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Solid-phase Microextraction (SPME) with Stable Isotope Calibration for Measuring Bioavailability of Hydrophobic Organic Contaminants

Xinyi Cui, Lianjun Bao, and Jay Gan*

Department of Environmental Sciences, University of California, Riverside, CA 92521

Abstract

Solid-phase microextraction (SPME) is a biomimetic tool ideally suited for measuring bioavailability of hydrophobic organic compounds (HOCs) in sediment and soil matrices. However, conventional SPME sampling requires the attainment of equilibrium between the fiber and sample matrix, which may take weeks or months, greatly limiting its applicability. In this study, we explored the preloading of polydimethylsiloxane fiber with stable isotope labeled analogs (SI-SPME) to circumvent the need for long sampling time, and evaluated the performance of SI-SPME against the conventional equilibrium SPME (Eq-SPME) using a range of sediments and conditions. Desorption of stable isotope-labeled analogs and absorption of PCB-52, PCB-153, bifenthrin and *cis*-permethrin were isotropic, validating the assumption for SI-SPME. Highly reproducible preloading was achieved using acetone-water (1:4, v/v) as the carrier. Compared to Eq-SPME that required weeks or even months, the fiber concentrations (C_f) under equilibrium could be reliably estimated by SI-SPME in 1 d under agitated conditions or 20 d under static conditions in spiked sediments. The C_f values predicted by SI-SPME were statistically identical to those determined by Eq-SPME. The SI-SPME method was further applied successfully to field sediments contaminated with PCB 52, PCB 153, and bifenthrin. The increasing availability of stable isotope labeled standards and mass spectrometry nowadays makes SI-SPME highly feasible, allowing the use of SPME under non-equilibrium conditions with much shorter or flexible sampling time.

Introduction

Bioavailability of hydrophobic organic contaminants (HOCs) in sediments or soils has been the subject of many studies over the last two decades. Conceptually, in a sediment or soil, a HOC must be in the freely dissolved form to be bioavailable, because the freely dissolved concentration (C_{free}) controls diffusive mass transfer processes such as evaporation, sorption, and uptake into macro- and microorganisms.¹ The determination of C_{free} is therefore essential for estimating HOC bioavailability.

Passive samplers have been explored for C_{free} determination, which include semipermeable membrane devices (SPMDs) and polyethylene devices (PEDs). However, sorbent-based samplers operate under the presumption of equilibrium, which may take weeks or even months to achieve for strongly hydrophobic compounds. One approach to circumvent this constraint is to preload the passive sampler with a performance reference compound (PRC).

*Corresponding author: Jay Gan, Department of Environmental Sciences, University of California, Riverside, CA 92521, U.S.A., Tel: 951-827-2712, Fax: 951-827-3993, jgan@ucr.edu.

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In such an application, the desorption rate constant of the preloaded PRC is used to approximate the absorption rate constant of the target analyte, a condition that should be readily met if the PRC is an isotope labeled analogue.^{2,3} The PRC-based calibration approach has been applied for PEDs and SPMDs to calibrate sampling and eliminate the effect of environmental factors for in situ sampling.⁴⁻⁷

When compared to SPMDs or PEDs, the small size and non-depletive nature of a solid phase microextraction (SPME) fiber make it much more applicable for evaluating bioavailability, especially in solid matrices such as soils and sediments.⁸⁻¹¹ For instance, at equilibrium, C_{free} in a sediment may be derived from the concentration on the fiber (C_f) through the use of a fiber-water partition coefficient (K_{fiber}). There are two general types of SPME configurations, i.e., injector-type SPME and disposable SPME. In particular, disposable SPME makes use of only the fiber without the accessories of an injector-type SPME assembly, lending much increased flexibility, as well as compatibility with bench-scale bioassays. The concurrent exposure of disposable fibers and organisms in the same bioassay chamber is considered to be representative of organism exposure, and consequently a highly accurate approach for evaluating bioavailability.¹²⁻¹⁴ However, the requirement for equilibrium is still a significant technical barrier limiting the usefulness of SPME in bioavailability assessment,¹⁵⁻¹⁸ Options such as the use of thinner coating have been proposed;¹⁹ however, thinner polymer coating generally coincides with a smaller sampling volume that makes the sampling less sensitive. The use of PRC kinetic calibration may be used to overcome the dilemma of requiring long exposure time in SPME sampling, thus greatly expanding its applicability. In studies from Pawlitzyn's group, injector-type SPME preloaded with labeled standards was successfully used to analyze the total analyte concentration (not C_{free}) in river water,²⁰ wine,²¹ and blood samples.²² In a recent study, a C18-coated fiber with PRC calibration was used to determine C_{free} of pharmaceuticals in fish tissues,²³ highlighting the potential for other novel applications, such as in situ deployment in complex environmental matrices.

