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Solid-Phase-Microextraction Measurement of 62 Polychlorinated Biphenyl Congeners in Milliliter Sediment Pore Water Samples and Determination of K_{DOC} Values

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Determining dissolved concentrations of polychlorinated biphenyls (PCBs) in sediment pore (interstitial) water with conventional solvent extraction methods is problematic because obtaining large (liter) quantities of pore water, separating it from the sediment, and removing the influence of colloids can be very difficult. However, solid-phase-microextraction (SPME) can achieve similar detection limits using milliliter water samples as achieved with organic solvent extraction requiring a liter of pore water. Five different SPME sorbents were evaluated for their ability to yield the best detection limits for di- to octachlorobiphenyl congeners, both with GC/ECD and with GC/MS (both positive ion EI and negative ion CI). SPME using the 7 μm PDMS fiber with GC/MS (positive ion EI) yielded the best combination of signal-to-noise and selectivity using a 30 min extraction, although ECD was also suitable. Pore water was obtained by centrifuging wet sediment followed by flocculation to remove colloids. Quantitative calibration was simplified by adding dichloro- to hexachlorobiphenyl internal standards chosen to be compatible with either ECD or MS detection. Calibration curves and relative response factors (including the SPME and GC steps) were determined for all 62 PCB congeners that are present in above-trace quantities in commercial Aroclors. Calibrations were linear (r^2 typically >0.995) from low pg/mL to ng/mL concentrations, with near zero intercepts. Detection limits for all individual PCB congeners ranged from <1 to 3 pg/mL using 1.5 mL water samples. Dissolved organic matter (DOM) had no measurable effect on dichloro- and trichlorobiphenyls, but did contain about 10 to 25% of the tetrachlorobiphenyls and up to 60% of the hexachlorobiphenyl congeners. Log DOC/water partitioning coefficients (log K_{DOC}) ranged from 3.6 to 4.6 for 2,3,5,6-tetrachlorobiphenyl and from 4.2 to 5.5 for 3,3′,4,4′,5,5′-hexachlorobiphenyl.

Recent investigations into the biological effects of legacy pollutants such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) increasingly demonstrate that dissolved concentrations in the sediment pore water (interstitial water) are better at predicting toxicity and uptake than the use of equilibrium partitioning models based on sediment concentrations and organic carbon (K_{OC}) partitioning coefficients.[1−7] However, measuring dissolved concentrations of hydrophobic organic compounds (HOCs) in sediment pore water is much more daunting than measuring sediment concentrations since water concentrations will be several orders of magnitude lower than sediment concentrations. Obtaining and preparing large volumes of pore water needed to obtain suitable detection limits can be very difficult,[8,9] and interferences from analytes associated with colloidal material must be eliminated. In addition, the method should be able to distinguish between "freely-dissolved" (in the free water phase only) and "total dissolved" analytes (in the free water phase and associated dissolved organic matter, or "DOM").

There are a variety of approaches to measure dissolved PCBs, but the methods usually address only a few selected PCB congeners and there is little standardization in methodology, which can make results from different laboratories studying environmental and biological effects of impacted sediments difficult to interpret. The various methods can be divided into two general approaches; "direct" (or "ex-situ") and "indirect" (or "in-situ"). Direct methods involve separating the pore water from the sediment, eliminating interferences from colloids (e.g., by flocculation or an air-bridge),[3,10,11] then determining the PCB concentrations in the prepared water with conventional extraction and analysis methods. Indirect approaches involve inserting a "non-

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depletive" sorbent into the sediment/water slurry (in the lab or in the field), waiting for the sediment/water/sorbent equilibrium to be achieved, analyzing the PCB concentrations in the sorbent and finally, calculating the dissolved concentrations with previously determined sorbent/water partitioning coefficients (which must be experimentally determined for each target analyte).[2,5−7,12−16] However, these indirect methods require several weeks (or even months) for the equilibration step and, therefore, are not suitable for widespread use in the routine survey of contaminated sites.

Direct measurement of dissolved PCBs and related HOCs has been performed using air-bridge techniques[3,10,11] in which the PCBs in a contaminated sediment are left to come to equilibrium with a receiver vessel of pure water. After equilibration, the PCB concentrations in the receiver are equal to their concentrations in the sediment/water slurry, and can be determined without interferences from colloids. Unfortunately, equilibrium can take several months to achieve, so the method is not suited for routine use. An alternate approach is to collect pore water by centrifugation and remove the colloids by flocculation.[3,10,17] This yields a water sample free from colloids that can be analyzed using conventional methods such as organic solvent extraction.

Although it is relatively trivial to obtain liter volumes of most types of water samples, it can be prohibitively difficult to obtain and prepare (e.g., centrifugation of liters of sediment and flocculation of the water) the hundreds of milliliters of sediment pore water required to get sufficient sensitivity, especially for sandy sediments.[8,9] The large volumes (often several liters) of sediment samples that need to be shipped to the lab could make field surveys with multiple sediment samples impractical. Fortunately, SPME can be used to replace solvent extraction and achieve similar detection limits with a few milliliters of water. Since SPME is an equilibrium extraction, the mass of a particular PCB congener extracted from 1 L of water is only moderately more than the mass extracted from a milliliter of water.[17] Thus, small water samples are as good as large water samples in terms of detection limits using SPME. Fortunately, SPME compensates for its inability to quantitatively extract large water samples by transferring all of the extracted PCBs directly into the GC injection port, while only a tiny fraction of PCBs in a solvent extract are actually injected into the GC.

