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DEVELOPMENT OF NEGLIGIBLE DEPLETION HOLLOW FIBER-PROTECTED LIQUID-PHASE MICROEXTRACTION FOR SENSING FREELY DISSOLVED TRIAZINES

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Abstract—A new sampling method, termed negligible depletion hollow fiber-protected liquid-phase microextraction, was developed for sensing the freely dissolved concentration (C_{free}) and evaluating the availability of atrazine (ATR), desethyl atrazine (DEA), and simazine (SIM) in water. The sampling device was prepared by impregnating 1-octanol to both the pores and the lumen of a piece of polypropylene microporous hollow fiber membrane. After equilibrium and negligible depletion extraction, the 1-octanol in the lumen of the hollow fiber (10 μL) was collected for determination of triazines. Determination of C_{free} and the distribution coefficient to 1-octanol (D_{OW}) can be performed with this technique. A wide linear working range (1–200 $\mu\text{g/L}$) and low detection limits (0.1–1 $\mu\text{g/L}$) were obtained for triazines. Measured log D_{OW} values of DEA (1.44 ± 0.04), SIM (2.06 ± 0.06), and ATR (2.33 ± 0.05) agreed well with those reported in the literature. The measured D_{OW} values were independent of the chemical concentration and sample pH (pH 3–10) and negligibly affected by the sample salinity (0–500 mM), suggesting that environmentally relevant pH and salinity have no significant effects on the availability of triazines. Although a slight ($\leq 31\%$) increase of C_{free} was observed, one-way analysis of variance indicated the C_{free} of triazines were not significantly affected by the presence of Aldrich humic acid, Acros humic acid, and bovine albumin V (dissolved organic carbon [DOC], 0–100 mg/L). From 3 to 36% of the spiked triazines, however, were found to associate with the dissolved organic matter (DOM) in surface-water samples (DOC, 32.0–61.9 mg/L), suggesting the origin of the DOM is a key parameter in determining its association with and, thus, the availability of triazines.

Keywords—Liquid-phase microextraction Freely dissolved concentration Triazines Dissolved organic matter

INTRODUCTION

The freely dissolved concentration (C_{free}) of a pollutant generally is believed to be the effective concentration that is related more to the bioavailability and the toxicity than to the total concentration [1]; thus, various methods have been developed for sensing the C_{free} [2]. As examples, semipermeable membrane devices [3], Empore disk [4], polyethylene devices [5], triolein-embedded cellulose acetate membrane [6], and solid-phase microextraction (SPME) [2] have been developed for sampling hydrophobic organic compounds, whereas diffusion gradients in thin films, Donnan membrane technique, permeation liquid membrane, and gel-integrated microelectrode were developed for the free/labile fraction of metals [7,8]. Negligible depletion SPME is very efficient in sensing the C_{free} of organic compounds [2], but it suffers from low sensitivity for analytes with a log octanol–water partitioning coefficient (K_{OW}) of less than three [9–11].

Recently, the environmental fate and toxicity of polar organic compounds including priority pollutants, such as phenoxy acid and triazine herbicides, as well as those of emerging contaminants, such as pharmaceuticals and their metabolites, pesticide degradation products, perfluorooctanoic acid, and perfluorooctanesulfonate, are of increasing concern [12,13]. Most of these compounds are hydrophilic or low hydrophobic, with log $K_{\text{OW}} < 3$. Methods for sampling the freely dissolved polar organic pollutants, however, are very limited. Two kinds

of passive samplers were specifically developed for the sampling of polar organic compounds [14,15]. These samplers are operated in the kinetic uptake regime, where sampling efficiency is prone to be affected by environmental parameters, such as the flux of flow, and calibrations usually are needed. Recently, equilibrium sampling techniques were developed to avoid the calibration of dynamic parameters, such as uptake rates. Equilibrium sampling through membranes, based on a three-phase hollow fiber-protected liquid-phase microextraction (HF-LPME) with an organic phase sandwiched between two aqueous phases, was developed for sensing the C_{free} of ionogenic organic compounds [16]. Thin liquid film extraction [17], based on supporting submicroliters of organic solvent ($\sim 0.2 \mu\text{L}$) in the pores of a hollow fiber membrane wall, was developed for organic compounds with log $K_{\text{OW}} > 3$. Two-phase HF-LPME technique, based on organic solvent filled in the lumen of a hollow fiber, has the potential to be adapted for sensing the C_{free} of organic compounds with log $K_{\text{OW}} < 3$. The large volume of sampling phase can enhance the method sensitivity, and the microporous hollow fiber membrane also can serve as a barrier for preventing the transport of dissolved organic matter (DOM)-associated analytes into the sampling phase. To our knowledge, however, all the developed two-phase HF-LPME methods have focused on the analysis of total concentration, and their application in sensing the C_{free} has not been reported [18,19]. The modification of samples and the nature of exhaustive extraction in these procedures could disturb the species (the freely dissolved and the associated) of the analytes and, therefore, prohibit the measurement of C_{free} .