In this study, we explored the preloading of disposable SPME fibers with stable isotope-labeled HOCs as PRCs and tested stable isotope-SPME (SI-SPME) against equilibrium SPME (Eq-SPME) for bioavailability evaluation in sediments under different conditions. We anticipate that coupling stable isotopes with fiber will greatly improve the feasibility and capability of disposable SPME as a biomimetic tool because of the removal of the stringent sampling time requirement.

Theoretical Considerations

Loading of Labeled Compounds

The absorption of the isotope labeled PRC from the preloading solution onto the fiber could be described as:²⁴

$$C_f = K_{fs} C_w (1 - \exp(-k_e t)) \quad (1)$$

with the rate constant k_e defined as:

$$k_e = \frac{k_w A}{K_{fs} V_f} \quad (2)$$

where C_f is the PRC concentration in fiber, K_{fs} is the partition coefficient between the fiber and PRC solution, C_w is concentration of PRC in the preloading solution, k_w is mass transfer

coefficient for the aqueous boundary layer, A is the fiber surface area, and V_f is the fiber coating volume.

From Eq. 1, an increase in k_e would increase the impregnation rate of PRC onto the fiber. A few studies on PEDs showed that using mixtures of water-miscible solvents as the preloading solution decreased K_{fs} or increased k_e and led to faster loading. However, if the solvent fraction is too high or pure solvent is used, there may be large variations due to rapid solvent evaporation. Moreover, the partition of HOCs into the polymer phase may be inhibited if the solvent ratio is too high because of the decreased K_{fs} .^{24, 25} Therefore, based on previous studies, a mixture of acetone-water (20/80, v/v) was selected in this study as the preloading solution to achieve fast loading and good preloading reproducibility.

Kinetic Calibration

Absorption of HOCs from sediment matrix by SPME fiber can be described as:²⁶

$$n = n_0(1 - \exp(-k_{\text{abs}}t)) \quad (3)$$

where n and n_0 are the amounts of HOC absorbed onto the fiber at time t and at equilibrium, respectively and k_{abs} is the absorption rate constant. Likewise, desorption of a preloaded PRC from the fiber may be described as:^{3, 21}

$$q = q_0 \exp(-k_{\text{des}}t) \quad (4)$$

where q_0 is the preloaded amount of the labeled analog on the sampler, q is the amount of labeled analog remaining at time t , and k_{des} is the desorption rate constant.

When stable isotope-labeled analogs are used as PRCs, the desorption of PRCs from passive samplers into the sample matrix may be assumed to be isotropic to the absorption of the native HOCs onto the sampler from the sample matrix.^{2,3} Therefore, Eq. 3 and 4 may be combined as:

$$\frac{q}{q_0} + \frac{n}{n_0} = 1 \quad (5)$$

The sum of q/q_0 and n/n_0 being 1 at any sampling time would suggest isotropy of absorption and desorption processes, i.e., $k_{\text{abs}} = k_{\text{des}}$. In the operation of SI-SPME, n_0 may be obtained using Eq. 5 after q and n are determined from the mass spectrometric analysis of the fiber, which can then be used to calculate C_f , i.e., $C_f = n_0/V_f$, where V_f is the fiber coating volume.