SPME determinations of PCBs in water have been reported using different approaches including direct insertion of the fiber into the water sample,[18−20] and headspace sampling assisted by heat and microwaves,[21−23] although all of these methods have been limited to 20 or fewer congeners. Headspace methods directly avoid interferences from PCBs on colloids, and should reflect freely dissolved concentrations. Previous methods using direct SPME sampling of the water have not differentiated freely dissolved, total-dissolved, and colloidally associated PCBs.[18−20] However, this can be done by flocculating the water samples to remove colloids and adding appropriate internal standards to determine both total-dissolved and freely dissolved concentrations.[17,24,25] The internal standards are also used to compensate for the short (non-equilibrium) SPME sorption time required for reasonable sample throughput. This approach has recently been used to develop a robust method to determine total- and freely dissolved-PAHs (including 34 parent and groups of alkylated PAHs), and has obtained provisional approval from the American Society for Testing and Materials as Method D7363 (subject to final round-robin testing planned for late-2009). SPME is used rather than organic solvent extraction, since similar sensitivities can be achieved with 1.5 mL samples using SPME, as can be achieved with a liter of pore water using conventional solvent extraction.[17] This low sample volume requirement makes shipping large numbers of samples from the field to the lab practical, and the method was designed so that (once the colloids are removed) the SPME extraction and GC/MS analysis are coordinated so that a new sample is run every hour. The goal of the present work is to develop a similar approach for PCBs that includes all relevant PCB congeners and differentiates freely dissolved and total-dissolved concentrations, and eliminates contributions of colloidally associated PCBs.

EXPERIMENTAL SECTION

Sediment Samples. Freshwater sediments were collected using either a Ponar grab sampler or a shovel. Samples were sieved through a 2 mm screen to remove debris, and briefly mixed before sub-sampling into new glass jars. Storage was at 4 °C in the dark. Two clean sediments (non-detectable PCBs) were used for spiking experiments. One marine sediment was obtained from the National Institute of Standards and Technology (NIST SRM 1944). Washed sea sand (Fisher Scientific, Pittsburgh, PA) was used as an organic-free matrix for spiking experiments. Sediment characteristics are given in Table 1. Black carbon (BC) was determined using 375 °C oxidation for 24 h.[26] Dissolved organic carbon (DOC) in pore water was determined after flocculation using EPA method 5310 C. Sediments that had been air-dried (e.g., NIST SRM 1944) were reconstituted with distilled water by mixing for a minimum of 72 h. Sediment PCB concentrations were determined using 18-h. Soxhlet extractions with 1:1 acetone/iso-octane.

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Table 1. Sediment Characteristics

	sediment PCB concentration, μg/g dry wt				
	sediment A	sediment B	sediment C	sediment D[b]	sediment E[b]
di + tri + tetra	0.2	0.5	0.1	2.1	3.2
penta + hexa	26.6	48.6	2.6	0.9	1.4
hepta + octa	24.7	38.1	2.2	0.5	0.8
total PCBs	51.4	87.2	4.9	3.6	5.4
sediment TOC, dry wt.%	2.4	3.3	3.1	3.1	1.2
sediment BC, dry wt.%	2.0	1.2	2.6	1.0	0.8
pore water DOC, mg/L[a]	5.5	3.8	5.1	8.2	4.8

[a] Dissolved organic carbon was determined after flocculation. [b] Concentrations on the uncontaminated sediments D and E after spiking with the Aroclor mix described in the text.

Pore Water Preparation. Pore water samples were prepared fresh daily by transferring about 40 mL of the sediment/water slurry to a "certified clean" 40 mL glass "VOA" vial and centrifuging for 30 min at 1000*g*. (Higher speed caused the glass vials to break). This typically resulted in 10 to 15 mL of pore water that could be gently collected with a pipet. Flocculation of the water samples was performed twice using a 10 wt % solution of alum (aluminum potassium sulfate) added to the water at a 1:40 ratio.[3,10,17] A few drops of 1 M NaOH was added, and the vial was mixed to cause the flocculation. The vial was centrifuged again for 30 min, and the supernatant water was collected with a pipet.

Pore water samples were split into triplicate 1.5 mL aliquots which were placed into new 2 mL silanized glass autosampler vials (Agilent, Wilmington, DE), and immediately spiked with 10 μL of acetone containing about 100 pg of each internal standard. All samples were analyzed within 18 h of their preparation. Daily blank and calibration water samples were prepared in the same manner using 1.5 mL of HPLC-grade water (Fisher Scientific, Pittsburgh, PA). Blank water samples were analyzed between each different pore water sample to determine if any carryover of PCBs occurred on the SPME fiber.

Hexane extracts of water samples were prepared to allow comparison of the SPME data with conventional solvent extraction. Approximately 100 mL of the sediment water was centrifuged and flocculated. PCB internal standards were immediately added to the water followed by extraction three times with 20 mL of *n*-hexane. Extracts were concentrated to about 200 μL and analyzed by GC/MS.

SPME Analysis. Five commercially available SPME fibers were initially evaluated to determine which fiber gave the best combination of sensitivity for a range of PCB congeners, low background artifacts, and lack of carryover. All fibers were evaluated using both GC/MS and GC/ECD for the analyses. Fiber coatings tested included polydimethylsiloxane (PDMS) in both 7 and 100 μm film thicknesses, 60 μm PDMS/divinylbenzene (DVB), 65 μm Carbowax/DVB, and 30 μm DVB/Carboxen/PDMS (Supelco, Bellefonte, PA). Evaluations were performed with several concentrations of a commercial mix of 20 di- to decachlorobiphenyl PCB congeners (Accustandard Inc., New Haven, CT) in 1.5 mL water samples. Serial dilutions in acetone were spiked into the water samples until the PCB congener peaks could no longer be detected over the MS or ECD background signal (typically ca. 1 pg/mL).