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In the present study, negligible depletion HF-LPME (nd-HF-LPME) was developed for sensing the C_{free} and, thus, for evaluating the availability of polar organic compounds with $\log K_{\text{OW}} < 3$. This new sampling method was adapted from two-phase HF-LPME by prohibiting any manipulation of the samples and, thus, preserving the analyte speciation and sorption equilibria in the samples; extracting less than 5% of the freely dissolved amount of an analyte (negligible depletion extraction) for retaining the equilibrium between the free and the bound form undisturbed; and adopting sufficient extraction time to ensure equilibration between the sampling phase and the sample. The herbicides simazine (SIM) and atrazine (ATR), as well as its degradation product, desethyl atrazine (DEA), were adopted as model compounds because of their low $\log K_{\text{OW}}$ values (< 3) and the well-documented biological exposure results for comparison. Although two-phase [20] and three-phase [21] HF-LPME, as well as single-drop LPME [22], have been developed for the analysis of triazines, they are directed at determinations of total concentration that are not suitable for evaluating the availability of triazines. With the C_{free} determined by this newly developed method, the effects of DOM, pH, and salinity on the availability of ATR, SIM, and DEA were evaluated in the present study.

MATERIALS AND METHODS

Materials and chemicals

The Accurel Q 3/2 polypropylene hollow fiber membrane (inner diameter, 600 μm ; wall thickness, 200 μm ; pore size, 0.2 μm) was purchased from Membrana (Wuppertal, Germany). Solid DEA, SIM, and ATR standards were purchased from AccuStandard (New Haven, CT, USA). Individual standard stock solutions of DEA (400 $\mu\text{g}/\text{mL}$), SIM (200 $\mu\text{g}/\text{mL}$), and ATR (200 $\mu\text{g}/\text{mL}$) were prepared in methanol and stored in a refrigerator at 4°C. The working solutions were prepared daily by appropriate dilution of the stock solution with water. The high-performance liquid chromatography (HPLC)-grade 1-octanol and di-*n*-hexyl ether were purchased from Sigma-Aldrich (Steinheim, Germany). Terrestrial-origin humic acid sodium salts from Acros Organics (lignite, technical, 50–60% as humic acid; Acros Organics, Morris Plains, NJ, USA) and Aldrich humic acid salt (technical lot S15539-384; Sigma-Aldrich) were used as obtained. Bovine albumin V (BSA) was purchased from Biodee Biotechnology (Beijing, China). The dissolved organic carbon (DOC; determined with a Phoenix 8000 UV-persulfate total organic carbon analyzer; Tekmar-Dohrmann, Cincinnati, OH, USA) for Acros humic acid, Aldrich humic acid, and BSA were 45.0, 35.1, and 24.9%, respectively. High-performance liquid chromatography-grade methanol and acetonitrile were purchased from J.T. Baker (Phillipsburg, NJ, USA).

HPLC separation

The HPLC system consists of an Agilent (Santa Clara, CA, USA) 1100 Series isocratic pump (IsoPump) and an Agilent 1100 Series UV/Vis detector set at 220 nm. Chromatographic data were processed with an Agilent ChemStation program for the HPLC system. The separation of DEA, SIM, and ATR was conducted on a C_{18} column (length, 150 mm; inner diameter, 4.6 mm; pore size, 5 μm ; Zorbax RX- C_{18} ; Agilent) using a mixture (50:50, v/v) of acetonitrile and phosphate buffer (5 mM, pH 7.0) as mobile phase at a flow rate of 0.8 mL/min. To quantify the amount of analytes taken up into the sampling

device, external calibration curves were prepared by direct injection of 20- μL aliquots of standard solution (0.01–10 mg/L) prepared in methanol.