Materials and Methods

Chemicals and Materials

Four HOCs, including two PCBs (PCB52 and PCB153) and two pyrethroid insecticides (bifenthrin and *cis*-permethrin) were used as model HOCs in the method development and validation experiments. Non-labeled bifenthrin (98.8%) and *cis*-permethrin (97.0%) were obtained from FMC (Princeton, PA, USA) and non-labeled PCB52 and PCB153 were purchased from AccuStandard (New Haven, CT). Deuterated d_5 -bifenthrin (99%) was purchased from Toronto Research Chemicals (North York, Ontario, Canada), and phenoxy- $^{13}\text{C}_6$ -*cis*-permethrin (99%), $^{13}\text{C}_{12}$ -PCB52 (99%) and $^{13}\text{C}_{12}$ -PCB153 (99%) were obtained from Cambridge Isotope Laboratories (Andover, MA). Disposable PDMS fiber (430 μm glass core with 35 μm PDMS coating, Polymicro Technologies, Phoenix, AZ) was

cut into 1.0-cm long pieces with a razor blade and cleaned using Soxhlet extraction with ethyl acetate for 72 h before use. The volume of PDMS per 1-cm length of fiber was 0.51 μL . All solvents and other chemicals used in the study were gas chromatography (GC) or analytical grade.

Sediments

Five sediments were used, including EMP37 sediment (White Slough, Sacramento, CA), EMP73 sediment (Franks Tract, Sacramento, CA), Glen Charlie Pond sediment (GCP) (Wareham, MA), Sandy Creek sediment (SC) (San Diego County, CA), and a formulated sediment (FS) prepared according to OECD guidelines 218.²⁷ Selected sediment properties including total organic carbon content (TOC), soot carbon content, pH, cation exchange capacity (CEC), and particle size distribution were characterized using standard methods. The specific measurement procedures and selected properties are given in the Supporting Information (Table S1).

To generate sediment samples for method development, all five sediments were spiked with non-labeled HOCs at 0.1 mg/kg (dry wt) for PCBs and bifenthrin, and 0.4 mg/kg (dry wt) for permethrin (due to lower signal response on GC-MS/MS). Spiked sediment jars were capped and rolled for 48 h at 10 rpm to achieve homogenization.

Fiber Preloading Procedure

The method for fiber preloading was developed using non-labeled compounds. The acetone-water mixture (20:80, v/v) containing each HOC at 0.1 mg/L was used for the preloading kinetic test and solutions of 0.01, 0.1, and 1 mg/L were used for the preloading reproducibility test. For the preloading kinetics experiment, one 1-cm PDMS fiber was placed in 5 ml of the preloading solution in a 20-ml glass vial and mixed on a shaker at 80 rpm at room temperature. After 0.5, 1, 2, 4, 6, 10, 24, or 48 h, triplicate fibers were retrieved for analysis. In the reproducibility test, 10 pieces of 1-cm fibers were placed in 50 ml of preloading solution (0.01, 0.1, or 1 mg/L) in a 125-ml glass jar and mixed at 80 rpm. The fibers were retrieved after 24 h and analyzed. The reproducibility of the preloading procedure was evaluated by calculating the relative standard deviation (RSD) of the loaded amount on each fiber.

Isotropy Validation Experiment

The isotropy between absorption of HOCs and desorption of their labeled analogs was validated by the simultaneous determination of desorption time profile of the labeled PRCs and absorption time profile of the non-labeled HOCs. Briefly, one 1.0-cm piece of PRC-impregnated PDMS fiber was introduced into a 20-mL glass vial containing 1.0 g (dry wt equivalent) of spiked SC sediment and 1.0 mL of 0.2% NaN_3 solution (for inhibition of microbial biodegradation of HOCs). The vials were agitated on a shaker at 80 rpm, and triplicate fibers were retrieved after 6, 12, 24, 36, 48, 72, 96, 144, 192, 288, and 336 h. The concentrations of labeled and non-labeled HOCs on the fiber were determined by GC-MS/MS following solvent extraction.