All SPME determinations were performed using a Leap Technologies "Combi-Pal" autosampler (Carrboro, NC) equipped to oscillate the 2 mL autosampler vials during the SPME sorption step (thus eliminating the need for a stir bar and associated carryover of the PCBs). All GC analyses were performed using Agilent model 6890N gas chromatographs with either MS (Agilent model 5973) or ECD (Agilent G2397A) detectors. Separations were performed using a 60 m Agilent HP-5 MS column (0.25 μm film thickness, 250 μm i.d.). The oven temperature was held at 40 °C for 5 min during the SPME desorption, then programmed at 50 °C per min to 110 °C, followed by a temperature ramp of 12 °C per min to 320 °C (hold for 10 min). Since one goal of the method was to allow sample analyses to be performed with a per analysis time of an hour or less, SPME sorption was performed for 30 min. After the sorption period, the fiber was immediately desorbed into the GC/MS injection port in the splitless mode at the temperature specified by the supplier for 5 min. After an additional 15 min cleaning of the fiber, sorption of the next water sample began. This sequence corresponded to the about 50 min GC cycle time, and allowed a new sample to be analyzed every 50−60 min. Desorption and cleaning temperatures were 320 °C (7 μm PDMS), 250 °C (100 μm PDMS), 250 °C (PDMS/DVB), 230 °C (Carbowax, DVB), and 300 °C (DVB/Carboxen/PDMS). The extraction efficiencies of the fibers were determined by SPME extraction and analysis of a single water sample five times, and comparing the raw peak areas of two sequential determinations for each congener to determine the percent of each congener removed with each SPME sorption.

GC/MS was performed using selected ion monitoring (SIM). Because of the 35-Cl and 37-Cl isotope pattern, several intense mass spectral peaks occur at and above the nominal molecular weight of each congener, especially for more chlorinated congeners. Ions which show similar intensities in their mass spectra would be expected to give similar sensitivities, except that the background noise level (e.g., from GC column bleed, or artifacts from the SPME fiber) could decrease the signal-to-noise ratio (and the sensitivity) for some particular mass ion (m/z). Therefore, all of the intense m/z values in the molecular ion region of each congener were compared using a SPME injection of PCB standards, to select the m/z which yielded the best signal-to-noise ratio for each group of congeners. A similar approach was also used to determine the best m/z value to monitor as a confirmatory ion (corresponding to the loss of Cl_2) for each congener. The two ions which yielded optimal signal-to-noise for each congener are given in Table 2. Conventional positive ion electron impact MS was performed at 70 eV and the MS was tuned daily to the manufacturer's specifications. Negative ion chemical ionization (NICI) was performed according to the manufacturer's specifications, with methane as the reagent gas.

Table 2. Selective Ion Monitoring Masses for SPME-GC/MS Based on Optimized Signal-to-Noise

	nominal mass (amu)	quantitative ion $(m/z)^a$	confirmatory ion $(m/z)^b$	detection limit pg/mL
diCl	222	222	152	0.7
triCl	256	256	186	0.07
tetraCl	290	292	220	0.07
pentaCl	324	326	254	1.3
hexaCl	358	360	290	1.3
heptaCl	392	396	324	1.3
octaCl	426	428	358	3.3

[a] Ion mass in the molecular ion region showing the highest signal-to-noise ratio by SPME-GC/MS analysis. [b] Ion mass in the mass region of Cl_2 loss showing the highest signal-to-noise ratio by SPME-GC/MS analysis.

Selection of Target PCB Congeners and Internal Standards. Although there are 209 possible PCB congeners, only a subset of the possible congeners were produced in significant amounts during the synthesis of commercial Aroclors. Frame et al. have reported a complete breakdown of congener distributions in all commonly used commercial Aroclors.[27] On the basis of their report, we selected all congeners that had a concentration of 1 wt % or greater in any of the commercial Aroclor products. This resulted in a list of 62 congeners, ranging from dichloro- to octachloro-substituted congeners (Table 3). After the fiber selection, all methods development, calibrations, and evaluations were performed using these 62 congeners. In addition, several mixtures were obtained from Accustandard which, when combined, contained all 209 congeners. These standard mixtures are produced so that no single mixture contains coeluting congeners with the GC conditions used in this study. Separate analyses of these mixtures were performed to determine the retention behavior of all individual 209 congeners. The resultant retention times were used to set the time windows for the MS SIM program and determine which congeners could not be chromatographically resolved.

Ideally, isotopically labeled internal standards would be available for the most important PCB congeners.[17,24] However, in contrast to perdueterated PAHs, perdueterated PCBs do not show enough mass shift to overcome the isotope pattern caused by the chlorine isotopes. Although 13-C labeled PCB congeners are available, they are prohibitively expensive. In addition, one goal of this method was to be compatible with both MS and ECD detectors, which eliminates the use of isotopically labeled internal standards. Therefore, the congener distributions in commercial Aroclors reported by Frame et al.[27] and the retention times of all 209 congeners we determined were used to select internal standards from the list of PCB congeners that (1) were not present in significant concentrations in commercial Aroclors, and (2) were well-resolved from all other PCB congeners using the GC conditions reported above. These criteria lead to the selection of 3,5-dichlorobiphenyl (congener no. 14), 2,4,6-trichlorobiphenyl (no. 30), 2,3,5,6-tetrachlorobiphenyl (no. 65), 2,2′,4,5′,6-pentachlorobiphenyl (no. 103), and 3,3′,4,4′,5,5′-hexachlorobiphenyl (no. 169). Internal standards for the SPME analyses were diluted to 10 ng/mL in acetone, with 100 pg (in 10 μL) added to each 1.5 mL water sample, calibration water, and the water blanks.

