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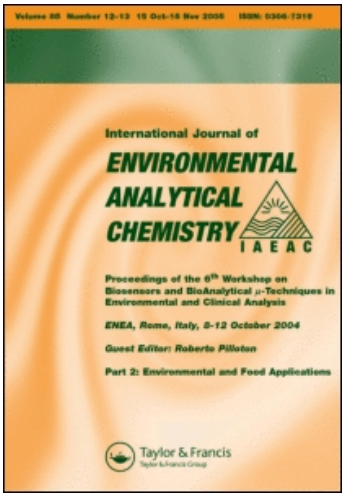

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Hollow-fibre liquid-phase microextraction of polychlorinated biphenyls: dynamic aspects and analytical challenges associated with their speciation

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Hollow-fibre liquid-phase microextraction of polychlorinated biphenyls: dynamic aspects and analytical challenges associated with their speciation

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In the present article, the hollow-fibre liquid-phase microextraction of polychlorinated biphenyls (PCBs) was conducted under non-equilibrium conditions, to investigate the bioavailability of PCBs in natural waters. The study was conducted for 12 PCB congeners ($\log K_{ow}$ ranging from 5.2 to 8.2) in the ng L^{-1} range. This appeared as a major challenge since aqueous solutions in this concentration range tend to evolve rapidly due to adsorption of PCBs on glass walls. The average aqueous diffusion layer was measured to be 43 ± 2 μm at 500 rpm. Aqueous diffusion coefficients of PCBs estimated from experimental data were found to be about two times lower than those predicted by the Hayduk-Laurie equation, possibly due to the underestimation of the molar volume of PCBs, the aggregation of PCBs in the aqueous phase, or a decrease of the actual aqueous concentration during the time of extraction. The presence of Aldrich humic acid (AHA) in the solution decreased, as expected, the mass transfer of PCBs to the fibre, but the flux was not linked either to the total or to the free PCB concentration. This suggests a semi-labile behaviour for the AHA–PCB complex, which was confirmed by the effect of stirring speed on the amount of PCBs extracted in the presence and in the absence of AHA. The whole of these observations suggests that diffusion in solution is not only one of the limiting process for the extraction of PCBs but also supports the need for more experimental data to understand in detail the mechanism of extraction of hydrophobic compounds, and their bioavailability in the presence of aquatic complexants.

Keywords: bioavailability; diffusion coefficient; polychlorinated biphenyl; hollow-fibre liquid-phase microextraction; extraction kinetics; glassware adsorption; PCB–humic interaction

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1. Introduction

In the last decades, the list of organic contaminants detected in natural waters has continuously lengthened, including persistent organic substances (www.chem.unep.ch/pops), organophosphorus insecticides [1], organotin compounds [2], volatile organic compounds [3], pharmaceuticals [4] and synthetic musk flagrances [5]. Total concentrations of organic contaminants are generally monitored as the indicator for water quality and regulations. Nonetheless, natural waters are complex matrices, in which organic

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pollutants are known to associate with suspended particulate matter [6], and dissolved organic matter [7]. In these conditions, a 'free concentration' is defined as the fraction of the contaminant unbound in the aquatic system. This concept of speciation plays a major role for environmental risk assessment, as it may be related to the bioavailability of the contaminant [8,9].

From the analytical point of view, a wide range of analytical tools are available for the quantification of the levels of organic contaminants in natural waters. These methods often rely on the isolation and the pre-concentration of the analyte using conventional liquid–liquid extraction (LLE), solid phase extraction (SPE), *in situ* passive samplers such as semi-permeable membrane devices (SPMD), and microextraction techniques, such as single-drop microextraction (SDME), hollow-fibre liquid-phase microextraction (HF-LPME) or solid phase microextraction (SPME) [10–12]. From a kinetic point of view, the profile of extraction versus time can be divided into three regimes: the kinetic, the near-equilibrium and an intermediate regimes [10,13].

In most monitoring studies, extraction is generally performed under depletive conditions to target the total (dissolved and/or particulate) concentrations of the organic pollutants, even though the influence of speciation on the analytical results is not well documented [14]. However, attempts to probe the free concentrations of organic contaminants in natural waters are less frequent. Equilibrium sampling with the various extraction tools mentioned above is a common way to access to the free concentration, and advantages/disadvantages have been reviewed by Heringa and Hermens [9]. Reaching equilibrium, however, is not always feasible because of technical challenges (e.g. solvent drop stability for SDME, or fouling of the system) or because time to reach equilibrium is too long [15,16]. Parameters that influence the time to reach equilibrium include the partition coefficient of the analyte (between the extracting phase and water), the stirring conditions, the sample volume and the dimension of the extraction device [17]. Time to reach equilibrium may vary from a few minutes up to several days/months depending on sampling conditions. From an environmental perspective, excessive sampling times are not compatible with the understanding of speciation under fluctuating conditions.

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In this context, it is interesting to examine non-equilibrium (or kinetic) sampling as a speciation tool, but the literature in this field is scarce. Jeannot and Cantwell [18] reported the use of SDME as a speciation tool for the determination of free progesterone in the presence of a binding protein. In 2000, Oomen *et al*. [19] investigated the non-equilibrium extraction of polychlorinated biphenyls (PCBs) using SPME in the presence of chyme to evaluate the availability of PCBs for intestinal uptake. These two studies showed that, under specific conditions (uptake process controlled by aqueous diffusion, high concentration of sorbed analyte, fast desorption from the matrix), the flux of analyte into the extracting device will not be proportional to the free concentration, but will be enhanced by a contribution of sorbed analytes. The complex formed by the analyte sorbed onto the matrix is qualified as labile under these conditions. More recently, the environmental speciation of polyaromatic hydrocarbons was investigated using non-equilibrium SPMDs in both laboratory [20] and field experiments [21].