Procedure of nd-HF-LPME

The sampling device was prepared as follows: The porous polypropylene hollow fiber was manually cut into a 5.2-cm length, and the two ends were sealed with heated tweezers. Then, the two-end-sealed hollow fiber was immersed and sonicated in 1-octanol for 2 h to impregnate the lumen with 1-octanol. After that, the hollow fiber was taken out, immersed into water, and then shaken for approximately 1 min to wash out surplus organic solvent in the water. Finally, one sealed end of the hollow fiber was clamped with a stainless-steel clamp to sink the hollow fiber into the water sample. Generally, the prepared HF-LPME device had an effective fiber length of approximately 5.0 cm, with a sampling phase of approximately 12 μL in the lumen and approximately 16 μL in the micropores of the wall (66% porosity). The schematic illustration of the HF-LPME sampling device is shown in Figure S1 (<http://dx.doi.org/10.1897/08-235.S1>).

For sampling, the hollow fiber sampler, prepared as described above, was put into 250-mL sample solution held in a 500-mL capped flask and shaken properly on a shaker (Model SA-31; Yamato, Tokyo, Japan). After extracting for the prescribed time, the hollow fiber sampling device was removed from the aqueous solution, and subsequently, the two sealed ends were cut open to flush the organic solvent ($\sim 11 \mu\text{L}$) in the lumen of the hollow fiber into a 200- μL glass vial with a microsyringe. Then, 10 μL of the collected organic solvent were manually aspirated into a HPLC microsyringe with 10 μL of preaspirated methanol, and the mixture obtained (total volume, 20 μL) was injected into the HPLC system for triazine analysis. The used fiber was discarded, and a fresh one was used for the next experiment. Because the analyte concentrations were very low ($\mu\text{g}/\text{L}$ level), there should be no difference in the partitioning behavior between single-compound and multi-compound systems. Therefore, standards with mixed triazines were used in the present study.

Sample collection and analysis

Three surface-water samples, two from a natural basin (Xingtang, Hebei, China) and one from a lake (Shijiazhuang, Hebei, China), were collected into glass bottles and stored at 4°C. The samples were analyzed with nd-HF-LPME after being filtered through a nylon membrane (pore size, 0.45 μm). The C_{free} of the studied chemicals also was determined after spiking 5 or 50 $\mu\text{g}/\text{L}$ each of the triazines standards and 100 mg/L of sodium azide, which was to avoid biodegradation. Before conducting the nd-HF-LPME procedure, the spiked samples were shaken for 1 h to allow the equilibration of the studied triazines with the sample matrix.

Effect of DOM on availability of triazines

With humic acid and BSA as models, the effect of DOM on C_{free} was studied using synthetic sample solutions (100 mM phosphate buffer, pH 7.0) with various concentrations of DOC (0–50 mg/L for humic acid and 0–100 mg/L for BSA). The solutions were shaken for 1 h before sampling with nd-HF-LPME to ensure equilibrium between the target compounds and DOM.

Data processing and calibration

All experiments were duplicated, and the mean values were reported. Because the present experiments demonstrated that the distribution coefficient between the 1-octanol and the aqueous solution (D_{OW}) remains constant and is independent of the environmentally relevant salinity and pH, the C_{free} of an analyte can be calibrated as follows:

$$C_{free} = C_{octanol}/D_{OW} \quad (1)$$

where $C_{octanol}$ is the analyte concentration in 1-octanol filled in the lumen of the hollow fiber and C_{free} is the analyte concentration of sample, which can be regarded as a constant equal to its initial concentration in negligible depletion extraction.

RESULTS AND DISCUSSION

Equilibration time

The analyte concentration in the sampling phase increases until the sampler and its surroundings have reached a thermodynamic equilibrium. To minimize biased-low measurements, it is very important to confirm that the thermodynamic equilibrium is reached [23]. Two requirements should be satisfied to evaluate the equilibrium: One is the constant $C_{octanol}$, and the other is the constant D_{OW} ($D_{OW} = C_{octanol}/C_{free}$), which also is consistent with the relevant partition coefficient between the 1-octanol and aqueous solution (K_{OW}). Several parameters that influence the equilibration time, including agitation, sampling phase, and environmentally relevant sample salinity and pH, were investigated in detail.