An Aroclor solution was prepared using 27 mg of Aroclor 1242, and 18 mg of Aroclor 1260 in 30 mL of acetone. This mix of Aroclors was used to provide a spiking solution with a more realistic congener distribution than the 62 congener calibration

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Table 3. Target PCB Congener List

PCB name	congener number	retention time, min	relative response factor[a]
2,2′-dichlorobiphenyl	4	17.23	0.64
2,3′-dichlorobiphenyl	6	17.88	0.89
2,4′-dichlorobiphenyl	8	17.99	0.93
4,4′-dichlorobiphenyl	15	18.80	0.82
2,2′,3-(2,4′,6-)trichlorobiphenyl	16 + 32	19.09	0.69
2,2′,4-trichlorobiphenyl	17	18.79	0.69
2,2′,5-trichlorobiphenyl	18	18.74	0.63
2,3,4′-trichlorobiphenyl	22	19.86	0.88
2,3′,5-trichlorobiphenyl	26	19.37	0.84
2,4,4′-trichlorobiphenyl	28	19.55	1.06
2,4′,5-trichlorobiphenyl	31	19.42	0.83
2′,3,4-trichlorobiphenyl	33	19.73	0.71
3,4,4′-trichlorobiphenyl	37	20.55	0.74
2,2′,3,4′-tetrachlorobiphenyl	42	20.54	0.69
2,2′,3,5′-tetrachlorobiphenyl	44	20.48	0.65
2,2′,3,6-tetrachlorobiphenyl	45	19.96	0.72
2,2′,4,4-(2,2′,4,5′-)tetrachlorobiphenyl	47 + 48	20.25	0.86
2,2′,4,5′-tetrachlorobiphenyl	49	20.19	0.85
2,2′,5,5′-tetrachlorobiphenyl	52	20.10	0.78
2,3,3′,4′-(2,3,4,4′-)tetrachlorobiphenyl	56 + 60	21.47	1.55
2,3,4′,6-tetrachlorobiphenyl	64	20.68	1.13
2,3′,4,4′-tetrachlorobiphenyl	66	21.17	0.96
2,3′,4′,5-tetrachlorobiphenyl	70	21.10	0.93
2,4,4′,5-tetrachlorobiphenyl	74	21.05	0.86
2,2′,3,3′,4-pentachlorobiphenyl	82	22.34	0.86
2,2′,3,3′,6-(2,2′,4,4′,6-)pentachlorobiphenyl	84 + 101	21.56	0.96
2,2′,3,4,4′-pentachlorobiphenyl	85	22.06	0.84
2,2′,3,4,5′-pentachlorobiphenyl	87	21.98	0.76
2,2′,3,5′,6-pentachlorobiphenyl	95	21.21	1.03
2,2′,3′,4,5-pentachlorobiphenyl	97	21.90	0.72
2,2′,4,4′,5-pentachlorobiphenyl	99	21.62	0.91
2,3,3′,4,4′-pentachlorobiphenyl	105	22.96	0.92
2,3,3′,4′,6-pentachlorobiphenyl	110	22.13	1.02
2,3′,4,4′,5-pentachlorobiphenyl	118	22.52	1.02
2,2′,3,3′,4,4′-hexachlorobiphenyl	128	23.75	1.22
2,2′,3,3′,4,6′-hexachlorobiphenyl	132	22.95	1.29
2,2′,3,3′,5,6′-hexachlorobiphenyl	135	22.39	1.80
2,2′,3,3′,6,6′-hexachlorobiphenyl	136	22.12	2.48
2,2′,3,4,4′,5′-(2,3,3′,4′,5,6-)hexachlorobiphenyl	138 + 163	23.30	3.60
2,2′,3,4,5.5′-hexachlorobiphenyl	141	23.06	1.34
2,2′,3,4′,5,5′-hexachlorobiphenyl	146	22.76	1.42
2,2′,3,4′,5′,6-hexachlorobiphenyl	149	22.50	1.63
2,2′,3,5,5′,6-hexachlorobiphenyl	151	22.32	1.55
2,2′,4,4′,5,5′-hexachlorobiphenyl	153	22.85	3.03
2,3,3′,4,4′,5-hexachlorobiphenyl	156	24.08	1.40
2,2′,3,3′,4,4′,5-heptachlorobiphenyl	170	24.81	0.51
2,2′,3,3′,4,4′,6-heptachlorobiphenyl	171	24.08	0.65
2,2′,3,3′,4,5,6′-heptachlorobiphenyl	174	23.92	0.78
2,2′,3,3′,4′,5,6-heptachlorobiphenyl	177	24.01	0.71
2,2′,3,3′,5,6,6′-heptachlorobiphenyl	179	23.13	1.55
2,2′,3,4,4′,5,5′-heptachlorobiphenyl	180	24.31	0.55
2,2′,3,4,4′,5′,6-heptachlorobiphenyl	183	23.62	0.87
2,2′,3,4′,5,5′,6-heptachlorobiphenyl	187	23.54	0.97
2,3,3′,4,4′,5′,6-heptachlorobiphenyl	191	24.42	0.54
2,2′,3,3′,4,4′,5,5′-octachlorobiphenyl	194	25.85	0.45
2,2′,3,3′,4,5,6,6′-octachlorobiphenyl	199	25.02	0.34
2,2′,3,4,4′,5,5′.6-octachlorobiphenyl	203	25.56	0.40

[a] Relative response factors (rrf) were calculated for each congener based on the internal standard having the same molecular weight by the equation; rrf = (peak area standard/conc. standard)/(peak area internal standard/conc. internal standard). Hepta- and octachlorobiphenyl congeners were based on the hexachlorobiphenyl internal standard. All values were determined using SPME-GC/MS, and include both the SPME efficiencies and GC/MS response.