The aim of the present article is to investigate analytical aspects of the non-equilibrium HF-LPME technique, and its potential as a tool to study environmental speciation of PCBs. As pointed out by Oomen *et al*. [19], the potential of this technique as a speciation tool can only be assessed if uptake mechanisms are identified. Therefore, extraction kinetics of PCBs by HF-LPME was investigated to find out whether uptake is controlled by diffusion in the solution or inside the extracting device. Then, the interaction

of PCBs with Aldrich humic acid (AHA) was investigated, to illustrate the effect of the lability degree of the sorbed PCBs on the analytical results.

In the present work, the study range for PCB concentration was voluntarily chosen in the $ng\,L^{-1}$, which is most relevant for environmental systems. As developed in this article, the study of PCBs in aqueous samples in the $ng\,L^{-1}$ range do face analytical challenges linked with adsorption of PCBs on every type of material. To overcome this issue, strategies such as measurement of the actual concentration and silanisation have been tested. Understanding the possible influence of these strategies on speciation results is another goal of this article.

2. Experimental

2.1 *Reagents*

Hydrophobic hollow-fibre membranes (polypropylene, Accurel ppq 3/2, 0.2 μm pore size, 100 μm thickness, 600 μm inner diameter) were obtained from Membrana GmbH (Wuppertal, Germany). *n*-Nonane, acetone and *n*-hexane were of analytical grade and ultrapure deionised water ($R > 18\,M\Omega\,cm$) was obtained with a MilliQ water purification system (Millipore, Billerica, MA, USA). Sodium phosphate, sodium chloride and hydrochloric acid were high-purity reagents. All glassware was cleaned with detergent solution (A-110204, Milian, Switzerland) and rinsed with solvent before use.

The PCBs (Table 1) were purchased from Supelco (Belafonte, PA, USA). PCB 209 was used as internal standard. A working PCB solution (congeners 18, 28, 31, 44, 52, 101, 118, 138, 149, 153, 180, 194) was prepared in acetone for the spiking experiments and analysed by gas chromatography to measure the exact PCB concentration. Octanol–water partition coefficients (K_{ow}), dissolved organic carbon/water partition coefficients (K_{DOC}) and aqueous solubilities from the literature are presented in Table 1.

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2.2 *Sample preparation*

Well-defined artificial water samples were used to make easier the characterisation of extraction kinetics. Artificial water samples in this study consisted in 200 mL phosphate buffer solutions (10^{-2} M, pH = 7.4 ± 0.1, $I = 0.03$ M). Ionic strength and pH were adjusted with sodium chloride and hydrochloric acid. Phosphate buffer (pH range representative of natural waters) was selected as it was previously used with immunoassays for PCBs [22] and in culture media (cells/microorganisms) for PCB exposure [23,24]. A 100 mL of this solution was transferred to a 250 mL blue-cap glass bottle, the PCBs in acetone (10–150 μL) were spiked with a syringe and the remaining 100 mL of solution was added. The samples were allowed to equilibrate for 24 h before extraction, in a horizontal shaker (150 rpm) in the dark. All experiments were conducted at room temperature (22 ± 1°C).

AHA (sodium salt, technical grade) was obtained from Sigma Aldrich (Steinheim, Germany). A 150 mg of this salt was dissolved in 7.5 mL of a NaOH 0.1 M to prepare a stock AHA solution. The total organic carbon of the stock AHA solution was measured using a TOC analyser (Shimadzu TOC-5000, Kyoto, Japan) and the carbon content in AHA was found to be 45.2 ± 1.4%, which is consistent with other reports (e.g. 42.5% reported by Jonassen *et al.* [25]). The influence of AHA was studied by adding volumes of the AHA stock solution to the artificial water to obtain final concentrations of AHA ranging from 5 to 100 $mg\,L^{-1}$.

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Table 1. List of PCB congeners and physicochemical properties (octanol–water partition coefficient; dissolved organic carbon/water partition coefficient; aqueous solubility).

IUPAC number	PCB congener	Log K_{ow}[a] (25°C)	Log K_{DOC}[b] (L/kg)	Log K_{DOC}[c] (L/kg)	Aqueous solubility ($\mu g\,L^{-1}$)[d]	Theoretical diffusion coefficient ($cm^2\,s^{-1}$)[e]
PCB 18	2,2′,5-trichlorobiphenyl	5.24	4.57	n.a.	n.a.	5.4×10^{-6}
PCB 28	2,4,4′-trichlorobiphenyl	5.67	n.a.	5.83	261	5.4×10^{-6}
PCB 31	2,4′,5-trichlorobiphenyl	5.67	n.a.	n.a.	220	5.4×10^{-6}
PCB 44	2,2′,3,5′-tetrachlorobiphenyl	5.75	4.78	n.a.	n.a.	5.2×10^{-6}
PCB 52	2,2′,5,5′- tetrachlorobiphenyl	5.84	4.41–5.11	6.05	199	5.2×10^{-6}
PCB 101	2,2′,4,5,5′-pentachlorobiphenyl	6.38	5.31–5.41	6.51	34	5.0×10^{-6}
PCB 118	2,3′,4,4′,5-pentachlorobiphenyl	6.74	5.18–5.62	6.70	29	5.0×10^{-6}
PCB 138	2,2′,3,4,4′,5′-hexachlorobiphenyl	6.83	5.98	7.02	7.5	4.8×10^{-6}
PCB 149	2,2′,3,4′,5′,6-hexachlorobiphenyl	6.67	5.79	n.a.	n.a.	4.8×10^{-6}
PCB 153	2,2′,4,4′,5,5′-hexachlorobiphenyl	6.92	6.51	6.88	13.6	4.8×10^{-6}
PCB 180	2,2′,3,4,4′,5,5′-heptachlorobiphenyl	7.36	6.29	7.12	3.4	4.6×10^{-6}
PCB 194	2,2′,3,3′,4,4′,5,5′-octachlorobiphenyl	7.80	n.a.	n.a.	4.1	4.5×10^{-6}
PCB 209	decachlorobiphenyl	8.18	n.a.	n.a.	n.a.	4.2×10^{-6}

[a]K_{ow} values estimated by Hawker and Connell [41]. [b]Range of values from the review by Krop *et al.* [7]. [c]Values reported by Durjava *et al.* [42]. [d]Values at 25°C after adjustment from the review by Li *et al.* [43]. [e] Calculated using the Hayduk and Laudie equation [36].

