C_{water(eq)}$].

EXPERIMENTAL SECTION

Materials. The poly(dimethylsiloxane) (PDMS) elastomer was prepared from a Silastic MDX4-4210 BioMedical grade Elastomer kit (Dow Corning), purchased from Factor II Inc., with a relative density after curing of 1.11 g/cm^3. The following PAHs were selected as model hydrophobic compounds: naphthalene (NAPH) (>99%, Aldrich), phenanthrene (PHEN) (98%, Merck), anthracene (ANTH) (99%, Fluka), fluoranthene (FLU) (99%, Aldrich), pyrene (PYR) (>99%, Sigma), and benzo[*a*]pyrene (BaP) (98%, Cerilliant). HPLC-grade methanol (Merck, Darmstadt, Germany) and Milli-Q water (Super Q-treated, Millipore) were used as solvents, and ethanol (96%, Kemetyl, Køge, Denmark) was used for cleaning the PDMS. Humic acid sodium salt (AHA) (Aldrich, 38.28% organic carbon), sodium dodecyl sulfate (SDS) (99%, Sigma), hydroxypropyl-β-cyclodextrin (β-HPCD) (Wacker, Munich, Germany), and NaCl (Fluka) were used for preparation of the artificial aqueous solutions. Shaking of passive dosing vials was performed at 1000 rpm with an IKA Vibrax VXR rotation shaker (IKA-Werke, Staufen, Germany) at 20 °C.

Preparation of Passive Dosing Vials. Passive dosing vials were prepared by casting PDMS into 10 mL glass vials in a manner similar to that described in previous studies.[20,22] The PDMS prepolymer and catalyst were mixed, and 500 ± 5 mg of this mixture was added to gastight 10 mL vials with PTFE-lined screw caps (Supelco). Vials were left in the refrigerator for at least 48 h to eliminate trapped air from the PDMS, maintained at room temperature for at least 72 h, and subsequently placed in an oven at 110 °C for at least 48 h to complete the curing. The resulting PDMS elastomer had a thickness of 2 mm (±0.2 mm) at the center of the vial and a contact area for passive dosing of 2.8 cm^2 (assuming a flat surface). To remove impurities and oligomers, ethanol was added, and the vials were left for at least 72 h; the vials were then rinsed three times with ultrapure water to remove the ethanol.

Loading of Passive Dosing Vials. Passive dosing vials were loaded by equilibrium partitioning from a loading solution containing the PAHs. Saturated methanol solutions of each PAH were prepared and then combined to a mixture of five PAHs (NAPH, PHEN, ANTH, FLU, and PYR) each at 10% of their solubility in methanol. One milliliter of this loading solution was added to each passive dosing vial and later replaced with an additional 1 mL of fresh loading solution while ensuring a total loading time of at least 72 h. This sequential loading procedure was dimensioned to minimize the use and waste of the toxic PAHs, while at the same time avoiding significant depletion of the second solution. The vials were in this way loaded to 10% of the saturation level, which is high enough for the analytical measurements and well below solubility limits, which are best avoided when working with mixtures. Additional vials were loaded in a similar manner with BaP using two sequential 1 mL volumes of a saturated BaP methanol solution, for use in the kinetics experiment to extend

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the data set toward highly hydrophobic compounds and high-molecular weight compounds. This loading procedure yielded vials that were loaded with BaP at the saturation level. The loading solution was finally discarded and a small volume of water added to remove all methanol. After all vials had been loaded, the silicone and vial surfaces were wiped with lint-free tissue. These loaded vials were now ready to be used in sequential series of passive dosing measurements.

Passive Dosing Kinetics. Passive dosing kinetics with respect to water and a humic acid solution (200 mg/L) were determined for a mixture of NAPH, PHEN, ANTH, FLU, and PYR and separately for BaP. The kinetics were measured for a solution volume of 5 mL contained in a passive dosing vial that was shaken at 1000 rpm and 20 °C. All experiments were conducted in triplicate using three parallel vials. Subsamples (0.5 mL) were taken from each vial at predetermined time points and added to 0.5 mL of methanol for conservation prior to instrumental analysis (described later). The measured concentrations in the solution ($C_{solution}$) were then plotted versus time and fitted to a first-order one-compartment model [$C_{solution(t)} = C_{solution(eq)}(1 - e^{-kt})$] to obtain dosing−time profiles and release rate constants (k). Data were fitted by a least-squares method using GraphPad (San Diego, CA) Prism 5, and the time to reach 95% of the equilibrium level was calculated [$t_{95\%} = \ln(20)/k$].