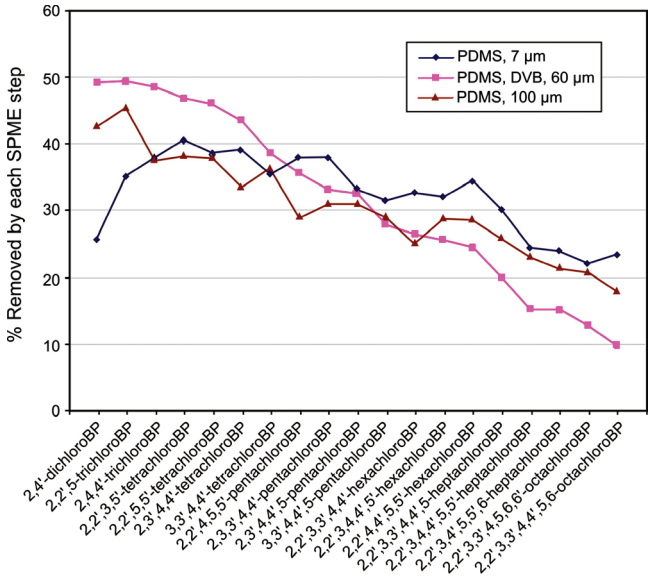

Figure 1. Percent of dissolved PCB congeners removed from a 1.5 mL water sample using a 30 min sorption with different SPME fiber materials.

solutions. This solution was used to spike the background sediments and the sea sand to a total PCB concentration of about 5 μg/g, followed by the addition of water to a wet slurry and 72 h of mixing by inversion to homogenize the samples.

Detection Limits and Calibration. Preparation of water calibration standards for the 62 target congeners is complicated by the fact that water solubilities of individual PCB congeners vary by more than 4 orders of magnitude. Because of the range in solubilities, two separate stock solutions were made so that water calibration standards could be prepared that covered a large range in PCB concentrations while not exceeding the solubility limit of the higher-molecular-weight congeners. Four-point calibration solutions were prepared in 1.5 mL of water, which contained from 6.7 to 1670 pg/mL for the lower-molecular-weight congeners (congeners 4 to 49 in Table 3), and 1.3 to 333 pg/mL for the higher-molecular-weight congeners. Detection limits were determined by analyzing increasingly dilute solutions until the signal-to-noise ratio no longer exceeded at least 3 to 1.

RESULTS AND DISCUSSION

Selection of SPME Fiber Phase. The five SPME stationary phases listed in the Experimental Section were first tested on both ECD and MS detectors using the Accustandard mix of 20 congeners (100 pg each congener in 1.5 mL of water). These initial experiments showed that the 65 μm Carbowax/DVB and 30 μm DVB/Carboxen/PDMS phases were unsuitable for PCB analyses because of low extraction efficiencies and/or unreasonably high background peaks with the MS, and especially with the ECD. The remaining three fibers all had reasonable extraction efficiencies, with about 10 to 70% of each PCB congener being extracted in 30 min from a 1.5 mL water sample (Figure 1). Overall, with the 30 min sorption period used, the 7 μm fiber was slightly more sensitive for higher-molecular-weight PCBs than the 100 μm fiber, but the opposite case was true for lower-molecular-weight PCB congeners. An earlier report that used heated headspace SPME for 30 min to compare the sensitivity of the same three fibers found the PDMS/DVB fiber to be much more sensitive for low-molecular-weight congeners, and the 100 μm PDMS fiber to be more sensitive for high-molecular-weight congeners than the other fibers.[21] Using the heated headspace method, the 7 μm PDMS fiber was the least sensitive for all congeners. In contrast, our comparison using direct insertion into the water showed that the same three fibers had no large differences in sensitivity based on congener molecular weight, and that the 7 μm had similar (or better) sensitivity than the other two fibers for most congeners. Clearly, optimizing fiber selection must consider the sorption mechanism used (headspace vs direct insertion) as well as the fiber characteristics.

On the basis of extraction efficiencies only, similarly low detection limits could be obtained from any of the three fibers. However, the 60 μm PDMS/divinylbenzene (DVB) showed higher background peaks (especially for the ECD detector), and thus was not as useful for PCB determinations as the 7 or the 100 μm PDMS fibers. In addition, the 100 μm fiber had slightly higher background peaks than the 7 μm fiber with both MS and ECD detectors. Since our previous experience with PAHs using SPME has shown that the 7 μm fiber is more robust (one fiber typically lasts for several hundred analyses) and has less carryover from highly contaminated waters than the 100 μm fiber,[17] we chose the 7 μm fiber for the remainder of the investigations.

Comparison of MS and ECD Detectors. ECD is typically more sensitive than MS for highly halogenated analytes. With the models of detectors used in this study, injected quantities of about 0.1 pg can be detected by ECD, while about 1 pg is needed for MS detection in the conventional positive ion electron impact (EI) mode. However, as discussed above, the SPME stationary phase (as well as sediment pore water samples) can yield chromatographic peaks that interfere with the detection of PCB peaks in the chromatogram. Unfortunately, this was found to be a significant problem for the ECD detector, even with the best of the fibers (the 7 μm PDMS fiber), presumably a result of PDMS bleed from the sorbent fiber. Because of these interferences, ECD had similar detection limits to MS. Since MS is a much more selective detector than ECD, MS was chosen as the detector of choice for the remainder of these studies. However, it should be noted that the same SPME approach can be used with an ECD detector as long as the water samples do not contain significant organics that would interfere with particular PCB congeners.