2.3 *Conventional liquid–liquid extraction of PCBs*

For some solutions, the actual aqueous concentration was measured following the conventional liquid–liquid extraction of 200 mL of water with 3×30 mL hexane in a 500 mL separation funnel. Extracts were dried using anhydrous sodium sulfate and were concentrated to 0.5 mL using rotary evaporation. Extracts were further concentrated to 100 μL under a gentle flow of purified nitrogen using 100 μL of *n*-nonane containing PCB 209 as internal standard (100 ng/mL).

In preliminary experiments, the adsorption on glass walls of the blue-cap bottles was confirmed. Briefly, artificial water spiked with PCBs was left for equilibration in blue-cap bottles for 24 h as described previously. The aqueous phase was removed and the extraction with 60 mL of *n*-hexane for 24 h on a horizontal shaker was done on the glass walls and extracts were treated as done for water extracts.

2.4 *Hollow-fibre microextraction of PCBs*

Hollow fibres were cut into 15 mm long sections and were cleaned in acetone prior to use. Hollow fibres were fitted on GC syringes as described by Basheer *et al*. [26]. Triplicate HF-LPME was performed simultaneously in a 250 mL blue-cap bottle under stirring. The volume of the solvent inside the fibre was evaluated by comparing the weight of the dry fibre and impregnated fibre and adding the volume inside the lumen (3.5 μL). The total volume of nonane inside the fibre was about 10 μL. Adsorption on the polypropylene membrane was assumed to be insignificant as compared to the partitioning with the nonane inside the pores of the membrane. Basheer *et al*. [27] coated the polypropylene hollow fibres with polymers to obtain significant adsorption properties.

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2.5 *Gas chromatography quantification*

Quantification of PCBs was performed by gas chromatography equipped with an electron capture detector (Autosystem XL, Perkin-Elmer, Wellesley, MA, USA). Compounds were separated on a Elite 5 (Perkin–Elmer) capillary column (30 m length, 0.25 mm internal diameter) with helium as the carrier gas (constant inlet pressure of 100 kPa). The GC oven program was as follows: initial temperature of 100°C, 20°C min^{-1} to 200°C, 2.5°C min^{-1} to 250°C, 20°C min^{-1} to 300°C held for 13.5 min. PCB 28 and 31 were not totally resolved with the present system and were quantified as a sum of the two congeners (28 + 31). A six-point calibration curve was obtained with PCB standards prepared in the range of 10–200 ng mL^{-1} in *n*-nonane, and a linear response was observed ($r^2 > 0.99$). There were no differences in the chromatogram between PCB standards and extracts, in terms of retention time or background. All statistical analysis was performed using SigmaStat 3.01 software (Systat Software, San Jose, CA, USA). For the sake of clarity, some figures were made for only four PCB congeners, representative of different levels of chlorination.

2.6 *Measurement of the aqueous diffusion layer thickness*

The thickness of the aqueous diffusion layer at the hollow-fibre surface is a key parameter to interpret extraction kinetics. The measurement of the aqueous diffusion layer of the HF-LPME system could not be performed with PCBs because, to the best of our knowledge,

their diffusion coefficients have not been accurately measured. As a substitute, 2-chlorophenol was chosen as the model compound as it has a relatively high solubility ($24\,g\,L^{-1}$ at pH = 7, $T = 25^{\circ}C$) and its acidic form ($pK_a = 8.5$) will accumulate in *n*-nonane ($\log K_{ow} = 2.15$). The diffusion coefficient of 2-chlorophenol was measured by Niesner and Heintz [28] for three different temperatures. Using linearity of Niesner and Heintz data, a D_w value of $9.0 \times 10^{-10}\,m^2\,s^{-1}$ was obtained for the temperature of the experiment (22°C).

To measure the aqueous diffusion layer thickness, solutions were prepared as explained earlier (phosphate buffer, pH = 7.5), spiked with 2-chlorophenol (Supelco; $152\,\mu g\,L^{-1}$) and extracted for various time. The HF-LPME extracts were quantified for 2-chlorophenol on a gas chromatography equipped with a flame ionisation detector (Autosystem XL, Perkin–Elmer, Wellesley, MA, USA).

3. Results and discussion

3.1 *Adsorption of PCBs on glassware*

Glass adsorption of PCBs from water has been reported previously and represents a significant source of error at low PCB concentration [14,29,30]. In a preliminary experiment, the adsorption of PCBs on glass walls from spiked solutions was tested with 100 mL bottles (volume to surface ratio of 1.0 cm). The extraction of the glass walls of the bottles confirmed that between 40 and 85% of the spiked PCBs were adsorbed on the glass walls after 1 day. Further absorption of PCBs (2, 4 and 7 days) is illustrated in Figure 1 for PCB 153. Actual concentration of PCBs in the aqueous phase was observed to further decrease over time, even below 10% of the initial spiked concentration within a week for some congeners. Using 250 mL bottles (volume to surface ratio of 1.3 cm), an average (for all PCBs) of $34 \pm 7\%$ of spiked PCBs was adsorbed on glass walls after 24 h of equilibration. As expected, bottles with a higher volume-to-surface ratio presented lesser adsorption. In the following experiments, 250 mL bottles were equilibrated for 24 h exactly to achieve an optimum reproducibility between samples.