Sequential Incubation and Calibration Strategy. Each passive dosing vial was first equilibrated with pure water [$C_{water(eq)}$ before], then with the solution [$C_{solution(eq)}$], and then again with pure water [$C_{water(eq)}$ after]. Average concentrations of the two water equilibrations [$C_{water(eq)}$] were used for the calculation of the free fraction and E values, except for situations of significant silicone depletion [$C_{water(eq)}$ after < $0.9C_{water(eq)}$ before], in which case only $C_{water(eq)}$ after was used. In this study, we developed and applied this sequential incubation strategy (water−solution 1−water−solution 2, etc.) instead of the parallel incubation strategy applied previously.[22] The sequential incubation scheme has the important advantage of being able to account for the possible depletion of the silicone source as well as for vial-to-vial differences in loading levels. Even though both error sources are easily kept at an acceptable minimum,[22] this sequential incubation approach is still expected to improve the overall robustness and precision of the analytical passive dosing method.

Application to Aqueous Solutions. Passive dosing was applied to four solutions with different medium constituents that either bind the PAHs or affect their activity coefficients in the solution. In this work, we emphasize the new measurement end point of "enhanced capacity (E)" that quantifies the capacity of the solution for HOCs relative to clean water:

$$E = \frac{C_{solution(eq)}}{C_{water(eq)}} \quad (1)$$

This E has some similarities to the well-established concept of "solubility enhancement" but should not be confused with it. Solubility enhancement is determined at and applies only to the saturation level of the HOCs, whereas the E can be determined at or below the saturation level. The three medium constituents that enhance the capacity of the solution ($E > 1$) by different mechanisms are (1) Aldrich humic acids, which enhance capacity by partitioning of PAHs into DOC aggregates; (2) β-cyclodextrin, which hosts PAHs in its hydrophobic cavity; and (3) sodium dodecyl sulfate (SDS), which hosts PAHs in the formed micelles. Finally, passive dosing was also applied to solutions of sodium chloride (NaCl), which was expected to reduce the capacity ($E < 1$) by what is known as the salting out effect.

Aldrich Humic Acid (AHA). Aldrich humic acid sodium salt (AHA, 38.8% organic carbon) was chosen as a model dissolved organic carbon (DOC), because it has been used in several other speciation studies with PAHs.[24−28] The binding of PAHs to AHA is expected to be a partitioning process, where K_{AHA} is the humic acid to water partition ratio that can be calculated according to

$$K_{AHA} = [C_{solution(eq)} - C_{water(eq)}]/[C_{AHA}C_{water(eq)}]$$

$$\frac{C_{solution(eq)}}{C_{water(eq)}} = K_{AHA}C_{AHA} + 1 \quad (2)$$

The PAH mixture was dosed to water and solutions with humic acid concentrations (C_{AHA}) of 6.5, 13, 25, 52, 104, and 208 mg/L at 20 ± 1 °C. Three passive dosing vials were used for each concentration in this and all subsequent experiments.

Hydroxypropyl-β-cyclodextrin (β-HPCD). Cyclodextrins are cyclic oligosaccharides containing six to eight glucose units and can host hydrophobic molecules within their hydrophobic cavity. Cyclodextrins have a broad range of applications. They are, for instance, deployed as supramolecular carriers in organometallic reactions, for the production of HPLC columns for chiral enantiomer separation, and for drug release in pharmaceutical applications. Hydroxypropyl-β-cyclodextrin (β-HPCD) is a water-soluble compound with a cavity size reported to be 0.262 nm.[3,29] Partitioning to the cavity can be described as being analogous to partitioning to DOC by eq 2. Loaded vials were equilibrated with water containing β-HPCD concentrations ($C_{\beta\text{-HPCD}}$) of 100, 200, 400, 800, 1500, and 3000 mg/L at 20 ± 1 °C. Depletion of the silicone source was observed only at the highest β-HPCD concentration and was corrected with the sequential calibration strategy as described above.

