SOLID PHASE ENHANCED DIRECT SPECTROFLUOROMETRIC DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCB's) IN NATURAL WATERS

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A method of detection of PCBs in water, based on extraction/concentration with chips cut from an SPE disk-shaped C18 inverse phase coupled with a fluorescence detection and quantification directly on the phase has been evaluated. Experiments were performed with PCBs congeners IUPAC No 3, 35, 67 and 169. Within the experimental conditions used: Hitachi F-4500 fluorometer, band-paths: 2.5 nm and extraction time: 60 min, the limits of detection were estimated to be respectively: 0.07, 0.4, 0.3 and 0.2 ppb. These values are within the range of PCB concentrations found in highly polluted waters and thus confer some interest to the method. However, its sensitivity is not sufficient for application to moderately polluted natural water and the method needs some further improvements. Moreover, fluorescence quenching by humic substances was shown to substantially affect PCB’s fluorescence intensity and must be taken into consideration for quantitative analysis.

\textbf{Keywords:} Polychlorobiphenyls; Solid Phase Extraction; Fluorometry.
INTRODUCTION

Because of the persistency and ubiquitous presence of PCB's in natural media, there is a continuous interest in the development of reliable, practical and sensitive methods for their analysis in natural media. Most of the methods developed so far for the detection and determination of PCB's usually include an extraction step (liquid-liquid extraction or adsorption on a solid phase) followed by a chromatographic analysis [1]. All these methods require sophisticated instruments and several steps which are known to introduce substantial loss of the analytes and possible errors in the measurements. Moreover, both extraction methods require the use of organic solvent whose utilization is more and more subject to law limitation.

In the case of solid phase extraction which is actually receiving increasing attention for the analysis of organic pollutants in natural aqueous media [2-13], recent works have shown that it is possible to avoid elution of the solid phase and chromatographic steps by performing a direct spectroscopic analysis of polycyclic aromatic hydrocarbons from the aqueous medium directly on an appropriate solid phase after extraction. [2, 7, 10-13]. To our knowledge such type of application has yet received little attention for the detection of PCBs in water. Only recently was reported the detection of PCBs performed by room-temperature phosphorimetry by Arruda et al. [13].

In the present work, we have evaluated a method of detection of PCB's in water, based on extraction with a SPE disk-shaped C18 inverse phase coupled with a fluorescence detection and quantification directly on the sorbent.

EXPERIMENTAL

Materials
The sorbent material was prepared as 5x10 mm rectangular chips cut from SPE disks made of octadecyl silica phase enmeshed on fiber glass support (Supelco ENVI-Disk™). As shown in Figure 1, the sorbent chip is attached to a stainless steel holder and the ensemble is immersed into 150 mL of an aqueous solution of PCB (thermostated at 20°C) for a fixed period-of time. Preliminary conditioning of the sorbing phase with acetonitrile or methanol was found to not improve significantly the efficiency of the analyte adsorption.
FIGURE 1. Schematized representation of the experimental process used for detection of PCBs in water based on a direct fluorometric analysis on the sorbing phase.

PCBs were obtained from Dr. Ehrenstorfer GmbH, Augsburg. For testing the method only four congeners which were exhibiting suitable fluorometric properties were used, namely IUPAC N°3, 15, 37 and 169. These congeners are substituted in para and/or meta position and are capable of assuming a planar conformation. According to the literature they are suspected to present a dioxin like toxicity [1]. Stock solutions were first prepared in acetonitrile (spectroscopic grade) at a concentrations of $10^{-3}$ M. The working solutions containing no more than 0.1 % of acetonitrile were obtained by appropriate dilution with milli-Q water followed by about 24 hours of stirring to ensure total dissolution.

The aqueous solutions containing PCBs at trace level were only in contact with stainless steel material to avoid adsorption onto the walls. This material comprises essentially: a 200 mL Becher, the sorbent holder and a rod-like agitator driven with a small motor set above the Becher (Figure 1).

**Fluorescence measurements**

After adsorption, the sorbent holder was placed in the sample compartment of an HITACHI F-4500 spectrofluorometer which was used for measuring the emission intensity from the surface of the sample as schematised in the Figure 1.

A band-path of 2.5 nm was selected for excitation. For emission, band-paths of either 1 or 2.5 nm were selected depending on the
concentration range. Fluorescence emission and excitation spectra were corrected for variation with wavelength of the lamp intensity and photomultiplier sensitivity. The apparatus was interfaced to a microcomputer for instrument operation and spectra processing.

Fluorescence quantum yields of PCBs in acetonitrile were estimated [14] by comparing the integrated intensity of the fluorescence spectra with respect to the emission spectra of a degassed solution of biphenyl ($\Phi_f = 0.18$, [15]) in cyclohexane. In these measurements the liquid solutions were contained in 1x1 cm fused silica optical cell and the concentrations were adjusted to give an absorbance lower than 0.1 at the wavelength of excitation.