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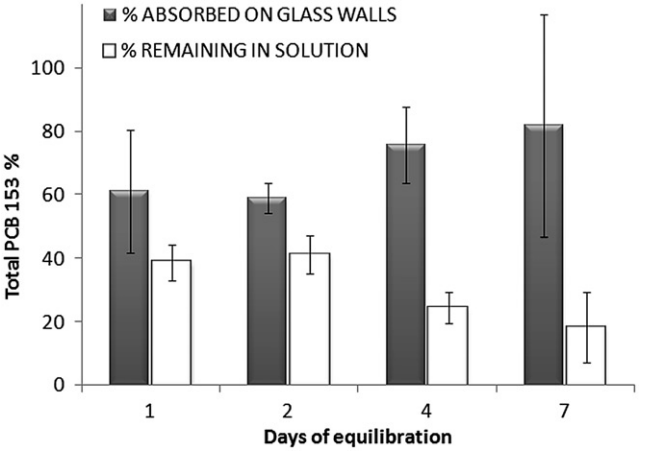

Figure 1. Evolution of the amount of PCB adsorbed and remaining in solution for a 100 mL bottle spiked at $200\,ng\,L^{-1}$. Both adsorbed and soluble PCB were determined experimentally.

3.2 *Depletion of the solution*

When the bioavailability of a chemical is investigated, it is essential to minimally disturb equilibriums that exist between the hydrophobic contaminant and natural aquatic colloids to determine specifically the free and the total concentration. In these conditions, depletive extraction can not be used [13,31]. Depletion of the solution by the hollow fibre was calculated according to the following equation:

$$\text{Depletion} = \frac{C_o \cdot V_o}{C_w \cdot V_w} \quad (1)$$

where C_w is the initial actual concentration of the analyte, C_o is the extract concentration, and V_o and V_w are the volumes of the organic and aqueous phases, respectively. Depletion of the solution by the hollow fibre was evaluated for five spiking levels (10–150 ng L^{-1}). As the volume of nonane in the fibre is 10 µL, the average depletion was calculated to range from 0.2 to 5.4% amongst PCB congeners. Thus, in the present conditions, the HF-LPME technique for PCBs appears as non-depletive for a 200 mL solution over 60 min.

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3.3 *Effect of extraction time*

The effect of extraction time was first evaluated for a solution spiked at 87 ng L^{-1} of each PCB congener (i.e. about 2 to 5×10^{-10} mol/L depending on the congener), continuously stirred at 500 rpm during 15, 30, 45 and 60 min ($n = 2$). The concentration in the fibre continuously increased with time for all PCB congeners over 60 min (Figure 2). Basheer *et al.* reported the effect of extraction time for the extraction of organochlorine pesticides from seawater [26] and rainwater [32] using HF-LPME. In their studies, an equilibrium was reached after 30 min using toluene as an extraction solvent (5 mL of sample). In the present study using n-nonane (200 mL of sample), the extraction was still in the kinetic

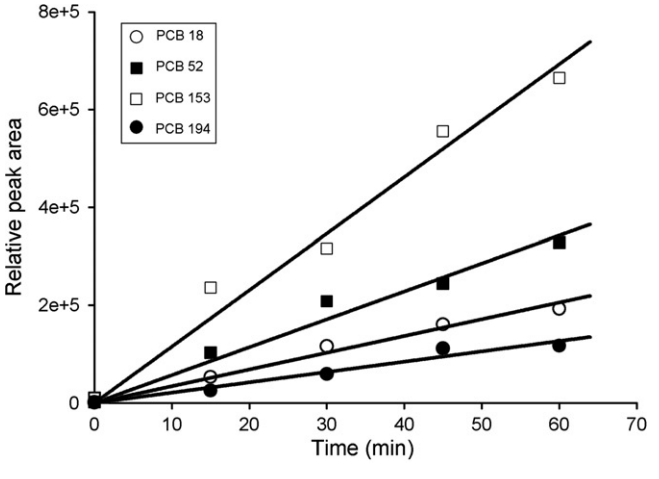

Figure 2. Analyte peak area *vs.* extraction time for PCB congeners 18, 52, 153 and 194, for a solution spiked at 87 ng L^{-1} of each PCB congener, continuously stirred at 500 rpm. Average for $n = 2$. The linear regressions are plotted and fit the data.

phase after 60 min for all PCB congeners. Slower kinetics of the current system was the result of different organic phases in combination with different sample volumes. Although *n*-nonane is less volatile and soluble in water than toluene, losses of organic solvent are observed after 2–3 h.

3.4 *Effect of sample agitation*

The effect of sample agitation on HF-LPME was evaluated for a solution spiked at 98 ng L^{-1} of each PCB congener, stirred for 60 min at 0, 100, 200, 300, 400 and 500 rpm ($n = 3$). Higher stirring rates were not suitable with the present device due to vortex formation. No air bubble was observed at the surface of the fibre, even for the highest stirring rates. The concentration in the fibre increased with agitation for all PCB congeners in the range of 0–500 rpm, which is typical of a system limited by aqueous diffusion as discussed later in this article. The extracted amount increased by a factor of 5–27 between 0 and 500 rpm, depending on the PCB congener. No obvious trend amongst level of chlorination was observed for this effect. The stirring rate was set to 500 rpm for the rest of the study to obtain the highest sensitivity.

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3.5 *Calibration*

In a first experiment, five bottles were prepared as described previously for five spiking levels (10–150 ng L^{-1}). After 24 h of equilibration, the solution was transferred to a separation funnel and extracted using conventional liquid–liquid extraction to measure the actual aqueous concentration. As mentioned previously, spiking concentrations were one to three order of magnitude lower than the aqueous solubility (at 25°C) for the various PCB congeners (Table 1). Despite spiking levels below solubility, the actual concentrations in the bottles equilibrated for 24 h were lower than the spiked concentration, due to glass wall adsorption as discussed earlier. The slope of the measured PCB concentration versus the spiked concentration were $63.8 \pm 5.9\%$, $71.3 \pm 3.7\%$, $77.0 \pm 7.6\%$ and $68.4 \pm 2.5\%$ for PCB 18, 52, 153 and 194, respectively. Silanisation of glassware is often suggested as a way to minimise the adsorption of organic substances onto glass surfaces. For that reason, the measurement of the actual concentration versus the spiked was also carried out in silanised glassware (including silanised glass stirring bars), even though it might be expected that PCB adsorption might be higher on the more hydrophobic silanised glassware. The actual concentrations in the bottles equilibrated for 24 h were also lower than the spiked concentration, and in fact, the actual concentrations were even lower than for non-silanised glassware. The slope of the measured PCB concentration versus the spiked concentration for silanised glassware were $62.3 \pm 1.9\%$, $65.8 \pm 4.3\%$, $55.1 \pm 3.1\%$ and $15.4 \pm 0.7\%$ for PCB 18, 52, 153 and 194, respectively.