Sodium Dodecyl Sulfate (SDS). SDS is an anionic surfactant used in many cleaning and hygienic products. Dissolved individual SDS molecules are expected to behave as cosolutes, whereas SDS micelles will act as a partitioning phase for the PAHs. No capacity enhancement is thus expected for SDS concentrations up to the critical micelle concentration (cmc), and a linear increase with SDS concentration is expected above the cmc. Partitioning to SDS micelles can thus be described as

$$\frac{C_{solution(eq)}}{C_{water(eq)}} = K_{SDS}(C_{SDS} - \text{cmc}) + 1 \quad (3)$$

Loaded vials were equilibrated with water with SDS concentrations (C_{SDS}) of 0.4, 0.8, 1.6, 2.0, 4.0, 6.0, 8.0, and 10.0 g/L at 20 ± 1 °C. Depletion of the silicone source was observed for SDS concentrations from 4 to 10 g/L.

Sodium Chloride. As previously mentioned, the addition of sodium chloride reduces rather than increases the capacity of a solution for PAHs by increasing their activity coefficients (γ). This phenomenon is known as salting out and can be described by the empirical salting out coefficient model $\gamma^s = 10^{k_S I}\gamma^o$, where γ^s is the aqueous activity coefficient in saline water, k_S is the salting out or Setschenow constant (liters per mole), and I is

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the ionic strength of the solution (moles per liter). With passive dosing, the chemical activity of the sample is controlled, meaning chemical activities are equal in equilibrated pure water [$a = \gamma^{o} C_{water(eq)}$] and saline water [$a = \gamma^{o} \times 10^{k_S I} C_{solution(eq)}$]. This yields a linear relationship between log E and ionic strength (I) with a negative slope equal to the Setschenow constant (k_S).

$$\log\left[\frac{C_{solution(eq)}}{C_{water(eq)}}\right] = -k_S I \quad (4)$$

Sodium chloride solutions of 0, 10, 20, 30, and 40 g/L were tested, corresponding to ionic strengths of 0, 0.17, 0.34, 0.51, and 0.68 M, respectively. Additionally, natural seawater was tested. This sample had a low organic carbon content (70 μmol of C/L) and salinity (34.94 g of NaCl/L) and was collected from the North Atlantic at position 53′29.41N, 23′19.81W and 3000 m depth during the Danish Galathea 3 expedition. At the end, the PDMS from the passive dosing vials used for dosing the NaCl solutions was extracted with methanol to determine the concentrations in the PDMS. These were divided by the corresponding $C_{water(eq)}$ to obtain PDMS−water partition ratios.

Chemical Analysis. Aliquots of the aqueous solutions were taken directly from the passive dosing vial and then mixed in a 1:1 ratio with methanol for preservation and dissolution from the binding matrix. Samples were then kept at −18 °C until they were analyzed by HPLC with multiband fluorescence detection (Agilent 1100 HPLC system equipped with a G1321A FLD operated at an excitation wavelength of 260 nm and emission wavelengths of 350, 420, 440, and 500 nm). A sample volume of 30 μL was injected at 28 °C, and the PAHs were separated on a CP-EcoSpher 4 PAH column (Varian Inc., Palo Alto, CA), operated at a flow rate of 0.5 mL/min. Methanol and water were used as the mobile phase: 50% methanol between 0 and 2 min, a linear gradient from 50 to 75% methanol between 2 and 7 min, a linear gradient from 75 to 100% methanol between 7 and 35 min, and 100% methanol from 35 to 50 min. PAH concentrations in the samples were quantified using a nine-point external standard calibration line. Signal integration was done using the HP Chemstation software (B.03.01, Agilent Technologies, Palo Alto, CA). The absence of effects of the matrix on the direct injection analysis by HPLC was checked for SDS, humic acids, and cyclodextrin. Pure water and aqueous solutions with the highest used concentration of constituents were mixed in a 1:1 ratio with methanol and then spiked with equal amounts of the PAHs. The measured concentrations in these solutions were between 90 and 100% of those for the methanol/water mixture (Table S1 of the Supporting Information).