Suwannee River humic acid from the IHSS was used for testing interference with PCBs.

RESULTS AND DISCUSSION

Fluorescence spectra in the sorbing phase.

FIGURE 2. Fluorescence spectra of the four investigated PCBs adsorbed from the water solution on a sorbent ship (5x10 mm) cut from an octadecyl silica phase SPE disk (Supelco ENVI-Disk™). C = 100 ppb, Time of immersion : 30 min; $\lambda_{exc}$: 260 nm. Excitation band-path: 2.5 nm; Emission band-path: 1 nm.

In the Figure 2 are shown the fluorescence spectra of the four investigated PCBs adsorbed on the sorbing phase after an immersion time of 30 min in a water solution containing 100 ppb of each. They are all exhibiting broad band emission located in the same wavelength
range with maxima ranging from 310 nm to 330 nm. Their fluorescence excitation spectra (not shown) display also very similar feature. It follows that selective excitation as well as synchronous fluorescence technique for individual identification and quantification, if even possible, should require special and complex data processing to be applied to this class of pollutants. Nevertheless, as evidence by their relatively noticeable fluorescence quantum efficiency reported in Table 1, their detection by using fluorometric technique appears to be potentially favorable and deserves for further development if a global fluorometric detection is envisaged.

<table>
<thead>
<tr>
<th>PCB's congeners</th>
<th>$\Phi_i^a$ x 10^{-2}</th>
<th>Log $K_{nr}^b$</th>
<th>Calib. slope$^c$ (u.FL ppb$^{-1}$)</th>
<th>LOD$^d$ (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB3</td>
<td>2.4±0.2</td>
<td>4.7</td>
<td>872.7</td>
<td>0.07</td>
</tr>
<tr>
<td>PCB15</td>
<td>2.5±0.2</td>
<td>5.1</td>
<td>144.8</td>
<td>0.40</td>
</tr>
<tr>
<td>PCB37</td>
<td>0.95±0.05</td>
<td>5.5</td>
<td>224.2</td>
<td>0.30</td>
</tr>
<tr>
<td>PCB169</td>
<td>0.97±0.05</td>
<td>6.7</td>
<td>301.5</td>
<td>0.20</td>
</tr>
</tbody>
</table>

TABLE 1: Photophysical and analytical figures of merit of the four investigated PCBs. $^a$: Fluorescence quantum yields in aerated solution in acetonitrile. $\Phi_i$ have been estimated by comparing integrated fluorescence intensities with that of a biphenyl degassed solution in cyclohexane and assuming $\Phi_i (cyclohexane) = 0.18$ after reference [15]. $^b$: In reference [1] after Shiu and Mackay. [22]. $^c$: In fluorescence unit (u.FL) in the experimental conditions of our measurements: slits: 2.5 nm, PM voltage: 950 V. $^d$: Limit of detection estimated from the calibration slope and a minimum signal equal to three times the rms. noise signal taken as 60 u.FL$^{-1}$ in the conditions of our measurements.

**Adsorption kinetic of PCBs into the sorbent phase followed by fluorometry**

The growth of the signal in function of the immersion time for several concentrations in the solutions were recorded for the four PCBs. In concentration conditions where the signal recorded directly from the liquid solution could be measured, a signal with intensity about two
orders of magnitude larger than in the solution was obtained from the sorbent.

FIGURE 3. Kinetic of the adsorption of PCB169 followed by fluorometry on the sorbing phase. for different concentrations in aqueous solution. \( \lambda_{	ext{exc.}} \): 260 nm.

As an example, in Figure 3 are shown the variations of the fluorescence intensity of PCB169 in function of the immersion time and for concentrations ranging from 1 ppb to 100 ppb. In all cases, a regular variation following commonly observed adsorption kinetic law has been obtained [10, 16]. As a rule, for all PCBs, the equilibrium appears to be attained in more than one hour for concentrations larger than 10 ppb. For concentrations lower than 10 ppb which is the concentration range of interest, the equilibrium time appears exceedingly long (Figure 3b). In this case, an optimum immersion time of 60 min was adopted as a compromise between sensitivity and a reasonable time for analysis.

**Calibration curves and limits of detection**

The calibration curves (Figure 4) were constructed with concentrations ranging from 1 to 10 ppb. The intensities were measured at the maximum of emission after subtraction of the blank spectrum recorded in the same conditions. In spite of the reduced number of experimental points, due in part to the long time required for preparing spiked solutions (\( \approx \) 24 hours), good correlation factors were obtained. The main source of error was found to arise from the alteration of the sorbent surface during stirring which is tough to induce some damage in the fiber glass structure.