Method Validation Experiment in Spiked Sediments

Performance of SI-SPME was validated by comparing against measurements made with the same samples using Eq-SPME in two different experimental settings. In the first experiment, desorption of PRCs and absorption of non-labeled HOCs were simultaneously measured in the spiked sediments under continuous mixing conditions. One 1.0-cm piece of PRC-preloaded fiber, 1.0 g (dry wt) of the spiked sediment, and 1.0 ml of 0.2% NaN_3 solution in 20-mL glass vials were mixed at 80 rpm on a shaker and fibers were removed after 1, 2, or 3

d of mixing. The fibers were analyzed to derive n_0 using Eq. 5 and then C_f . In a parallel experiment, C_f was measured using Eq-SPME. Briefly, one 1.0-cm piece of clean fiber was introduced into a 20-mL glass vial containing 1.0 g (dry wt) of the same spiked sediments and 1.0 mL of 0.2% NaN_3 solution. The vials were agitated at 80 rpm, and fibers were retrieved after 6 d of mixing. Preliminary experiments showed that an apparent equilibrium was attained after 6 d agitation for all HOCs (Figure S1). The retrieved fibers were extracted and analyzed for C_f at equilibrium. The C_f values obtained from the two different methods were statistically compared.

In the second experiment, SI-SPME was applied to a sediment-water system similar to that used for sediment chronic toxicity tests. Briefly, simulated test vessels were prepared in 300-ml glass jars by adding 10 to 50 g (dry wt, depending on the sediment type) to form a 1.5-cm sediment layer and hard water to form 6-cm overlaying water. One 1.0-cm piece of preloaded fiber was inserted into the sediment layer. The test vessels were kept at room temperature with a 16:8 h light:dark photoperiod under static conditions. Hard water was periodically added to maintain the overlaying water at the same level. Exposure duration of 20 d was selected to assure adequate desorption of PRCs and absorption of HOCs for reliable analysis, and also to simulate the time length used typically in sediment toxicity tests.^{28, 29} After 20 d, the fibers were removed for analysis to derive n_0 and then C_f . For comparison, C_f was also measured by Eq-SPME. While it would be ideal to measure C_f under static conditions, previous studies suggested that it may take more than 80 d to achieve equilibrium and that degradation loss may compromise C_f measurement.¹⁸ Moreover, it has also been shown that the equilibration method (e.g., agitation on shaker, static coexposure with *Lumbriculus variegates*) had no significant effect on C_f at equilibrium.¹⁶ Therefore, the C_f for Eq-SPME was measured under agitated conditions. Briefly, the simulated test vessels were prepared in the same way as that in the SI-SPME treatment, but without PDMS fiber. After 20 d, the overlaying water was carefully removed, and 1 g (dry wt) of sediment was removed from the test vessel and mixed with one piece of 1.0-cm clean PDMS fiber under agitated conditions for 6 d to reach equilibrium. The fibers were retrieved and analyzed for C_f . The C_f values derived from the two different methods were statistically compared.

Method Validation Experiment with Field Contaminated Sediments

The performance of the SI-SPME method was further tested using field samples. A PCB-contaminated sediment sample was collected from the ocean floor at the Palos Verdes Shelf (a Superfund site) along the Los Angeles coast in southern California. The bulk sediment concentrations of PCB 52 and PCB 153 were determined to be 205 ± 13 and 47 ± 1.2 ng/g (dry wt), respectively. A bifenthrin-contaminated sediment sample was collected in a drainage channel of a commercial nursery in Lake Forest, California, and the bulk sediment concentration was 13 ± 5.1 ng/g (dry wt). To measure C_{free} of PCBs, 2 g sediment (dry wt), 1 ml water with 0.2% NaN_3 , and 1 piece of 1-cm ^{13}C -PCB-preloaded fiber were placed together in a 20-ml glass vial and replicate vials were mixed on a shaker at 80 rpm. For bifenthrin, a similar setup was used except that 12 g (dry wt) sediment and 2 ml 0.2% NaN_3 solution were used due to the overall low bifenthrin level. The fibers were retrieved at 6, 12, and 24 h for PCB52, 1, 2, and 3 d for PCB153, and 17, 25, and 31 d for bifenthrin. Simultaneously, the Eq-SPME method was used to obtain C_f by mixing the samples till equilibrium. Time durations for reaching equilibrium were chosen as 2, 6, and 41 d for PCB52, PCB153, and bifenthrin based on preliminary kinetic test results (Figure S2). Extraction and analysis of fibers followed the same procedure as described above.

Chemical Analysis

The fibers were extracted using a simple solvent extraction method.⁹ Briefly, the retrieved fibers were gently wipe cleaned with a damp tissue paper to remove solids and were placed in 300- μ L conical glass inserts positioned in 2-mL GC vials. After addition of 200 μ L hexane, the vials were sonicated for 15 min in a sonication water bath. Preliminary experiments showed that recoveries of the tested HOCs were 91.3-119.8%.