For halogenated organics like PCBs, there are two useful ways in which to operate the MS detector, that is, conventional MS (which uses electron impact ionization [EI] and positive ion detection, and is used for virtually all standard GC/MS methods for organic compounds) and negative ion chemical ionization (NICI)MS. For highly halogenated compounds, NICI is more sensitive than conventional positive ion EI, but the opposite is true for less halogenated compounds. Therefore, we compared the sensitivity of both modes of ionization with several dilutions of the 20 standard dichlorobiphenyl to decachlorobiphenyl congeners. In essence, NICI and positive ion EI had similar sensitivities for the tetrachloro congeners (on a signal-to-noise basis), but NICI was much more sensitive for the more highly chlorinated congeners. Unfortunately, the sensitivity of NICI for lower-molecular-weight PCBs was increasingly poor as the degree of chlorination dropped. Thus, the detection limits for NICI for the dichloro- and trichloro- congeners were orders of magnitude

poorer than positive ion EI, although NICI was more sensitive for the higher-molecular-weight PCBs. For pore water determinations of PCBs, we decided that conventional positive ion EI was the method of choice for four reasons: (1) the sensitivity is similar for all PCB congeners, making calibration and routine operation more robust and simpler; (2) most PCBs found in pore water will be the lower-molecular-weight congeners, for which NICI has much poorer sensitivity; (3) positive ion EI has a greater range of detector linearity than NICI, and (4) many more laboratories are familiar with positive ion EI than NICI. However, it should be noted that, if the goal for a particular sediment pore water is to get trace determinations of the more highly chlorinated PCB congeners, than NICI would be the MS method of choice.

Blanks and Carryover. Previous applications of SPME to PAHs demonstrated that atmospheric PAHs (e.g., from diesel exhaust) could cause significant concentrations of lower-molecular-weight PAHs in blank water samples.[17] In addition, small amounts of carryover from one SPME determination to the next could occur when a highly contaminated water was analyzed.[17] To monitor such possible interferences for PCBs in the present study, we analyzed two water blanks between each contaminated sample. In contrast to PAHs, no atmospheric contamination of the SPME fiber was observed. The desorption/cleaning procedure used was also successful in avoiding detectable carryover of PCBs on the 7 μm PDMS fiber, even after the analysis of highly contaminated samples.

Flocculation to Remove Colloidal Material. Colloids in water samples from sediments can contain high concentrations of PCBs, and must be removed before determining dissolved PCB concentrations for two reasons. First, colloids can contaminate the SPME sorbent, and result in large amounts of carryover between samples.[17] Second, substantial amounts of PCBs can be associated with colloidal material, even after centrifugation.[3,10,17] For example, duplicate water samples from sediments A and B that were contaminated with higher-molecular-weight congeners were extracted with hexane with and without flocculation (but after centrifugation. Note that DOM is not removed by centrifugation and flocculation.). While the amounts of lower-molecular-weight congeners with and without flocculation were similar, the concentrations without flocculation of higher-molecular-weight congeners were about two to four times higher for pentachloro- and heptachloro- congeners, and three to six times higher for heptachloro- congeners. While these two sediments were fairly sandy, the clean sediment E consisted of fine clays, and when water generated by spiking this sediment with the Aroclor spiking solution (and mixed for 3 days) was extracted before and after flocculation, nearly 60% of the low-molecular-weight congeners and up to 95% of the higher-molecular-weight congeners were associated with the colloids. Clearly, the colloids must be removed to obtain accurate dissolved PCB concentrations.

Ghosh et al. have previously used flocculation to remove colloidal PCBs and PAHs from sediment water samples, and have reported no significant losses of PCBs or PAHs. The flocculation method was validated by comparing PCB and PAH concentrations in the water after flocculation to those obtained using an air bridge and several months to obtain equilibrium.[3,10] In the previous development of a similar SPME method for PAHs, we observed that two flocculation steps were necessary to fully remove colloidal PAHs, but did not remove any measurable amounts of dissolved PAHs.[17]

In the present study, we validated the use of two flocculation steps for PCBs by spiking 50 g of washed sea sand with the mixed Aroclor solution (total PCB sediment concentration of 3 μg/g) and mixing overnight with 100 mL of water to let the PCBs equilibrate between the sand and the water. This sand was selected since it contains no measurable carbon, and should contain no colloidal material. The resultant water was collected, centrifuged, and then split into two aliquots. One aliquot was flocculated twice, and the other was not flocculated. The unflocculated and the flocculated aliquots were analyzed with the SPME method. Twenty-eight congeners were detected at quantifiable levels and ranged from dichloro- to heptachloro- PCBs. As shown in Table 4, the concentrations measured for each PCB congener averaged 95 ± 13% for the flocculated versus unflocculated water samples, which is within the reproducibility of the SPME method. It is also useful to note that there is no trend in PCB concentration differences with congener molecular weight since, if the flocculation procedure did remove PCBs, the higher-molecular-weight congeners would show much higher losses than the lower-molecular-weight (more water-soluble) congeners.

Potential losses of PCBs during flocculation were also investigated with an uncontaminated sediment E that had been spiked with the Aroclor mix by performing the SPME analyses on the sediment pore water after two and after three flocculation steps. There was no significant change in the dissolved concentrations with the additional flocculation step, with the concentrations after three flocculation steps averaging 94 ± 8% of those measured after two flocculation steps. As was the case for the spiked sand discussed above, there was no trend in PCB concentration changes with congener molecular weight.

Method Sensitivity, Linearity, and Reproducibility. GC/MS detection limits were determined by performing SPME analyses on increasingly dilute 1.5 mL water calibration samples of the 62 target congeners. This procedure was repeated with 3 different previously used 7 μm PDMS fibers on 3 different days (several weeks apart), so that the detection limits reported in Table 2 reflect routine operating conditions. Detection limits were based on the lowest concentration that showed at least a 3:1 signal-to-noise ratio for all congeners of a particular molecular weight, and range from 0.7 pg/mL for dichloro-, trichloro-, and tetrachloro-congeners, to 3.3 pg/mL for octachloro- congeners.