The limits of detection (LOD) for each PCBs (Table 1) has been estimated from the slope of the calibration curves and the lowest detectable signal taken equal to three times the rms. value of the noise signal in the condition of our measurements with the spectrofluorometer.
we used. From the data in Table 1, no simple correlation between the LOD values and the fluorescence quantum yields or the $K_{ow}$ values seems to be in evidence. Other factors such as geometrical

\[ y = 872.37x \\
R^2 = 0.9246 \\
\]  

\[ y = 144.78x \\
R^2 = 0.9992 \\
\]

\[ y = 224.71x \\
R^2 = 0.9913 \\
\]

\[ y = 301.51x \\
R^2 = 0.9875 \\
\]

FIGURE 4. Calibration curves of the fluorometric signal measured on the sorbing phase for concentrations ranging from 1 to 10 ppb in the aqueous solutions. $\lambda_{exc}$: 260 nm. Band-paths: 2.5 nm. Photomultiplier voltage 950 V. Immersion time: 60 min.

conformation or polarity may also control the efficiency of the solute adsorption process. In practice, the individual LODs determined for the four congeners appear to be close to the highest concentration values (< 0.5 ppb) of total PCBs found in highly polluted rivers [1]. In view of these results, the sensitivity of the method, although attractive, needs to be substantially improved before being applicable to moderately polluted natural waters.

**Interference with humic substances**

It is now well established that colloidal matter, suspended solids and dissolved organic material as humic substances may significantly affect the PCB's partition and adsorption process from liquid to solid sorbent [8, 17]. Low recoveries of PCB's in natural waters, were mostly attributed to the humic substances capability to trap the analytes within
its complex structure or to block active sites [18] thus competing with the solid phase sorbent. Interaction of PCB3 with humic substances is confirmed by the Stern-Volmer plot shown in Figure 5a which demonstrates that a significant fluorescence quenching of the PCB fluorescence in water occurs in the presence of Suwannee River humic acid. The fact that this plot is linear indicates that an unique process is operating [19]. In the case of polycyclic aromatic hydrocarbons most of the studies published so far have concluded that fluorescence quenching by humic substances is best interpreted by assuming a ground state association as the dominant process [20, 21]. Therefore, the same process may be though also to be predominantly operating in the case of PCBs.

![Stern-Volmer plot of PCB3 fluorescence signal from the sorbing phase as a function of concentration of Suwannee river humic acid in the aqueous solution.](image1)

![Calibration curves for PCB3 for pure water and in water in the presence of 10 mg/L of Suwannee river humic acid. Same experimental conditions as in FIGURE 4.](image2)

**FIGURE 5.** (a) Stern-Volmer plot of PCB3 fluorescence signal from the sorbing phase as a function of concentration of Suwannee river humic acid in the aqueous solution. (b) Calibration curves for PCB3 for pure water and in water in the presence of 10 mg/L of Suwannee river humic acid. Same experimental conditions as in FIGURE 4.

In Figure 5b are compared the calibration curves obtained for PCB3, from measurements on the sorbing phase after extraction, i: from pure water and ii: from water containing 10 mg/L of humic acid. In the presence of humic acid, a calibration curve with a lower slope was obtained which indicates, on one hand, that as expected, the presence of humic acid induces a significant fluorescence quenching and, in the other hand, that important errors could be made if quantitative measurement of PCBs in natural water is based on an external calibration curve built from pure water solutions.
CONCLUSIONS

In the present work, we have shown that fluorescent PCBs can be efficiently trapped in SPE octadecyl silica phase disks thus allowing their detection in water at concentration where they are not detectable in the aqueous solution. Accordingly, the method appears suitable to determine the presence of a mixture of PCBs in natural waters and may be proposed as an alert method to detect ground-water contamination by contaminated soils or sediments or to determine the PCB’s distribution in a contaminated area. Compared to most common method, its main advantage lies in its simplicity. However, owing to the low concentrations usually found in most environmental waters, usually largely below the ppb range, its sensitivity as it has been estimated within the experimental and instrumental conditions used in this exploratory work, needs to be substantially enhanced. Obviously the fluorometric detection must be improved to satisfy for sufficient sensitivity. Moreover several other points not considered in this preliminary work, call for further investigation.

In these first experiments, only planar PCBs have been tested. they are relatively good fluorophore and they present some interest in reason of their potential toxicity, however they are thought to be minor components in PCB mixture. Non planar PCBs (bearing chlorine atom in the position 2 and/or 2') are expected to be more abundant, but their fluorescence properties are not yet precisely known and further works are needed to know in which extent they can contribute to the total fluorescence signal of PCBs present in a given ground water sample.

The difficulties induced by interference with humic substances can be circumvented either by a previous destruction of these substances [8] or by using an internal calibration procedure as for instance the standard addition method. Interference with eventually present PAHs can be more difficult to eliminate and this point needs also to be investigated.

The octadecyl silica phase disks with fiber glass support used in our experiment reveal to be fragile and partial damage on their surface either during handling or during stirring has been observed to incidentally introduce important error in the fluorescence signal. For better reproducibility and also for improving the efficiency of the solute trapping by the sorbing phase different type of supports should be tested. Such a research objective may also contribute to improve the applicability of the method. Acknowledgements
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