In HF-LPME, diffusion through the aqueous diffusion layer usually is the rate-limiting process, and the extraction can be accelerated by stirring or shaking. The equilibration time for all the analytes was up to 2 d without agitation (data not shown), but it reduced to only 60 and 240 min with stirring (500 rpm) and shaking (90 rpm), respectively (Fig. 1). Although Figure 1 demonstrates that the dynamic equilibrium time with stirring was much shorter than that with shaking, shaking was adopted in further experiments, because it facilitated batch experiments and, thus, increased the overall sampling efficiency. The effect of shaking rate on equilibration time also was investigated. Extraction for 174 and 140 min ensured all the compounds reached equilibration at 115 and 140 rpm, respectively. The $C_{octanol}$ fell sharply after shaking 150 min at 140 rpm (Fig. S2 [http://dx.doi.org/10.1897/08-235.S1]), probably because strong shaking leads to little water entering into the extraction phase. In a subsequent study, sampling was carried out by shaking at 115 rpm.

The organic solvent filled in the lumen of the hollow fiber as the sampling phase dramatically influenced the equilibration time. By replacing the 1-octanol sampling phase with di-*n*-hexyl ether, one of the most frequently used polar organic solvents for supported liquid membrane extraction of polar organic compounds, the equilibration time reduced to less than 15 min for SIM and 60 min for ATR (Fig. S3 [http://dx.doi.org/10.1897/08-235.S1]). This is because di-*n*-hexyl ether is more hydrophobic than 1-octanol, and the partition coefficients to it are much lower than that of 1-octanol for the three triazines studied. Di-*n*-hexyl ether obviously can shorten the equilibration time, but the equilibrium concentration of analytes in di-*n*-hexyl ether was approximately 10% of that in 1-octanol. To satisfy the requirement of a low detection limit, 1-octanol was adopted in the following experiments.

To study the effect of environmentally relevant salinity (0–

Fig. 1. Effect of agitation on the uptakes of desethyl atrazine (DEA; □), simazine (SIM; ▽), and atrazine (ATR; △) to the negligible depletion hollow fiber-protected liquid-phase microextraction (nd-HF-LPME) sampling device: (a) Stirring at 500 rpm; (b) shaking at 90 rpm. Sample solution, 100 μ g/L of DEA and 50 μ g/L each of SIM and ATR in 1 mM phosphate buffer (125 mL, pH 7.0); sampling phase, 1-octanol filled in the hollow fiber.

500 mM) and pH (5.0–8.5) [11] on equilibration time, the respective equilibration time was examined using two paired solutions of 0 and 500 mM NaCl (in 1 mM phosphate buffer, pH 7.0) as well as at pH 5.0 and 8.5 (in 50 mM phosphate buffer), respectively. Table 1 shows the calculated uptake rate constant (k), equilibration time ($t_{90\%}$), and goodness of the fit (R^2), based on their uptake profiles in various sample matrices. Obviously, the equilibration time in all the tested cases had the order ATR > SIM > DEA, which was attributed to the same sequence of D_{OW} (Table 2). The significantly longer equilibration time in 500 mM NaCl can be explained by the slower diffusion of the analytes from the aqueous solution with higher salinity to the sampling phase. The longer equilibration time at pH 5.0 might be attributed to the slight protonization of triazines (alkaline organic pollutants) that reduced the rates of their uptake into the sampling phase. Because the $C_{octanol}$ of ATR for a 180-min extraction was comparable to that for a 240-min extraction in 500 mM NaCl solution (data not shown), 180 min, based on Table 1, was sufficient to ensure that all the analytes reached equilibrium and was adopted in the following studies.