Each of these five spiking levels (10–150 ng L^{-1}), left for equilibration for 24 h, were also extracted using the HF-LPME technique for 60 min. at a stirring speed of 500 rpm ($n = 3$). The concentration in the nonane extract in the fibre was plotted versus the spiking level for PCB 18, 52, 153 and 194 (Figure 3) and varied linearly with the spiking levels for all PCB congeners, for both silanised and non-silanised glassware. Although the actual aqueous PCB concentrations in the silanised bottles were lower than for non-silanised glassware, the concentrations in the nonane extract from silanised bottle were comparable

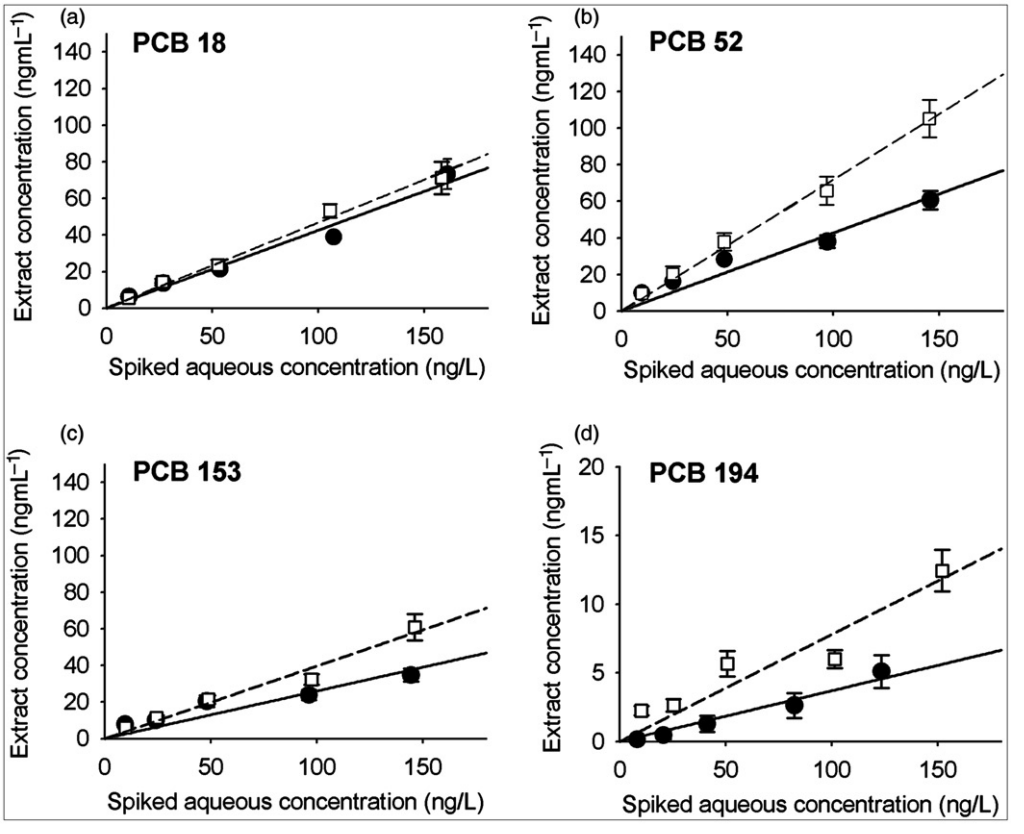

Figure 3. HF-LPME organic phase concentration *vs.* spiked aqueous PCB concentration for a range of standard solutions, after 60 min of extraction, for non-silanised (●) and silanised (□) glassware. Average ± standard deviation for $n = 3$.

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or slightly higher than from non-silanised bottles. These observations will be discussed later in this article.

3.6 *Kinetic approach*

In the kinetic regime of extraction, the concentration in the organic phase (C_o) will be related to the PCB flux (J) from the aqueous to the organic phase, the volume of the organic phase (V_o), the surface (S) of the fibre and the extraction time (Equation (2)). N represents the number of moles of solute passing through a surface of area S.

$$J = \frac{1}{S} \cdot \frac{dN}{dt} = \frac{1}{S} \cdot \frac{d(C_o \cdot V_o)}{dt} \tag{2}$$

At steady state, J is independent of time and:

$$C_o = \frac{S}{V_o} \cdot J \cdot t \tag{3}$$

In addition, it can be shown [33] that the flux (J) of PCB at the aqueous/organic phase interface is ruled by three processes (Equation (4)): (a) the transport of the PCB by diffusion in the aqueous phase, (b) its transfer at the interface and (c) its transport by diffusion in the organic phase. For diffusion at a planar surface:

$$J = \left[\frac{\delta}{D_w} + \frac{\delta_o}{D_o \cdot K_D} + \frac{1}{k_d}\right]^{-1} \cdot C_w \tag{4}$$

where δ and δ_o are the diffusion layer thickness in water and organic solvent, D_w and D_o are the diffusion coefficients of the solute in water and the organic solvent, respectively, K_D is the partition coefficient between water and the organic solvent and k_d is the rate constant of transfer at the interface. Because the radius of curvature of the hollow fibre (400 μm) is large compared to δ (~40 μm as measured below), Equation (4) is applicable to the present cylindrical hollow fibre.

