



## Analysis of Polybrominated Biphenyls in Environmental Samples by Porous Anodic Alumina-Based Solid-Phase Microextraction Combined with Gas Chromatography

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Porous anodic alumina was fabricated as solid-phase microextraction fiber coating by a two-step anodization process. The obtained coating presents a regular nanoporous array structure with pore diameter of 60 nm, characterized with scanning electron microscopy. It was employed in analysis of polybrominated biphenyls (PBB 9, 30, 52, 103 and 155) followed by gas chromatography detection. Key parameters affecting extraction procedures, including extraction temperature, time, salt concentration and desorption time, were optimized. Under the optimal conditions, the calibration curves were linear within the range of 0.05-3 µg/L and the detection limits were between 0.006 and 0.013 µg/L. The single fiber and fiber-to-fiber relative standard deviations were below 8.2 and 11.3 %, respectively. Finally, the developed method was applied to the analysis of polybrominated biphenyls in real samples and the reliability of method was proved by recovery experiments.

**Keywords:** Solid-phase microextraction, Porous anodic alumina, Polybrominated biphenyls, Gas chromatography.

### INTRODUCTION

Polybrominated biphenyls (PBBs) had been widely applied as additive flame retardants in plastics, textiles, electronic equipment and other combustible materials<sup>1</sup> for decades. Due to their lack of covalent link to these materials, polybrominated biphenyls are easily leach from the products and released into the environment. The risk of polybrominated biphenyls was recognized to be endocrine disruptors and potentially carcinogenic to humans<sup>2</sup>. Although the manufacturing of PBBs had been ceased for many years. The presence of these compounds in environmental and biological samples has been reported occasionally<sup>3,4</sup>. Polybrominated biphenyls are well-retained in water and soil samples in trace levels because of their great chemical and thermal stability. Therefore, it is of great significance to develop a sensitive, rapid and effective technique for the trace analysis of PBBs in environment.

The analysis of PBBs in environmental matrixes generally involves a step of sample preparation. The classical pre-concentration techniques of PBBs, such as solid phase extraction<sup>5,6</sup> and liquid-liquid extraction<sup>7,8</sup>, are usually time-consuming and require large amounts of solvents. Owing to the advantages of high sensitivity, rapidity, simplicity and free of solvents, solid-phase microextraction (SPME) has been widely applied in food<sup>9,10</sup>, environmental<sup>11,12</sup>, biological<sup>13,14</sup> and pharmaceutical<sup>15,16</sup>

analysis. The core of SPME is the sorbent coating and supporting substrate. Some polymeric coatings such as polydimethylsiloxane (PDMS), carboxen/polydimethylsiloxane (CAR/PDMS) and polyacrylate (PA) are commercially available. However, these polymeric coatings are unstable in thermal desorption process, they may therefore bleed from the fused silica especially when they are used to absorb semi-volatile compounds.

An oxidized metal-based fiber coating has improved the mechanical and thermal stability of traditional SPME coatings. In recent years, metallic oxide has received great attention in analytical chemistry. TiO<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub> have been successfully served as stationary phase of solid phase extraction and high-performance liquid chromatography owing to their excellent adsorptivity, stability and durability. Porous anodic alumina (PAA) has been used as important template material for fabrication of low dimensional nanostructures<sup>17,18</sup> due to their highly ordered arrays of nanopores. A porous anodic alumina based capacitive sensor was developed to detect trace levels of polychlorinated biphenyls (PCBs). It was found that the regular nanopores of PAA not only provide large surface area for PCB adsorption, but also benefit for the enhancement of capacitive response<sup>19</sup>. In SPME techniques, TiO<sub>2</sub> nanotube array was prepared in situ on Ti wire as SPME coating for extraction of polynuclear aromatic hydrocarbons in water samples. It

exhibited many advantages over commercial SPME fibers<sup>20</sup>. Porous anodic alumina has been also applied as SPME fiber coating to concentrate biological volatile organic compounds<sup>21</sup>, and it offered extremely high enhancement factors (EFs) for these analytes ranging from  $2.1 \times 10^6$  to  $16.1 \times 10^6$ . From the above, it is possible to enrich PBBs by using PAA as SPME fiber coating.