RESULTS AND DISCUSSION

Passive Dosing Kinetics. Equilibration times ($t_{95\%}$) for pure water samples ranged from 11 min for fluoranthene to 23 min for benzo[a]pyrene (Figure 2 and Figure S-1 of the Supporting Information). A longer time was required to equilibrate the humic acid solution (200 mg of AHA/L), with equilibration times ranging from 28.5 min for naphthalene to 375 min for benzo[a]pyrene (Figure 2). On the basis of these measured passive dosing kinetics, overnight incubations were found to be more than sufficient for the equilibration of even high-capacity solutions if high-speed agitation is applied. The improved kinetics not only make the method more time efficient but also provide the possibilities of applying passive dosing to less stable analytes, studying how speciation changes with time, and possibly even studying binding kinetics.

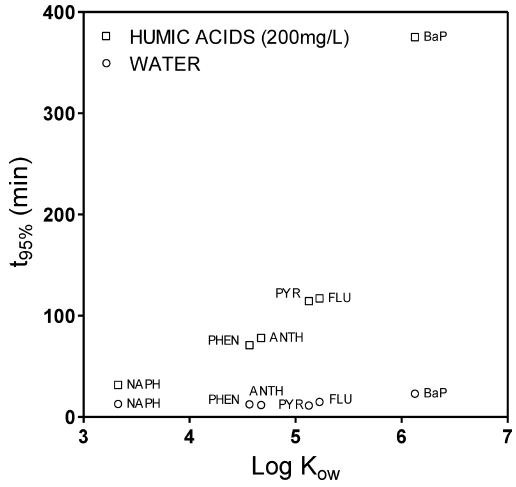

Figure 2. Time to reach equilibrium ($t_{95\%}$ in minutes) when shaken at 1000 rpm plotted vs log K_{ow} of the analytes. $t_{95\%}$ values ranged from 11 to 23 min in water and increased with hydrophobicity in the humic acid solution (200 mg/L).

The equilibration times for the passive dosing of pure water were rather similar for all PAHs, which is in contrast to those for equilibrium sampling methods where equilibration times increase with hydrophobicity.[13] This is in good agreement with the fact that passive dosing involves equilibration with a fixed volume of water, whereas equilibrium sampling requires a water volume that increases with the polymer to water partition ratio. Equilibration times for passive dosing increased with analyte hydrophobicity in the humic acid solution. This is due to the higher analyte mass that had to be transferred into a solution with a higher enhanced capacity. However, it has to be noted that the equilibration time for BaP increased by a factor of only 16, whereas the enhanced capacity and thus the mass to be transferred increased by a factor of 300. This agrees with the observation that humic acids not only bind HOCs but also enhance their diffusive release from the polymer.[21,30,31]

Aldrich Humic Acid (AHA). These E values for AHA solutions were very precise, with relative standard deviations (RSD) of typically <3% (mean RSD of 2.8%, range of 0.2−10.6%). This implies that even small enhancements of, for instance, only 5−20% can be measured with high precision, which can be difficult to do with other methods.[32] The E values were then plotted versus humic acid concentration and fitted with linear regression using eq 2 (Figure 3a). The slope of these regressions yielded partition ratios (K_{AHA}) that ranged from 1016 L/kg of C [standard error (SE) of 85 L/kg of C] for naphthalene to 152173 L/kg of C (SE of 2086 L/kg of C) for pyrene.

The partition ratios were plotted versus the octanol−water partition ratios (K_{ow}) of the PAHs (Figure 4). Log K_{AHA} increases linearly with log K_{ow} with a slope close to unity, which is in good agreement considering binding of PAH to humic acids as a partitioning process driven mainly by the hydrophobicity of the PAHs. The partition ratios obtained were also in good agreement with those measured by fluorescence quenching[27] and a solid phase depletion method.[28,32] Published partition ratios based on reverse phase separation and complexation−flocculation were systematically lower, indicat-