Usually, k_d is very large, so that the third term of Equation (4) is negligible. In addition, to evaluate importance of the first two terms for a hollow fibre, δ_o can be approximated as half of the fibre radius [34] (i.e. 200 μm). $D_o \sim D_w$ and $K_D \sim K_{ow}$ (i.e. ranging from 10^4 to 10^8) for the various PCBs can be used. δ ranges from 1 to 400 μm in the literature, with more typical values between 10 and 100 μm [35]. Under these conditions, the transport in the aqueous phase is dominant, and Equation (4) becomes:

$$J = \frac{D_w}{\delta} \cdot C_w \tag{5}$$

This equation illustrates the fact that, when stirring increases (i.e. when the aqueous diffusion layer thickness, δ decreases), the efficiency of the microextraction increases. δ can then be obtained combining Equations (3) and (5):

$$\delta = \frac{S \cdot D_w \cdot t}{V_o} \cdot \frac{C_w}{C_o} \tag{6}$$

The HF-LPME extraction of the 2-chlorophenol, was conducted to measure δ for the present system. The extraction profil with time for a solution spiked at $152\,\mu g\,L^{-1}$ is presented in Figure 4. Using Equation (6) and a D_w value of $9.0 \times 10^{-10}\,m^2\,s^{-1}$ for

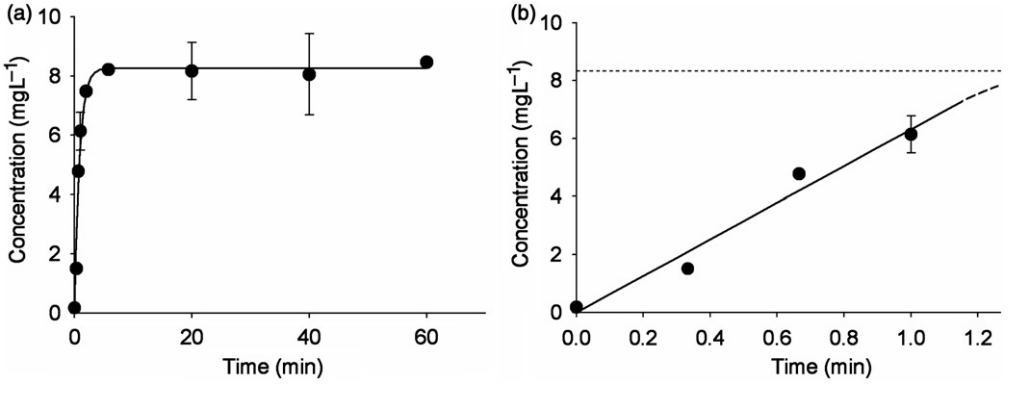

Figure 4. Accumulation of 2-chlorophenol with time using HF-LPME at long (a) and short (b) time scales. The slope of the kinetic (linear) regime of the extraction is $6.2 \pm 0.3\,mg\,L^{-1}\,min^{-1} = 4.8 \pm 0.2 \times 10^{-5}\,M\,min^{-1}$.

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2-chlorophenol, δ was calculated to be $43 \pm 2\,\mu m$ at a stirring rate of 500 rpm, which is a reasonable value for the system studied here. The present δ-value is in line with the literature, as 100 μm corresponds to a relatively quiescent system and 10 μm to a very efficient stirring [35]. Back-calculation using the measured value for δ in terms of Equation (3) confirms that the transport of hydrophobic PCBs to the hollow fibre is dominated by diffusion in the aqueous phase.

3.7 *PCB diffusion coefficients*

Since no experimental data exist on the diffusion coefficient of PCBs in water, the experimental values computed from the present data were compared with theoretical values. Theoretical values can be calculated from equations derived from the Stokes–Einstein relation, such as the Hayduk and Laudie equation [36]:

$$D_w(cm^2.s^{-1}) = \frac{13.26 \times 10^{-5}(g\,mol^{-1}\,s^{-2})}{\eta^{1.14} \cdot \overline{V}^{0.589}} \quad (7)$$

where η is the viscosity in centipoises and $\overline{V}$ is the molar volume of the PCB congener ($cm^3\,mol^{-1}$). In the literature, $\overline{V}$ values are derived from their molecular weights and liquid (or supercooled liquid) densities at room temperature. Values range from 230 to 320 $cm^3\,mol^{-1}$ from tri to octachlorobiphenyls [37]. Using Equation (7) and a viscosity of 1 centipoise (water), the theoretical diffusion coefficient is found to decrease from 5.4×10^{-6} to $4.4 \times 10^{-6}\,cm^2\,s^{-1}$ from tri to octachlorobiphenyls (Figure 5).

Equation (6) applied to HF-LPME experiments can also be then used to evaluate experimental values of the diffusion coefficients of PCBs, using $\delta = 43\,\mu m$. Results are shown on Figure 5 for both non-silanised and silanised glassware. Experimental diffusion coefficients decreased from tri to octachlorobiphenyls and were overall smaller than theoretical values by a factor of 2. Experimental diffusion coefficients in water with non-silanised glassware were smaller than with silanised glassware, particularly for most

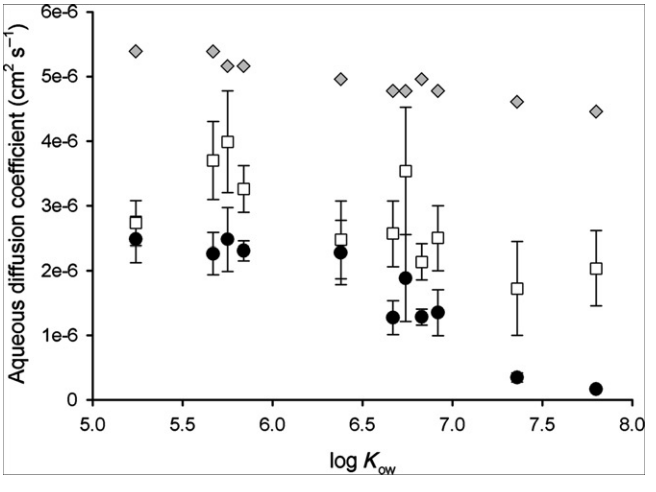

Figure 5. Experimental (●: non-silanised glassware, □: silanised glassware) and theoretical (◆) diffusion coefficient. Average ± standard deviation for $n = 5$.