Table 1. Effect of sample matrix on the uptake rate constant (k), equilibration time ($t_{90\%}$), and goodness of the fit of the data (R^2) from different sample solutions (100 $\mu\text{g/L}$ of desethyl atrazine [DEA] and 50 $\mu\text{g/L}$ each of simazine [SIM] and atrazine [ATR] spiked in different matrices) to the hollow fiber-protected liquid-phase microextraction (HF-LPME) sampling devices

Sample matrix	Compounds	k (1/min)	$t_{90\%}$ (min)	R^2
1 mM phosphate buffer (pH 7.0)	DEA	0.054	43	0.70
	SIM	0.025	92	0.87
	ATR	0.013	177	0.96
Mixture of 1 mM phosphate and 500 mM NaCl (pH 7.0)	DEA	0.041	56	0.83
	SIM	0.017	135	0.95
	ATR	0.0097	237	0.98
pH 5.0 (50 mM phosphate buffer)	DEA	0.042	55	0.89
	SIM	0.026	88	0.99
	ATR	0.014	164	0.98
pH 8.5 (50 mM phosphate buffer)	DEA	0.058	40	0.84
	SIM	0.032	72	0.90
	ATR	0.018	128	0.97

Sample depletion

For equilibrium sampling, it is very important to confirm that the sample volume is sufficiently large to avoid disturbing the equilibrium between the bound and the freely dissolved analytes. Usually, a depletion of less than 5% of the freely dissolved amount is adopted as a criterion of negligible depletion sampling [23]. Studies with samples prepared in 50 mM phosphate buffer (pH 7.0) showed that C_{octanol} increased with the sample volume up to 250 ml and then remained constant (Fig. S4 [http://dx.doi.org/10.1897/08-235.S1]), and the depletion was less than 5% for all the compounds at this volume. In the following studies, a sample volume of 250 ml was selected for negligible depletion sampling.

Calibration

Because C_{free} is calibrated with the determined C_{octanol} and D_{OW} according to Equation 1, reliable determination of C_{free} depends on the accurate determination of C_{octanol} and the calibration of D_{OW} in aqueous samples with various environmental matrices. The effect of environmentally relevant salinity (0–500 mM NaCl) [11] was studied using sample solutions with various concentrations of NaCl prepared in 5 mM phosphate buffer (pH 7.0). As shown in Figure 2a, the D_{OW} values increased gradually with salinity, and this increase in higher concentrations of NaCl (250–500 mM) exhibited a significant difference from the NaCl-free solutions ($p < 0.05$). This could be easily explained by the salting-out effect. The calculated relevant standard deviations (RSDs) of D_{OW} in the entire en-

Fig. 2. Effect of salinity on the distribution coefficients of desethyl atrazine (DEA; □), simazine (SIM; ▨) and atrazine (ATR; ■) to 1-octanol: (a) NaCl prepared in 5 mM phosphate buffer; (b) Phosphate. Sample solution, 250 ml of 100 $\mu\text{g/L}$ of DEA and 50 $\mu\text{g/L}$ each of SIM and ATR (pH 7.0); sampling phase, 1-octanol; sampling time, 180 min. Asterisks represent results that are significantly different from the 0 mM NaCl or 1 mM phosphate group ($p < 0.05$).

vironmentally relevant range (0–500 mM) were 13, 15, and 10% for DEA, SIM, and ATR, respectively. The effect of salinity also was studied using phosphate solutions with varied concentrations (1–200 mM, pH 7.0). As shown in Figure 2b, the phosphate concentration slightly influenced the D_{OW} values, with RSDs of 17, 14, and 5.0% for DEA, SIM, and ATR, respectively. A significant statistical difference of the D_{OW} values in higher concentrations of phosphate (50–200 mM)

Table 2. Nonlinear regression of regression coefficient (R^2), distribution coefficient to 1-octanol (K_{OW}), and the negative log of the acid dissociation constant ($\text{p}K_{\text{a}}$) of desethyl atrazine (DEA), simazine (SIM) and atrazine (ATR) based on data at different pH values (pH 1.25–10.0, 100 mM phosphate buffer) and literature values

Compounds	R^2	$\text{p}K_{\text{a}}$		$\log K_{\text{OW}}$	
		Fitted	Literature	Fitted	Literature
DEA	0.84	1.83 ± 0.38	1.65 ^a	1.41 ± 0.01	1.51 ^b
SIM	0.91	1.63 ± 0.30	1.62 ^c	2.12 ± 0.01	2.18 ^b
ATR	0.93	1.03 ± 0.60	1.71, ^a 1.70 ^{cd}	2.41 ± 0.01	2.61, ^b 2.3–2.7 ^d

^a Abate et al. [36].

^b Steen et al. [25].

^c Shen and Lee [20].

^d Xing et al. [26].