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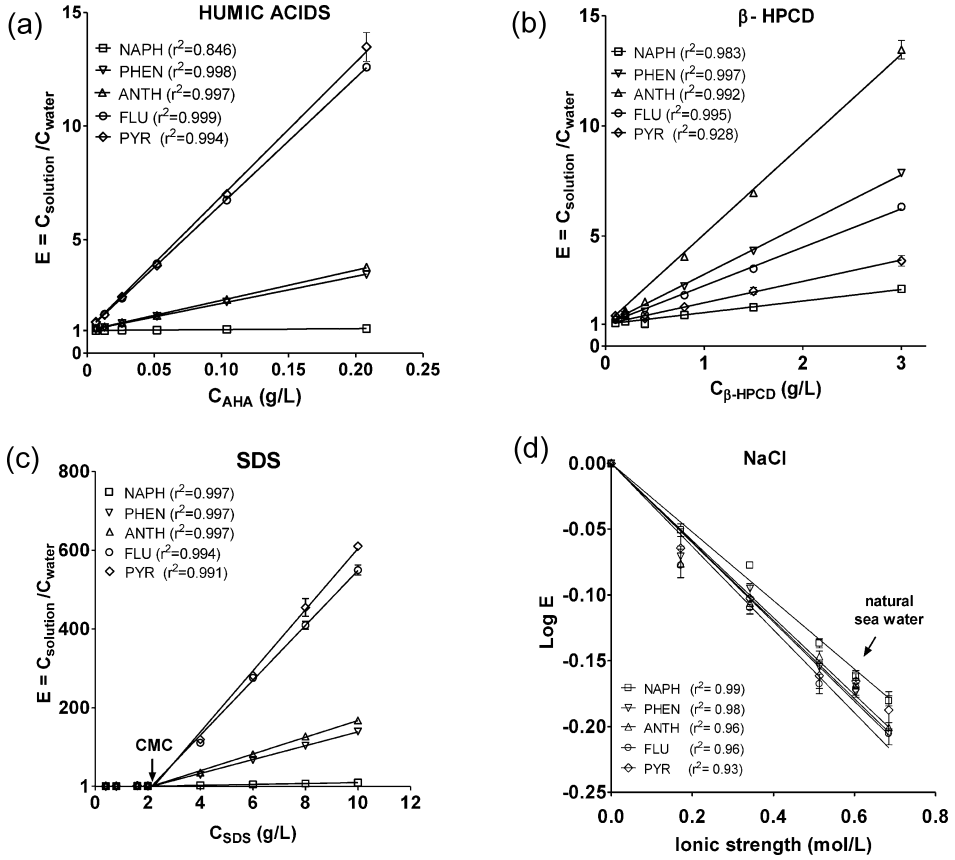

Figure 3. Enhanced capacity vs constituent concentration in four solutions containing (a) Aldrich humic acid (AHA), (b) hydroxypropyl-β-cyclodextrin (β-HPCD), (c) sodium dodecyl sulfate (SDS), and (d) sodium chloride (NaCl). Plots show mean values (n = 3) and the standard error of the mean (SE).

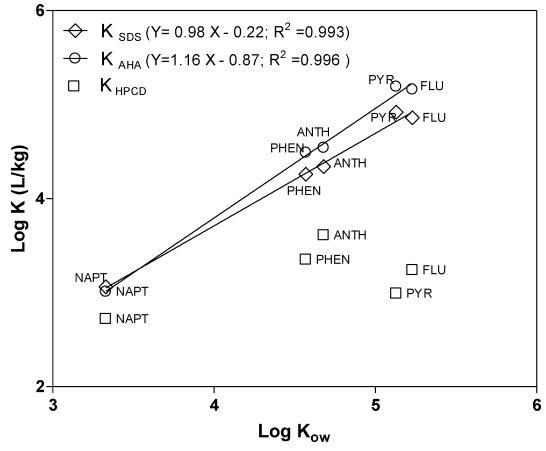

Figure 4. Partition ratios vs octanol–water partition coefficients. Log values of measured partition ratios to sodium dodecyl sulfate micelles (SDS; n = 8 × 3), Aldrich humic acid (liters per kilogram of C) (AHA; n = 6 × 3), and hydroxypropyl-β-cyclodextrin (β-HPCD; n = 6 × 3) were plotted vs log K_{ow}. Partition ratios for SDS and AHA were fitted by linear regression.

ing insufficient discrimination between dissolved and bound forms, which has been discussed and explained previously.[33]