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hydrophobic PCBs. Several factors may explain the difference between theoretical and experimental diffusion coefficients. First, theoretical values may be overestimated as $\overline{V}$ is based on the molecular weight and the sub-cooled density, and does not take into account the geometry and hydrophobic interactions of PCBs. Then, the actual aqueous concentration, C_w, although initially measured by conventional LLE, may decrease over the 60 min of extraction due to further adsorption on the glassware. Additionally, adsorption on the stirring bar is common source of error in studies of PCBs at low concentration [31,38], and this might have resulted in a lower PCB concentration in the aqueous phase during extraction. Finally, the actual diffusion coefficient of PCBs in the low ng L^{-1} range might be smaller than predicted due to aggregation of PCB molecules in the aqueous phase. Gustafson and Dickhut [39] measured D_w for a suite of polyaromatic hydrocarbons, and reported that measured values deviate exponentially from predicted D_w values with increasing molar weight. In their paper, Gustafson and Dickhut [39] proposed that the exponential deviation of PAH aqueous diffusivities may be due to hydrophobic interactions and the formation of dimers or trimers in aqueous solutions. In this context, experimental diffusion coefficients of hydrophobic chemicals in water, based on other techniques, are required for comparison for further interpretation of the present data.

The effect of silanisation on the extraction of PCBs is unclear. Hypothetically, amphiphile residues of silanisation might have been released in the solution and have affected the aqueous solubility of PCBs. It is also possible that PCBs adsorbed on silanised glassware might desorb faster than from non-silanised glassware and be supplied to the hollow fibre. More sophisticated analytical tools would be required to elucidate the real role of silanisation. It seems clear, however, that it may affect both the bulk concentration of PCBs in solution and their kinetic accumulation in HF-LPME.

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3.8 *Effect of Aldrich humic acid*

The effect of AHA on HF-LPME was evaluated by comparison of the GC-ECD peak area in the presence and in the absence of the humic substance (Figure 6). For lighter PCBs, no

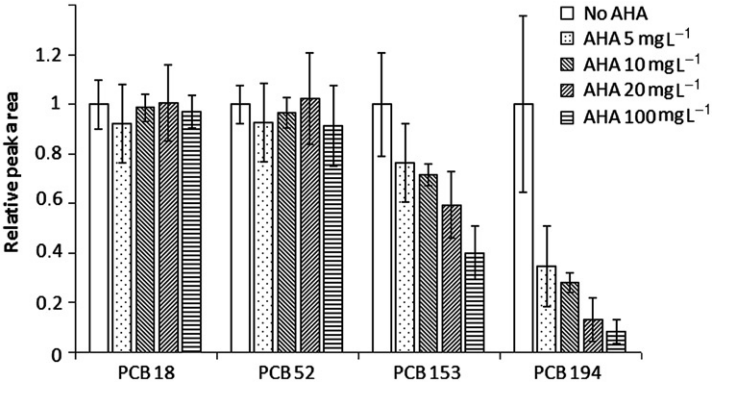

Figure 6. Relative PCB peak area, compared to the PCB peak area in the absence of AHA after 60 min of extraction, with ~70 ng L^{-1} of PCB congener in solution ($n = 11$ for no AHA, $n = 8$ for AHA 5 mg L^{-1}, $n = 2$ for AHA 10 mg L^{-1}, $n = 11$ for AHA 20 mg L^{-1}, $n = 8$ for AHA 100 mg L^{-1}).

significant difference could be observed in the absence or presence of AHA in the concentration range of 5–100 mg L^{-1} (i.e. a range of 2–45 mg C L^{-1} for dissolved organic carbon). However, with increasing molecular weight (i.e. increasing hydrophobicity), the peak area significantly decreased when AHA is added in the solution. The association of hydrophobic pollutants and dissolved organic matter is often illustrated by the K_{DOC} partition coefficient, defined as:

$$K_{DOC} = \frac{C_{DOC}}{C_{free}} \tag{8}$$

where C_{DOC} is the weight fraction of PCB in the DOC and C_{free} is the free PCB concentration. Some values for PCBs have been reported in the literature (Table 1). If only 'free' PCB was contributing to the microextraction, the flux of PCB in the presence of AHA (J_{HA}) should be easily computed using K_{DOC} values according to the following equation:

$$J_{AHA} = \frac{J_{noAHA}}{1 + K_{DOC} \cdot [DOC]} \tag{9}$$

with J_{noAHA} the PCB flux in the absence of AHA using similar experimental conditions. By combining Equations (3) and (9), C_o would be found to be inversely proportional to $(1 + K_{DOC} \times [DOC])$. However, the actual PCB concentration in the fibre was up to two orders of magnitude higher than what would be predicted if only 'free' PCBs were contributing to the extraction. In other words, the actual PCB flux, in the presence of AHA is smaller than that due to the contribution of all PCB species, but much larger than that which would be observed if only free PCBs were contributing. Thus, the complexes formed between PCBs and AHA appear to be labile or semi-labile towards the microextraction device under the present conditions.

To further investigate the possible role of the lability of the PCB-AHA complexes on the measured flux, the extraction of PCBs from a solution spiked at about 100 ng L^{-1} in the presence of AHA 100 mg C L^{-1} was performed at stirring rate between 0 and 500 rpm. The ratio between the PCB peak areas in the presence and in the absence of AHA was calculated for each stirring rate (Figure 7). Because of the complexation by AHA, this ratio is always ≤ 1. Stirring has no effect for lighter PCBs (e.g. PCB 18 and 52), and the ratio equalled 1, suggesting that either these PCBs are not complexed by AHA or the PCB-AHA complex is totally labile at all stirring conditions tested here. For larger mass PCBs (e.g. PCB 153 and 194), the ratio of the PCB peak areas in the presence and in the absence of AHA decreased with stirring speed. This confirms the formation of semi-labile complexes between those PCBs and AHA, whose contribution to the extraction flux will vary with stirring conditions. Indeed, at lower stirring rates (e.g. 0 rpm) the aqueous diffusion layer thickness, δ is larger and the contribution of PCB to the flux is larger due to higher probability of dissociation of the PCB–AHA complex. The semi-labile behaviour confirms that non-depletive non-equilibrium sampling of organic contaminants such as PCBs in a natural sample cannot be linked simply to either free or total PCB concentration.