Fig. 3. Effect of sample pH on the distribution coefficients of desethyl atrazine (DEA; $\square$), simazine (SIM; ∇), and atrazine (ATR; $\triangle$) to 1-octanol. The lines represent the fit of Equation 2 to the data. Sample solution, 100 $\mu\text{g/L}$ of DEA and 50 $\mu\text{g/L}$ each of SIM and ATR in 100 mM phosphate buffer (250 ml); sampling phase, 1-octanol; sampling time, 180 min.

from that in the 1 mM phosphate was observed ($p < 0.05$). The D_{OW} values measured at a solution of 100 mM phosphate were adopted to calibrate the effect of salinity in the present study. The slight increase of D_{OW} with salinity suggests the availability of triazines was enhanced with the presence of salt in the aqueous media.

The partitioning of ionizable hydrophobic chemicals to 1-octanol can be remarkably influenced by pH, and lower distribution coefficients in 1-octanol are obtained when part of these compounds are ionized [24]. The D_{OW} values of these compounds increased dramatically when the pH increased from 1.2 to 2.0, then slightly increased in the pH range of 2.0 to 3.0, and finally, remained almost constant in the pH range of 3.0 to 10.0 (Fig. 3). This result was consistent with those reported in the literature [20], and it could be explained by the $\text{p}K_{\text{a}}$ (the negative log of the acid dissociation constant) values of these compounds. With $\text{p}K_{\text{a}}$ values of approximately 2.0 (Table 2), these compounds were mainly protonated at pH 1.0 or less, partly protonated at pH 1.0 to 3.0, and completely in neutral molecular form at pH 3.0 or greater. Because the neutral molecular form has a higher K_{OW} than the protonated form, profiles of D_{OW} to pH as shown in Figure 3 were observed. The almost-constant D_{OW} values at pH 3 to 10 indicated that it was unnecessary to calibrate the effect of pH within the environmentally relevant pH range (5.0–8.5) [11]. Mean-

while, the constant D_{OW} indicated that the availability of triazines was not affected under the environmentally relevant pH.

Assuming both the molecular form (A) and the protonated form (HA) of DEA, SIM, and ATR can partition into 1-octanol with the respective partitioning coefficient of $K_{\text{OW,HA}}$ and $K_{\text{OW,A}}$, the D_{OW} at different pH was fitted to the following equation [11]:

$$D_{\text{OW}} = (K_{\text{OW,HA}} + K_{\text{OW,A}} \cdot 10^{\text{pH}-\text{p}K_{\text{a}}}) / (1 + 10^{\text{pH}-\text{p}K_{\text{a}}}) \quad (2)$$

The experimental results fitted well with Equation 2, with correlation coefficients (R^2) in the range of 0.84 to 0.93 (Fig. 3). The nonlinear regression values of K_{OW} ($K_{\text{OW,HA}}$ in Eqn. 2) and the $\text{p}K_{\text{a}}$ of HA represented in Table 2 demonstrated their agreement with those reported in the literature. The relatively large deviation of $\text{p}K_{\text{a}}$ might be attributed to the absence of data at pH 1 or less, which is difficult to measure precisely with a common pH meter.

Analytical performance and D_{OW} determination

To investigate the analytical performance and determine the D_{OW} , 100 mM phosphate solutions (pH 7.0) spiked with 0.1 to 200 $\mu\text{g/L}$ of analytes were adopted. Results demonstrated that the proposed method possessed good linearity ($R^2 = 0.9971\text{--}0.9997$) and excellent precision at the 5 $\mu\text{g/L}$ level (RSD, 1.7–2.1%, $n = 3$). The calculated detection limits (signal to noise ratio = 3) for DEA, SIM, and ATR were 1.0, 0.2, and 0.1 $\mu\text{g/L}$, respectively. Lower detection limits can be expected by injecting a larger volume of sampling phase into the HPLC system or using a more sensitive detector. This proposed method is simple, and the sample preparation/extraction is very rapid and not labor-intensive. The log D_{OW} values were independent of the chemical concentration (Table S1 [http://dx.doi.org/10.1897/08-235.S1]) and agreed well with the log K_{OW} values reported in the literature [25,26].