Hydroxypropyl-β-cyclodextrin (β-HPCD). The enhanced capacity for all measured PAHs increased linearly with β-HPCD concentration (Figure 3b). Precise results, with relative standard deviations of typically <4% (mean RSD of 3.5%, range of 0.5–11.6%), were obtained, showing that the method can also be effective for the case of inclusion complex binding. The slopes of these linear regressions (Figure 3b, eq 2) yielded β-HPCD–water partition ratios that ranged from 525 L/kg (SE of 12 L/kg) for naphthalene to 4083 L/kg (SE of 64 L/kg) for anthracene. These partition ratios were consistent with values measured with fluorescence quenching[34] and a solid phase depletion method.[32] Further, the linearity in Figure 3b indicates that the entire experiment was within the 1:1 stoichiometry regime.[35] This is in good agreement with the working principle of passive dosing, which ensures constant freely dissolved concentrations when HPCD concentrations are increasing. This is in contrast to conventional binding studies, in which an increase in HPCD concentration (grams of β-HPCD per liter) leads to a decrease in analyte concentrations in both the free form (nanograms per liter) and in the β-HPCD phase (nanograms per gram of β-HPCD).

The measured partition ratios were plotted versus K_{ow} but did not show the strong correlation observed for both humic acid and SDS (Figure 4). This clearly demonstrates that inclusion complexation with HPCD is driven not just by the hydrophobicity of the PAHs.

Sodium Dodecyl Sulfate (SDS). As expected, the enhanced capacity (E) of the solutions remained close to 1 at SDS concentrations below the critical micelle concentration (cmc), confirming that individual SDS molecules behave as cosolutes rather than as a binding phase (Figure 3c and Figure S-2 of the Supporting Information). Above the cmc, the E then increases linearly with SDS concentration (Figure 3c). The passive dosing method yielded precise measurements of E, with

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Table 1. Determined Partition Ratios and Salting Out Constants for the Five PAHs

compound	K_{AHA}[a,b] (L/kg of C)	$K_{\beta\text{-HPCD}}$[a,c] (L/kg)	K_{SDS}[a,d] (L/kg)	K_{PDMS}[a,e] (L/kg)	k_S[a,f] (L/mol)
naphthalene	1016 ± 85	525 ± 13	1157 ± 28	661 ± 34	0.259 ± 0.005
phenanthrene	30739 ± 195	2260 ± 24	18361 ± 225	6156 ± 372	0.302 ± 0.006
anthracene	34220 ± 359	4083 ± 64	22112 ± 383	7901 ± 521	0.299 ± 0.009
fluoranthene	143373 ± 736	1745 ± 22	72501 ± 1962	17232 ± 1116	0.316 ± 0.009
pyrene	152173 ± 2086	972 ± 43	82278 ± 2478	19064 ± 1139	0.293 ± 0.012

[a]Standard errors are given. [b]Aldrich humic acid−water partition ratios ($n = 6 \times 3$). [c]Hydroxypropyl-β-cyclodextrin−water partition ratios ($n = 6 \times 3$). [d]Sodium dodecyl sulfate−water partition ratios ($n = 8 \times 3$). [e]Poly(dimethylsiloxane)−water partition ratios ($n = 5$). [f]Salting out constant ($n = 5 \times 3$). All measurements were performed at 20 ± 1 °C.

RSDs typically being less than 4% (mean RSD of 3.4%, range of 0.5−11.1%). Linear regressions (eq 3 and Figure 3c) yielded partition ratios to the SDS micelles (K_{SDS}) that ranged from 1140 L/kg (SE of 17 L/kg) for naphthalene to 77620 L/kg (SE of 2008 L/kg) for pyrene. These K_{SDS} values were in a good agreement with values from solid phase dosing and sampling methods.[32] For instance, we obtained a log K_{SDS} for pyrene of 4.89 ± 0.01, whereas Kim[32] reported a value of 4.98. Additionally, the linear regressions for each of the PAHs yielded estimates of the cmc that all were within the range of 2.1−2.6 g/L, which is in good agreement with the value of 2.4 g/L reported by Ko[36] for low-ionic strength solutions. Finally, log K_{SDS} values were plotted versus log K_{ow} (Figure 4), and a linear regression yielded a slope of 0.98 ± 0.05 with an r^2 of 0.99. This indicates that binding of PAH to SDS micelles is driven mainly by the hydrophobicity of the solute.