4. Conclusions and implications

In the present article, extraction kinetics of PCBs in the ng L^{-1} by HF-LPME were investigated, and the transport of hydrophobic PCBs to the hollow fibre is dominated in the

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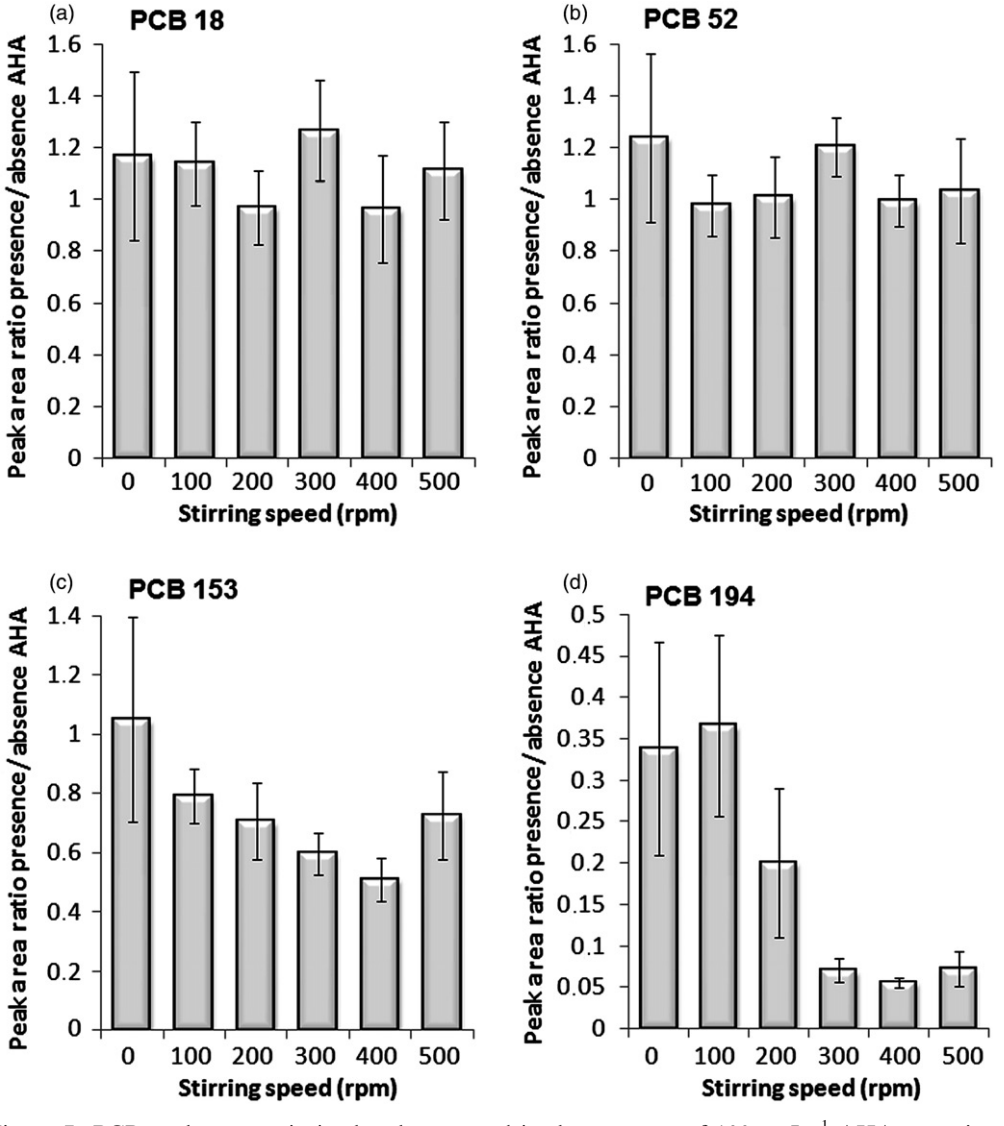

Figure 7. PCB peak area ratio in the absence and in the presence of 100 mg L^{-1} AHA at various stirring speeds, with ~70 ng L^{-1} of PCB congener in solution.

present conditions by diffusive transport in the aqueous phase. Under such conditions, the kinetics of association/dissociation of PCBs with complexants in the solution may influence the amount of PCB extracted in the kinetic phase of extraction. This is exemplified by the present experiments with AHA, in which the complexes formed between PCBs and AHA appear to be labile or semi-labile towards HF-LPME. Thus, the lability degree of the sorbed hydrophobic chemicals must be carefully considered in non-equilibrium sampling techniques, to properly interpret their speciation and availability.

In addition, the present investigation reveals that analytical measurement and ultimately understanding the bioavailability of PCBs in natural waters using HF-LPME

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faces some challenges. The interplay of analytical (very low concentrations, losses by adsorption on glassware) and physicochemical difficulties for the interpretation of flux must be emphasised. This is exemplified in this article by the effect of silanisation on the PCB flux in the hollow fibre. Due to very low concentrations and high hydrophobicity of PCBs, small physical or chemical changes in the analytical conditions may have significant effects and should be studied carefully.

This study clearly shows that dynamic factors (either diffusion in the aqueous solution or chemical kinetics of complexation with natural complexants like humics) play a major role in the results of HF-LPME. In order to study these effects quantitatively, however, well-controlled analytical and hydrodynamic conditions should be used. In particular, systems enabling the development of well-characterised diffusion layer at the membrane surface, such as flow-through cells would allow the control of the hydrodynamic conditions. However, these systems involve more surfaces where PCB can adsorb [40], which may also complicate the study of speciation.

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