Application in real sample analysis

As shown in Table 3, the C_{free} of all analytes in the studied real water samples were below their detection limits. Except for the measured C_{free} of ATR in the Shijiazhuang sample at the 5 $\mu\text{g/L}$ spiked level, which had relatively high deviation, the measured C_{free} of analytes were between 64 and 97% of the spiked concentration, suggesting that from 3 to 36% of the analytes were associated with DOM in these samples.

Effect of DOM on availability of triazines

The results of the effect of DOC are shown in Figure 4 after normalizing to the C_{free} without DOC. Except for DEA,

Table 3. Freely dissolved concentrations (C_{free}) of desethyl atrazine (DEA), simazine (SIM), and atrazine (ATR) in surface water samples^a

Sample	pH	DOC (mg/L)	Spiked ($\mu\text{g/L}$)	C_{free} ($\mu\text{g/L}$)		
				DEA	SIM	ATR
Xingtang 1	8.46	32.0	0	ND	ND	ND
			5	3.75 ± 0.11	4.48 ± 0.11	4.33 ± 0.22
			50	47.5 ± 2.8	37.5 ± 1.1	41.1 ± 0.86
Xingtang 2	8.55	34.3	0	ND	ND	ND
			5	3.76 ± 0.18	4.85 ± 0.07	4.72 ± 0.09
			50	43.9 ± 3.8	38.4 ± 2.5	40.4 ± 2.8
Shijiazhuang	8.57	61.9	0	ND	ND	ND
			5	3.69 ± 0.17	4.75 ± 0.38	5.19 ± 0.41
			50	42.1 ± 3.5	32.0 ± 2.5	36.5 ± 2.1

^a DOC = dissolved organic carbon; ND = not detected.

Fig. 4. Effect of dissolved organic carbon (DOC) concentration on the freely dissolved concentration (C_{free}) of desethyl atrazine (DEA; □), simazine (SIM; ▨), and atrazine (ATR; ■): (a) Aldrich humic acid; (b) Acros humic acid; (c) bovine albumin V (BSA). The C_{free} in the presence of DOC was normalized to that without DOC. Sample solution, 100 μ g/L of DEA and 50 μ g/L each of SIM and ATR in 100 mM phosphate buffer with various concentrations of DOC (250 mL, pH 7.0); sampling phase, 1-octanol; sampling time, 180 min. Asterisks indicate that C_{free} in DOC solutions is significantly elevated relative to corresponding DOC-free solutions ($p < 0.05$).

the normalized C_{free} of the triazines were slightly enhanced with the addition of DOC. For the tested humic acid solutions, statistical analysis with one-way analysis of variance indicated that the normalized C_{free} of each analyte in the DOC-present solutions generally was not significantly different from that in the DOC-free solutions ($p > 0.05$), except for SIM or ATR in higher concentrations of DOC (25 or 50 mg/L). For the BSA solutions, however, the normalized C_{free} of SIM and ATR in the DOC-present solutions were significantly enhanced compared to those in the DOC-free solutions ($p < 0.05$). This apparent enhancement was strengthened in high concentrations of DOC, such as 25 to 50 mg/L for humic acid and 100 mg/L for BSA. These results were contrary to those reported in literatures [20,22], in which a sensible decrease of the extracted triazines was observed with the presence of humic acid. One possible reason is that the presence of humic acid decreased the extraction rates of SIM and ATR; thus, the amount of extracted analytes reduced with the nonequilibrium extraction procedures adopted in the literature. Another possible cause is that the humic acids (unknown source) used in literature [20,22] are able to associate with triazines.

Whereas the Aldrich and Acros humic acids were seldom associated with triazines, an association between triazines and the DOM in real surface-water samples was observed (Table 3). These results suggest that the origin of the DOM is a key parameter to determine its association with and, thus, the availability of triazines. In fact, the effect of DOM on the availability of triazines remains controversial. Membrane dialysis results indicated that ATR could associate with DOM through interior adsorption sites on the DOM molecule by transitory hydrogen bonding and subsequent physical entrapment [27], suggesting that DOM might reduce the availability of ATR, which disagrees with exposure results reported in the literature [28,29]. Ultrafiltration results [30] demonstrated relatively low binding affinity of humic substances for ATR, and the reported K_{OC} values (partition coefficients for the binding affinities of ATR) to 16 different humic materials varied from 87 to 575 L/kg of carbon. A recent SPME study [31] indicated that no significant partitioning could be observed for ATR to three different DOM (including Aldrich humic acid and soil humic acid); the authors attributed it to the low sensitivity and the bad repeatability (RSD, 8–19%) of the SPME gas chromatography method for organic compounds with $\log K_{OW} < 5$.