Both non-labeled and labeled HOCs were analyzed on a Varian 3800 GC (Varian Instruments, Sunnyvale, CA) in tandem with a Varian 1200 triple-quadrupole mass spectrometer (MS/MS). Separation was achieved on a Factor Four-5MS (Varian) capillary column (30 m $\times$ 0.25 mm i.d.) with 5% diphenyl-95% dimethylsiloxane liquid phase (0.25 μ m film thickness). A 1.0- μ L aliquot of the sample was injected at 260 $^{\circ}$ C in the splitless mode at a constant flow of 1 mL min⁻¹. Helium (99.999%) was used as the carrier gas in the pressure-pulse mode (45 psi for 0.8 min). The oven temperature started at 90 $^{\circ}$ C, and increased at 15 $^{\circ}$ C min⁻¹ to 300 $^{\circ}$ C (held for 4 min). The MS/MS electron ionization source was 70 eV (EI), and the transfer line, manifold, and ionization source temperatures were 300, 40, and 170 $^{\circ}$ C, respectively. Argon (99.999%) was used as the collision gas, with resolutions of quadrupoles equal to 1.2 and 2 for Q1 and Q3, respectively. The scan time was 0.25 s for all planned segments.

Quality Control and Data Analysis

Data are shown as mean $\pm$ standard deviation. SPSS, version 16.0 (SPSS Software, Chicago, IL) was used for the one-way ANOVA analysis and *t*-test comparison.

Results and Discussion

Fiber Preloading Kinetics and Reproducibility

Accumulation of PCB-52, PCB-153, bifenthrin, and *cis*-permethrin from the acetone-water solution onto the PDMS fiber as a function of time is shown in Figure S3. The uptake of HOCs was fast, with the fiber impregnated level reaching a steady state in 10 h for PCB congeners and in 24 h for pyrethroids. The relatively fast equilibrium was similar to that found with PCBs and PAHs for polyethylene membranes in methanol-water mixture (80:20, v/v).²⁴ A preloading time of 24 h under agitated conditions was subsequently used for preparing PRC-preloaded fibers.

The preloading reproducibility was evaluated by calculating RSD of replicate fibers. The reproducibility when preloading 10 pieces of fiber in one batch was good, with RSD <8% for the four HOCs and three different loading solution concentrations. In fact, the RSD was <5% in 8 of the 12 treatments (Table 1). Although the reproducibility of within-batch preloading was good, the variation between different batches may increase due to the use of potentially different spiking solutions. Therefore, it is advisable to experimentally determine the preloaded amount (q_0) for each batch before use.

The value of q_0 can be adjusted by changing the chemical concentration of the preloading solution (Table 1) or the equilibration time (Figure S3). It must be noted that q_0 should not be too small to compromise analytical sensitivity. For subsequent experiments in this study, 0.01 mg/L was used as the concentration of the preloading solution. The fibers were preloaded just before use and 4 pieces of the preloaded fibers were randomly selected for quantifying q_0 for every batch.

Isotropy Validation

When plotted on a logarithmic scale, desorption of the preloaded PRCs into the SC sediment decreased linearly as a function of time (Figure S4), with the slope as $-k_{\text{des}}$ ($R^2 = 0.96$). The derived k_{des} values increased in the order of bifenthrin < permethrin < PCB153 < PCB52, which conformed to the decreasing order of PDMS-water partition coefficients (K_{PDMS}) or molecular size of the four HOCs from previous studies.^{8, 18, 30, 31} The absorption of non-labeled HOCs (n/n_0) from the spiked SC sediment mirrored the desorption of labeled PRCs (q/q_0), as shown in Figure 1 for all four HOCs. The sum of q/q_0 and n/n_0 was not statistically different from 1 for most of the time points in the plots for PCBs and permethrin, suggesting isotropy between the HOCs and their isotope labeled analogs preloaded onto the fiber. There were a few time points where the sum of q/q_0 and n/n_0 was greater than 1, especially for bifenthrin. Similar variations were also observed in several previous studies.^{3, 20, 21} For instance, elimination of PAHs from polyethylene sheets was found to be slightly slower than uptake.³² Additionally, slightly different physicochemical properties between labeled and non-labeled analogs may have also contributed to such a discrepancy.^{3, 20, 21} The increased discrepancy observed for bifenthrin may be also due to variations in measurements as a result of the very slow desorption of d₅-bifenthrin from the fiber. Further studies may be needed to determine if there is an upper cutoff in terms of molecular sizes or hydrophobicity for the use of SI-SPME analysis.