As noted above, preparing calibration solutions in water is complicated by the fact that the 62 target congeners have water solubilities that vary by four orders-of-magnitude. Four-point calibration solutions were prepared in 1.5 mL of water, which contained from 6.7 to 1670 pg/mL for the lower-molecular-weight congeners (congeners 4 to 49 in Table 3), and 1.3 to 333 pg/mL for the higher-molecular-weight congeners. SPME analyses of the four-point calibration solutions resulted in standard curves that were all linear (r^2 >0.99 for all 62 congeners, and >0.999 for the majority of congeners), and all y intercepts were essentially zero. The relative response factors for each of the 62 congeners showed no significant dependence on concentration (confirming calibration linearity and near-zero y intercepts). Average relative response factors for each of the calibration congeners versus their internal standards are given in Table 3.

Table 4. Effect of Flocculation on PCB Concentrations and Comparison of SPME with Hexane Extraction

		SPME, no floc[a]		SPME, 2× floc[a]		hexane[b]	
PCB	cong. no.	mean pg/g	SD	mean pg/g	SD	mean pg/g	SD
2,3′-dichlorobiphenyl	6	129	22	120	10	122	4
2,4′-dichlorobiphenyl	8	574	102	505	30	547	4
4,4′-dichlorobiphenyl	15	143	23	119	6	139	5
2,2′,3-(2,4′,6-)trichlorobiphenyl	16 + 32	297	34	287	6	314	1
2,2′,4-trichlorobiphenyl	17	179	22	169	6	189	2
2,2′,5-trichlorobiphenyl	18	524	62	501	18	578	1
2,3,4′-trichlorobiphenyl	22	121	11	135	19	141	3
2,3′,5-trichlorobiphenyl	26	61	7	59	6	65	1
2,4,4′-trichlorobiphenyl	28	227	27	279	82	265	3
2,4′,5-trichlorobiphenyl	31	318	38	292	33	300	2
2′,3,4-trichlorobiphenyl	33	270	29	250	24	265	6
3,4,4′-trichlorobiphenyl	37	66	7	57	5	72	2
2,2′,3,5′-tetrachlorobiphenyl	44	99	15	83	4	111	3
2,2′,3,6-tetrachlorobiphenyl	45	35	5	31	4	42	1
2,2′,4,5′-tetrachlorobiphenyl	49	72	12	56	2	67	2
2,2′,5,5′-tetrachlorobiphenyl	52	126	19	92	4	92	1
2,3,4′,6-tetrachlorobiphenyl	64	31	16	40	3	42	8
2,3′,4,4′-tetrachlorobiphenyl	66	52	10	53	15	49	2
2,3′,4′,5-tetrachlorobiphenyl	70	63	12	47	4	55	1
2,4,4′,5-tetrachlorobiphenyl	74	32	5	25	2	31	1
2,3,3′,4′,6-pentachlorobiphenyl	110	20	4	18	5	22	1
2,2′,3,3′,4,6′-hexachlorobiphenyl	132	14	1	14	1	19	1
2,2′,3,4′,5′,6-hexachlorobiphenyl	149	59	6	53	5	58	1
2,2′,3,5,5′,6-hexachlorobiphenyl	151	20	1	23	8	27	1
2,2′,4,4′,5,5′-hexachlorobiphenyl	153	55	11	46	10	52	3
2,2′,3,3′,4,4′,5-heptachlorobiphenyl	170	11	2	13	1	12	4
2,2′,3,4,4′,5,5′-heptachlorobiphenyl	180	29	2	27	3	31	3
2,2′,3,4′,5,5′,6-heptachlorobiphenyl	187	16	3	16	3	19	1

[a] Colloid-free water samples were generated using the mixed Aroclor spike as described in the text. SPME analyses were performed on samples before and after two flocculation steps. [b] Hexane extraction was performed on 100 mL of water after flocculation. Standard deviations are based on triplicate analyses of a single hexane extract.

The reproducibility of replicate SPME analyses of calibration solutions containing the 62 congeners typically have RSDs less than 5% for most individual congeners. However, SPME analyses of replicate pore waters from contaminated sediments or spiked with the Aroclor mixture have somewhat higher RSDs, at least partially because of the increased complexity of the PCB chromatograms. For such samples, RSDs for the low- and middle-molecular-weight congeners are typically in the 5−10% range, but tend to be in the 10−20% range for the higher-molecular-weight congeners. As would be expected, the reproducibility becomes poorer as the PCB concentrations approach the detection limit.

Comparison of SPME versus Hexane Extraction. The SPME method was validated by comparing quantitative results with those based on hexane extraction. Water was prepared as discussed above by spiking clean sand and mixing. Approximately 100 mL of the water was then collected, centrifuged, and flocculated, and the water was split for SPME analysis and hexane extraction. As shown in Table 4, the SPME method yielded generally good agreement with the concentrations determined by conventional solvent extraction, with the hexane extract concentrations averaging 104 ± 9% of the SPME values.