Sodium Chloride. PAH concentrations in the equilibrated solutions decreased with increasing NaCl concentrations without large differences between the individual PAHs. This decline was on average 15 and 35% in saline solutions with ionic strengths of 0.17 and 0.51 M, respectively. A linear regression was fitted to the experimental log E values (Figure 3d and eq 4), which yielded precise Setschenow constants (Table 1 and Figure S-3 of the Supporting Information) that were in good agreement with values obtained by SPME.[37] The linear regression was further used to predict the capacity of natural deep seawater with a salinity of 34.94 g of NaCl/L (equivalent ionic strength of 0.57 M). Predicted E values were ~0.67 for all PAHs, which were in very good agreement with actual experimental measurements on the deep seawater with deviations of only 1−4% (see Figure 3d and Table S-2 of the Supporting Information).

Freely Dissolved Concentration versus Chemical Activity. The application of passive dosing as an analytical technique is new and provides a high level of accuracy in the distinction between the two measurement end points, "freely dissolved concentration" and "chemical activity". These two are closely related parameters[38] but also show some fundamental differences. The freely dissolved concentration quantifies the concentration of unbound analyte molecules, whereas chemical activity quantifies the energetic state of the analyte relative to a chosen reference state.

In the case of binding to third phases such as humic acids, the freely dissolved concentration (C_{free}) and chemical activity remain proportionally related ($a = \gamma C_{free}$, where γ is the activity coefficient). In this situation, it is mainly a matter of convenience, tradition, and practicality whether to work with one or the other. Freely dissolved concentration is often the first choice, because it is conceptually easier to understand and is also more solidly established within the sectors of analytical chemistry, toxicology, and pharmacology.

However, the addition of an electrolyte to the solution will affect the activity coefficient, which is the proportionality factor between the two measurement end points. In this approach, the chemical activity will then still be controlled at the same level by partitioning from the preloaded polymer, whereas C_{free} will decrease because of the salting out effect resulting from the increase in the activity coefficient. The subtle but important difference between chemical activity and C_{free} is visible in the experiments with NaCl, where the chemical activity level remained unchanged but the freely dissolved concentration decreased with increasing NaCl concentrations (Figure 3d).

Changes in activity coefficients have the following implications with regard to analytical passive dosing. (1) When speciation in a high-ionic strength aqueous sample is being studied, the free fractions and binding constants should be keyed to water adjusted to the same ionic strength as the actual solutions. Otherwise, the free fraction will be overestimated and binding constants underestimated. (2) This is not necessary when working with the enhanced capacity end point, as long as the reference solution is well-defined and specified.

Enhanced Capacity as a New Measurement End Point. This study focuses on the capacity of a solution as a novel analytical measurement end point. This also has a physical meaning and can be utilized in many dynamic systems. Various medium constituents can act as HOC carriers and can therefore play an important role in the fate of HOCs at both the micro and macro scale. For instance, the enhanced capacity of blood for a given substance would be informative with regard to the convective mass transfer of the substance in the bloodstream. Additionally, the capacity of blood components (plasma, serum, and erythrocytes) to serve as drug carriers and depots provides helpful information for understanding pharmacokinetics as well as drug clearance and half-life.[39] The E is also important with respect to the environmental fate of HOCs. Colloid-facilitated transport of HOCs through soil macropores has been recognized as a possible transport pathway toward drain- and groundwater.[40,41] The E of soil leachates can be precisely determined and connected to the quantity and quality of the DOC present, ultimately leading to estimates of the potential leaching of PAH via this transport phenomenon.

The main advantage of E relative to the established concept of solubility enhancement is that it can be determined below the saturation level, and at defined levels of each mixture component. This feature provides new possibilities for working at low environmental concentrations and also relevant mixture compositions.

The combination of E measurements and simple linear regressions (Figure 3a−d) can yield partition ratios, critical micelle concentrations, and Setschenow constants. Compared to nonlinear regression of free fraction measurements, the

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linear regression is simpler to perform, and it is also easier to detect and deal with deviations. Chalumot and co-workers have used a similar approach in a recent SPME study, where they determined the binding constant based on $1/C_{free}$ instead of C_{free}.[35]

General Features and Future Developments. This study focuses on the development of passive dosing as a practical and high-performance analytical tool, which can be directed to the determination of different measurement end points in several application areas. Examples of such applications are given in Table 2. We used HPLC with a multiband fluorescence detector to measure PAH concentrations in solution and water. However, equilibrated solutions from the passive dosing vials can be extracted and analyzed with any method of choice, making the new technique easily adaptable to the available equipment. For rather complex solutions that may also contain particles, it can be cumbersome to measure total concentrations in the solutions, and a combination of radiolabeled analyte and liquid scintillation counting can then be an efficient way to deal with this challenge.[22] Passive dosing may also be used with very small sample volumes in contrast to diffusive sampling techniques that require a minimal sample volume for equilibration of the polymer without depletion of the sample.