Method Validation in Spiked Sediments under Agitation Conditions

The C_f values estimated using SI-SPME after 1 d of sampling are plotted against C_f values from Eq-SPME for all four HOCs in Figure 2. The C_f values measured by the two different approaches were significantly ($p < 0.0001$) correlated ($R^2 = 0.97$). Moreover, slopes of the correlation line were close to 1 (1.04 ± 0.04). Based on t -test, the slope was not significantly different from 1 ($p = 0.16$), indicating that SI-SPME provided statistically equivalent measurements of C_f as Eq-SPME.

The C_f values given by SI-SPME at different desorption intervals (1, 2, and 3 d) are listed in Table 2. There was no significant ($p > 0.05$) difference among the C_f values from the three sampling intervals in 15 of 20 treatments based on ANOVA analysis. This observation implies that the time duration for SI-SPME sampling may be somewhat flexible because quantitative analysis may be realized over a range of sampling time. In the event that the target analytes span over a wide range of molecular sizes or K_{ow} , such as PAHs and PCBs, two or more sampling intervals may be necessary, with a shorter sampling time for the less hydrophobic compounds and a longer sampling time for the more hydrophobic compounds. Similarly, a longer sampling time may be needed for strongly hydrophobic compounds like bifenthrin, as measurable desorption should occur prior to fiber analysis.

Method Validation in Spiked Sediments under Static Conditions

One unique application of SPME sampling is co-exposure of disposable fibers with test organisms in sediment toxicity or bioaccumulation tests, because the measurement is more representative of organism exposure.¹²⁻¹⁴ The performance of SI-SPME was further evaluated in a simulated sediment toxicity test under static conditions. The C_f values estimated by SI-SPME after 20-d exposure were significantly correlated with those given by Eq-SPME for all four HOCs ($R^2 = 0.89$, $p < 0.0001$) (Figure 3) in different sediments. The slope of the regression line was 0.93 ± 0.078 , and the value was not statistically different from 1 ($p = 0.53$ based on t -test).

When the conventional Eq-SPME is used for co-exposure in sediment samples, it is often impossible to attain equilibrium between the fiber and the sample matrix, even though a steady state for uptake and elimination in the test organisms may be reached rather

quickly.^{9,33,34} The lack of equilibrium is a source for uncertainties for the prediction of bioavailability using Eq-SPME.³⁵ Results from this study clearly indicated that SI-SPME can circumvent the requirement for equilibrium in static sediments, making it ideal as a biomimetic tool for bench-scale bioassays or *in situ* deployment (also under static conditions).

Method Application to Field-Contaminated Sediments

The SI-SPME method was further applied to field-contaminated sediments and the derived C_f was compared against C_f from the Eq-SPME analysis (Table 3). The C_f values derived from SI-SPME at three different sampling intervals are also listed in Table 3. Statistical comparison against C_f values given by Eq-SPME showed that SI-SPME produced similar results (Table S2). To evaluate the flexibility of sampling time for SI-SPME, one-way ANOVA was also performed on C_f values derived from the three sampling intervals. The p values from ANOVA test were 0.12, 0.063, and 0.056 for PCB 52, PCB 153, and bifenthrin, respectively, indicating that all of the different sampling intervals yielded similar C_f values for the field contaminated sediments. While the data for bifenthrin were relatively scattered, which may be again attributed to the strong hydrophobicity and slow desorption of bifenthrin, the SI-SPME approach was still capable of predicting C_f with acceptable accuracy. Fernandez et al. (2009) also observed relatively large deviations for more hydrophobic and larger PAH congeners such as benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene, and dibenz[a,h]anthracene) when using PRCs with PEDs for the estimation of pore water concentrations.⁴ It must be noted that PRC concentrations (18 and 22 mg/L for PCBs) were much higher than those in the sediment (205 ± 13 and 47 ± 1.2 $\mu\text{g/kg}$, dry wt). The observations suggested that it is not essential to keep the PRC loading levels close to those in the sampled matrix. From Eq. 5, n_0 is dependent on n and the ratio q/q_0 . The use of a large q_0 may result in improved measurements, although in practice it is important to make sure that n and q fall within the linear range of a calibration curve. This implies that when SI-SPME is used for analysis of field samples, it is unnecessary to know in advance the HOC levels in the sample.