Effect of Dissolved Organic Carbon (DOC) on PCB Concentrations. After centrifugation and flocculation to remove the colloidal material, PCBs are found partitioned between the water and the dissolved organic carbon phase (DOC). Poerschmann et al. have demonstrated that hydrophobic solutes such as PAHs and PCBs added to a water sample become equilibrated with the DOC fraction in 1 or 2 min.[24,25] Therefore, when an internal standard solution is added to a flocculated water sample, the internal standards equilibrate with the DOC fraction, and determining the concentration of an individual PCB congener based on its analogous internal standard (e.g., calculating the concentrations of sample hexachlorobiphenyl congeners based on the added hexachlorobiphenyl internal standard) will represent the total concentration in the water including the freely dissolved and DOC-associated PCBs.[17,24,25] This sum will be referred to as the "total dissolved" concentration. Ideally, the internal standard should be an isotopically labeled analogue of each PCB congener, but this is not practical for the present method targeting 62 congeners, nor does this approach work for ECD detectors. Therefore, the total dissolved concentrations assume that the internal standard and the related sample congeners have the same partitioning behavior to the DOC.

A comparison of the peak areas of the PCB internal standards from the water calibration standards and pore waters from all of the sediments used in this study shows that measurable loss of the higher-molecular-weight PCBs does occur to the DOC with contaminated sediment C and in Aroclor-spiked sediments D and E, but was not measurable in the other samples, perhaps because of relatively low DOC values. None of the samples showed measurable partitioning of the di- and trichlorobiphenyl internal standards into the DOC. For samples C, D, E, and NIST 1944 marine sediment, from about 9 to 16% of the tetrachlorobiphenyl internal standard was found in the DOC phase, while about 25 to 44% for the pentachlorobiphenyl and up to 60% of the hexachlorobiphenyl internal standard was associated with the DOC (Table 5).

Table 5. Partitioning to Pore Water DOM, and log K_{DOC} Values

PCB name	sediment cong. no.	% associated with DOM				log K_{DOC}			
		C	D	E	SRM 1944	C	D	E	SRM 1944
2,3,5,6-tetrachlorobiphenyl	65	16%	9%	10%	24%	4.56	4.09	4.36	4.51
2,2′,4,5′,6-pentachlorobiphenyl	103	44%	40%	25%	39%	5.19	4.91	4.84	4.80
3,3′,4,4′,5,5′-hexachlorobiphenyl	169	54%		61%	61%	5.37		5.50	5.19
DOC, mg/L		5.1	8.2	4.8	90				

As previously discussed, quantitation of pore water PCBs using the full range of internal standards gives the total dissolved concentrations in the combined freely dissolved and DOC phases.[17,24,25] Since partitioning of di- and trichlorobiphenyl congeners to the DOC phase was not measurable, the total dissolved and freely dissolved concentrations are effectively the same for these congeners. For the higher-molecular-weight congeners, freely dissolved concentrations can be calculated from the same analytical data set as used to determine total-dissolved concentrations by basing the calibrations and sample concentration calculations on a lower-molecular-weight internal standard (details of this approach are given in references 17, 24). Therefore, the trichlorobiphenyl internal standard (which shows no measurable partitioning to the DOC phase) was used as the internal standard for all of the higher-molecular-weight PCBs to calculate their freely dissolved concentrations. On the basis of these data and the DOC concentrations (Table 1), the DOC/water partition coefficients (K_{DOC}) can be calculated for the higher-molecular-weight PCBs. As shown in Table 5, log K_{DOC} values range from 3.56 to 4.56 for the tetrachloro- congener, and from 4.24 to 5.50 for the hexachlorobiphenyl congener 169. These values are in reasonable agreement with those reported by Poerschmann et al. for similar congeners including the tetrachloro- congener PCB-52 (log K_{DOC} = 4.94), the pentachloro- congener PCB-118 (log K_{DOC} = 5.78), and the hexachloro- congener PCB-153 (log K_{DOC} = 5.87)[25] (note that Poerschmann et al. reported K_{DOM} values, which were converted to K_{DOC} values assuming his DOM was 65% carbon). Although our values are all somewhat lower, these differences may be caused by different DOM used in the determinations. Poerschmann et al. performed their measurements in water containing 100 ppm of DOM that had been extracted from coal wastewater, while our measurements were based on natural DOM in sample waters at 5 to 8 ppm DOC (except for the SRM 1944, which had a DOC value of 90 ppm). Since different carbon types in sediment can show K_{OC} values that vary by as much as 3 orders of magnitude for a particular organic compound,[28] it seems reasonable to expect that different sources of DOM would have different partitioning properties resulting in a range of K_{DOC} values for a single PCB congener.

Practical Considerations of the Method. As noted above, one goal of this study was to develop the methodology to best fit into practical aspects of running large numbers of sediment pore water samples for all 62 relevant PCB congeners. Although flocculation is required prior to analysis to remove colloids, the simple flocculation step allowed all storage and preparation steps to be performed with readily available disposable "certified clean" glassware that is commonly used in trace environmental studies. In addition, removal of the colloids greatly increases the useful lifetime of the SPME fibers as was previously reported for PAHs.[17] With the 7 μm PDMS fiber and the cleaning procedures described, we have not observed detectable carryover of PCBs from one determination to the next, and the fibers typically can be used for hundreds of determinations without significant loss in performance. Even when a fiber loses sensitivity, the changes are accounted for by the internal standards added to the water samples.

Perhaps the greatest practical advantage of SPME is the ability to use small volume water samples. The ability of SPME to transfer all of the extracted analytes to the GC injection port (as opposed to only a tiny fraction being injected for a solvent extract) yields a great practical advantage for the analysis of field sediment pore waters. Conventional extraction and analysis methods require large volumes (up to a liter or more) of pore water to attain pg/mL detection limits, and such volumes can be very difficult or even impossible to obtain, even from several liters of wet sediment. Since the SPME method requires only a few milliliters of water, separating and preparing the pore water is greatly simplified, and the quantity of wet sediment that must be shipped to the lab can be reduced to a 100 mL. The difficulties in obtaining large numbers of field sediments, homogenizing and shipping sediments to the lab, and storing the samples for laboratory analyses are, therefore, greatly reduced.

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