This slight enhancement of C_{free} with DOC concentration, however, should not be neglected. This phenomenon also was observed for other polar compounds, such as dinitrophenolic compounds [32], bisphenol A [33], and alkylphenols [17]. Biological exposure experiments indicated that statistically, the bioavailability of ATR was not affected by the DOM [28,29], but enhanced uptake by *Daphnia magna* was observed in cases with low DOC concentrations [28]. Lorenz et al. [34] also reported that DOC increased the toxic effect of the triazine herbicide terbutylazine on zebrafish. Haitzer et al. [35] reviewed the comprehensive effects of DOM on the bioconcentration of organic chemicals in aquatic organisms. It was pointed out that DOM mostly caused decrease of bioconcentration but, in some cases, enhanced bioconcentration, and the enhancement (2–303%) only occurred at a DOC concentration of less than 10 mg/L. The enhancements of bioconcentration occurred in all classes of compounds (except organometallics) and for various types of DOM, but the mechanisms remained unknown.

CONCLUSION

The enhancement of ATR uptake with the presence of DOM observed in the present study agreed with that reported in some bioconcentration experiments, suggesting this nd-HF-LPME technique might be developed as a biomimic extraction method for assessing the bioavailability of polar organic compounds. This proposed method provides access to determination of 1-octanol to water partition coefficients and the freely dissolved analyte concentrations as well as the binding to DOM. Because 1-octanol was used as the sampling phase, the measured C_{octanol} is compatible with many environmental risk assessment and fate models, in which K_{OW} was used to estimate bioconcentration factors and environmental phase partitioning. In addition, the widely available K_{OW} values of most compounds provide a helpful reference to verify the method reliability. Furthermore, this proposed method is conducted in the equilibrium uptake range and with negligible sample depletion; thus, in principle, this method is applicable for field sampling. Further study, however, is needed to demonstrate the stability of the device and to determine the equilibration time under field conditions.

SUPPORTING INFORMATION

Table S1. The measured log distribution coefficient to 1-octanol (D_{OW}) of triazines under optimized sampling conditions (desethyl atrazine [DEA], simazine [SIM], and atrazine [ATR] were spiked in 250 ml of 100 mM phosphate buffer, pH 7.0).

Fig. S1. Schematic illustration of the hollow fiber-protected liquid-phase microextraction (HF-LPME) sampling device: 1 = sample bottle; 2 = sample; 3 = HF-LPME sampling device; 4 = the stainless-steel clamp; 5 = wall of the hollow fiber; 6 = lumen of the hollow fiber. Both the wall and the lumen of the hollow fiber were impregnated with 1-octanol.

Fig. S2. Effect of shaking rates on the uptake profiles of triazines. Sample solution: 125 ml of 100 $\mu\text{g/L}$ of desethyl atrazine (DEA) and 50 $\mu\text{g/L}$ each of simazine (SIM) and atrazine (ATR) in 1 mM phosphate buffer (pH 7.0), and shaken at (a) 115 rpm or (b) 140 rpm.

Fig. S3. The uptake profiles of simazine (SIM) and atrazine (ATR) to di-*n*-hexyl ether filled in the hollow fiber-protected liquid-phase microextraction (HF-LPME) sampling device. Note the analyte concentration in di-*n*-hexyl ether sampling phase (C_{DHE}), and desethyl atrazine (DEA) is not shown (DEA is less than the detection limit). Sample solution: 125 ml of 100 $\mu\text{g/L}$ DEA and 50 $\mu\text{g/L}$ each of SIM and ATR in 1 mM phosphate buffer (pH 7.0).

Fig. S4. Effect of sample volume on the sampling of desethyl atrazine (DEA), simazine (SIM), and atrazine (ATR). Sample solution: 100 $\mu\text{g/L}$ of DEA and 50 $\mu\text{g/L}$ each of SIM and ATR in 5 mM phosphate buffer (pH 7.0). Sampling phase: 1-octanol; sampling time: 180 min.

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