Application Considerations

Although Eq-SPME has become a popular method for bioavailability estimation in sediments or soils, the critical requirement for equilibrium greatly limits its application and also casts uncertainties to studies where sampling was done in the linear (non-equilibrium) range.^{36,37} The kinetic calibration approach proposed in this study, with the aid of stable isotope labeled analogs, may be used under non-equilibrium conditions with drastically reduced sampling time, lending SPME a much expanded capability for bioavailability assessment. Even though only four HOCs were tested in this study, SI-SPME should be equally applicable to other HOCs for which isotope labeled compounds are available. Nowadays there is an abundant supply of HOCs labeled with deuterium, ^{13}C or ^{18}O , including all of the environmentally important PAHs, PCBs, dioxins, and DDT derivatives. Unlike radioactive isotope labeled compounds, the use of stable isotope labeled HOCs poses no known health concerns. Concurrent to the increased availability of stable isotope labeled HOCs, sensitive GC-MS (or LC-MS) systems have also become routine devices in most laboratories. In fact, many laboratories are already using stable isotope labeled compounds as recovery surrogates and internal standards in analysis. Thus, the use of SI-SPME for HOC bioavailability measurement may only involve simple changes in sample handling protocols. The selectivity of mass spectrometry also allows the preloading of many HOCs onto the same fiber, enabling simultaneous bioavailability estimation of contaminant mixtures. To that end, mixtures of deuterium or ^{13}C labeled PAHs and PCBs are already available commercially. In addition, the possibility of using SPME for *in situ* monitoring has been reported in several previous studies.^{13,38} The method described in this study may

provide a great benefit due to the shortened and flexible deployment time, which is important because long-term placement as required by Eq-SPME would result in possible alterations of the sampler (e.g., biofouling, physical damages) and even the chance of losing deployment apparatus.

However, for practical use of SI-SPME, several issues deserve further discussion. When compared with Eq-SPME, the shorter sampling time used in SI-SPME would imply less analytes enriched onto the fiber from the matrix, leading to decreased sensitivity. Although analytical sensitivity may be improved by using fiber with thicker polymer coating, longer sampling intervals may be necessary due to slower desorption of PRCs from the thicker fiber.¹⁹ There is also the likelihood that stable isotope-labeled compounds are not available, especially for emerging contaminants. It is possible to use labeled PRCs that are of similar structures to the target analytes and extrapolate on the basis physicochemical properties (e.g., K_{ow}).^{3,4} In such an application, it would be critical to use PRCs from the same chemical class so that a correlation may be assumed. In addition, from results observed for bifenthrin in this study, HOCs with large molecular sizes or strong hydrophobicity may present a challenge due to slow desorption of PRCs from the fiber. Further research is needed to establish the upper limit (e.g., in K_{ow}) for SI-SPME application, and to investigate modifications that may lead to reduced uncertainties and improved method performance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The isotropy between absorption (■) and desorption (○) for PCB 52, PCB153, bifenthrin, and *cis*-permethrin. The sum of absorption ratio (n/n_0) and desorption ratio (q/q_0) at each time interval is represented by ▲. The symbols (■, ○, ▲) and error bars represent means and standard deviations for three replicates. The dash line means value of 1

Figure 2.

Correlation between fiber concentrations (C_f) at equilibrium derived by SI-SPME and measured by Eq-SPME in five different sediments under agitated conditions. The linear relation obtained was: $C_{f-Eq} = (1.04 \pm 0.040)C_{f-SI} + (1.21 \pm 0.70)$ with $R^2 = 0.97$ and $p < 0.0001$. The dotted line is 1:1 line. The open squares and error bars represent mean values and standard deviations. Three replicates were used for SI-SPME and duplicates were used for Eq-SPME.