Table 2. Measurement End Points of Passive Dosing, When It Is Applied as an Analytical Method

measurement end point	equation applied	examples of application
free fraction (ff)	$C_{water(eq)}/C_{solution(eq)}$	speciation in human fluids; estimation of free pollutant fraction in environmental solutions
enhanced capacity (E)	$C_{solution(eq)}/C_{water(eq)}$	environmental transport and pharmacokinetics of organic analytes
partition ratio (K) and binding constants	see eqs 2 and 3	study binding to, e.g., proteins, colloids, and carbon nanoparticles
k_S and $K_{polymer,water}$	see eq 4	studying the effect of electrolytes on partitioning calibration of passive samplers for marine environments

In the passive dosing approach presented here, the variable parameter that is measured is $C_{solution(eq)}$. This is in contrast to SPME, in which the amount of analyte associated with a micrometer thin polymer coating is measured. On one hand, this makes passive dosing potentially more robust with regard to surface-fouling artifacts,[14,15] which is critical for difficult matrices and constituents, such as proteins, colloids, and engineered nanoparticles. On the other hand, this somewhat limits the applicability domain of the passive dosing approach. Particularly for highly hydrophobic organic chemicals with aqueous solubilities of <1 μg/L, it will be difficult if not impossible to measure aqueous concentrations in small water samples with reasonable precision. Partitioning methods in which the analyte concentration is measured in the polymer rather than in the sample will then be better suited. This applies not only to diffusive sampling methods such as SPME but also to the more recently developed dynamic permeation methods[42] and solid phase dosing and sampling methods.[28]

Finally, the obtained partition ratios were characterized by very low relative standard errors of 0.5−8%, which is equivalent to 0.002−0.03 log unit. In addition to the high precision of the HPLC method, there are three main reasons for this high precision. (1) With passive dosing, freely dissolved concentrations and chemical activities were tightly controlled to a constant level, both within individual vials but also between vials. Consequently, all concentration ratios were based on solutions of the same chemical activity. (2) The passive dosing approach does not rely on a complete mass balance assumption. The approach is thus not affected by, e.g., analyte binding to surfaces of glass vials or Teflon septa, which can be sources of substantial error when determining partition ratios for hydrophobic analytes with a complete mass balance assumption.[16,17] (3) The estimations of the partition ratios were based on a large number of measurements that were used for the linear regressions, which reduces the error in the partition ratios.

While the results of this study clearly show that the approach of controlling chemical activity and measuring total concentration can provide very precise binding and speciation measurements, additional research is needed to determine the accuracy of such measurements. The SPME-based research of the past decade has already shown important improvements in accuracy compared to that of methods that include physical phase separation steps,[33] and this improvement also holds for the passive dosing approach. The recent research of Hsie and co-workers suggests that a further improvement in accuracy is possible when avoiding complete mass balance assumptions in cases where there is significant sorption of the analyte to glass surfaces.[17] For future binding and speciation research of HOCs, we envision that SPME, passive dosing, and other partition-based methods can complement each other, and that they can serve as reference methods for each other. With several independent methods to study and measure HOC speciation and binding without phase separation, these can be cross-validated, and any systematic errors, for instance, due to surface fouling or incomplete mass balances, can be detected and then minimized.

■ ASSOCIATED CONTENT

Supporting Information

Graphs showing passive dosing kinetics to water and humic acid solutions (200 mg/L) for two-, three-, and four-ring PAHs as examples (Figure S-1), the enhanced capacity of sodium dodecyl sulfate solutions for concentrations below the cmc (Figure S-2), experimentally determined Setchenow constants plotted versus the hydrophobicity for five PAHs (Figure S-3), matrix effect of each binding medium on the direct injection analysis by HPLC for the six PAHs (Table S-1), and experimental and predicted E values (eq 4) for natural deep sea water (Table S-2). This material is available free of charge via the Internet at http://pubs.acs.org.

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