Figure 3.

Correlation between fiber concentrations (C_f) at equilibrium derived by SI-SPME and measured by Eq-SPME in five different sediments under static conditions. The linear relation obtained was: $C_{f-Eq} = (0.93 \pm 0.078)C_{f-SI} + (0.073 \pm 0.28)$ with $R^2 = 0.89$ and $p < 0.0001$. The dotted line is 1:1 line. The open squares and error bars represent mean values and standard deviations for duplicates.

Table 1

Reproducibility of fiber preloading with the test compounds using different spiking concentrations in acetone-water (20:80, v/v) (Mean values are chemical concentration in the fiber polymer coating in mg/L.).

	0.01 mg/L			0.1 mg/L			1 mg/L		
	Mean	STD ^a	RSD ^b	Mean	STD	RSD	Mean	STD	RSD
Bifenthrin	15	1.1	7.2%	117	5.4	4.6%	1547	54	3.5%
Permethrin	8.5	0.57	6.7%	33	1.5	4.6%	695	26	3.8%
PCB-52	17	0.93	5.6%	180	5.1	2.8%	1553	48	3.1%
PCB-153	19	1.1	5.9%	271	11	4.3%	1855	65	3.5%

^a Standard deviation (10 replicates);

^b Relative standard deviation.

Table 2
 C_f values (mg/L) derived by SI-SPME after different desorption intervals under agitated conditions.

	PCB52			PCB153			Bifenthrin			Permethrin		
	1 d	2 d	3 d	1 d	2 d	3 d	1 d	2 d	3 d	1 d	2 d	3 d
FS	4.1±0.30	3.6±0.32	4.4±0.42	3.3±0.51	2.8±0.29	3.8±0.20	4.9±1.8	3.2±0.33	6.8±0.89	28±7.7*	14±1.1	24±1.3
SC	13±0.77	14±0.53	16±2.0	11±1.5*	18±1.4	20±2.1	13±1.1	57±26	51±30	69±13	127±31	115±36
EMP37	0.76±0.18	0.74±0.076	0.85±0.081	0.52±0.37	0.55±0.11	0.61±0.031	0.32±0.23*	0.39±0.073	0.81±0.014	4.1±5.1	3.4±1.3	3.6±0.86
EMP73	2.08±0.088*	1.8±0.055	1.2±0.066	2.7±0.25*	1.9±0.20	1.4±0.20	2.7±0.79	2.9±0.57	2.8±0.50	13±2.9	12±2.8	8.0±1.6
GCP	0.53±0.037	0.41±0.085	0.45±0.043	0.47±0.13	0.41±0.13	0.41±0.025	0.38±0.25	0.60±0.047	0.75±0.035	2.1±1.2	1.9±0.38	1.9±0.036

* Indicates significant difference among the three desorption intervals based ANOVA analysis ($\alpha = 0.05$).

Table 3

The chemical concentration on the fiber (C_f) derived from SI-SPME and Eq-SPME in field contaminated sediments.

	$aC_{\text{fiber-eq}}$	$bC_{\text{fiber-SI1}}$	$cC_{\text{fiber-SI2}}$	$dC_{\text{fiber-SI3}}$
PCB52	1.5±0.16	1.5±0.22	1.5±0.079	1.2±0.072
PCB153	0.39±0.10	0.39±0.040	0.43±0.017	0.53±0.093
Bifenthrin	12±1.0	17±2.4	12±0.86	18±3.2

^a Concentration on fiber derived from Eq-SPME approach after shaking for 2, 6, and 41 d for PCB52, PCB 153, and bifenthrin, respectively;

^b Concentration on fiber derived by SI-SPME approach after shaking for 6 hour, 1day, and 17 day for PCB52, PCB153, and bifenthrin, respectively;

^c Concentration on fiber derived by SI-SPME approach after shaking for 12 hour, 2day, and 25 day for PCB52, PCB153, and bifenthrin, respectively;

^d Concentration on fiber derived by SI-SPME approach after shaking for 1day, 3day, and 31 day for PCB52, PCB153, and bifenthrin, respectively.