In this study, a PAA was prepared by a simple two-step anodization method in oxalic acid electrolyte under a constant voltage. The as-prepared PAA was directly exploited as SPME fiber coating for adsorption and preconcentration of PBBs. The developed method involving headspace solid-phase microextraction (HS-SPME) and GC determination for several PBB compounds in environmental samples was established. Method validation was performed *via* recovery experiments in a real water sample collected from Yangtze river of China.

## EXPERIMENTAL

A stock solution containing 2,5-dibromobiphenyl (PBB9), 2,4,6-tribromobiphenyl (PBB30), 2,5,2',5'-tetrabromobiphenyl (PBB52), 2,4,6,2',5'-pentabromobiphenyl (PBB103) and 2,4,6,2',4',6'-hexabromobiphenyl (PBB155) with a concentration of 50  $\mu\text{g}/\text{mL}$  of each compound in isoctane, was purchased from AccuStandard, Inc. (New Haven, CT, USA). Aluminum wire (99.999 % purity, 0.25mm diameter) was purchased from Opp Coating Material Technology Co. Ltd. (Shenzhen, China). All chemicals were of analytical reagent-grade and obtained from Sinopharm Chemical Reagent Co. Ltd (Beijing, China). Commercial 85  $\mu\text{m}$  polyacrylate and 75  $\mu\text{m}$  CAR/PDMS SPME fiber coatings (Supelco, USA) were used for the comparison study.

### Preparation of porous anodic alumina fiber coating:

The preparation of the PAA fiber coating was performed following previous report<sup>21</sup> with some modifications. Aluminum wire (17 cm length) was cleaned with acetone and soaked in NaOH solution to remove the natural alumina. Subsequently, the tip 1.5 cm of the wire was electropolished in a mixture of ethanol and perchloric acid (4:1) at a constant voltage of 10 V for 3 min. The first anodization was performed in 0.3 M oxalic acid at 45 V for 2h. Then, it was immersed in a mixture of chromic acid (1.8 wt %) and phosphoric acid (6 wt %) at 70 °C for 3 h to remove the oxide layer. The second anodization was carried out under the same conditions for 5 h. The as-prepared fiber was assembled into a homemade SPME device modified from 5  $\mu\text{L}$  microsyringe. Finally, it was conditioned in the GC injector under nitrogen at 280 °C for 2 h.

**Headspace SPME procedure:** A series of PBBs standard solutions (0.02, 0.05, 0.1, 0.5, 1, 2, 3  $\mu\text{g}/\text{mL}$ ) were prepared by stepwise diluting of stock solution (50  $\mu\text{g}/\text{mL}$ ) with acetone. 5  $\mu\text{L}$  of the above standard solution was added to 5 mL of ultrapure water in a 15 mL vial. The vial was sealed with PTFE silicon septum and placed on a temperature controlled system under stirring at 800 rpm. The PAA fiber was exposed to the headspace over the sample for 10-50 min. Finally, the fiber was withdrawn and desorbed in the GC injection port for 5 min at 280 °C.

**GC analysis:** Polybrominated biphenyls separation was performed on a GC-2010 system (Shimadzu, Japan), equipped with a split/splitless injector and an ECD system. A RTX-5

capillary column (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu\text{m}$ ) (Restek, Bellefonte, PA, USA) was utilized. The injector and detector temperature was held at 280 °C and 300 °C, respectively. Splitless injection lasted for 1 min. Nitrogen (> 99.999 %) was used as the carrier gas with a constant pressure of 130.5 kpa and column flow rate was 1.5 mL/min. The oven temperature program was as follows: 120 °C held for 3 min, followed by increasing temperature to 240 °C at 20 °C/min and held for 2 min, then ramped to 290 °C at 10 °C/min and kept at this temperature for 3 min.

## RESULTS AND DISCUSSION

**Characterization of PAA fiber coating:** Fig. 1a shows the low-magnification surface morphological structure of the PAA fiber observed by scanning electron microscope (SEM), from which it can be seen that the surface of the coating is relatively intact after anodization for several hours. From Fig. 1b and 1c, it can be clearly found that tremendous regular nanopore arrays were generated on the surface. The highly ordered nanoporous structure of PAA is directly attributed to the large surface area and further the good extraction capability for PBBs molecules. The diameter of these nanopores is about 60 nm (Fig. 1d), which is consistent with literature reported<sup>22</sup>.

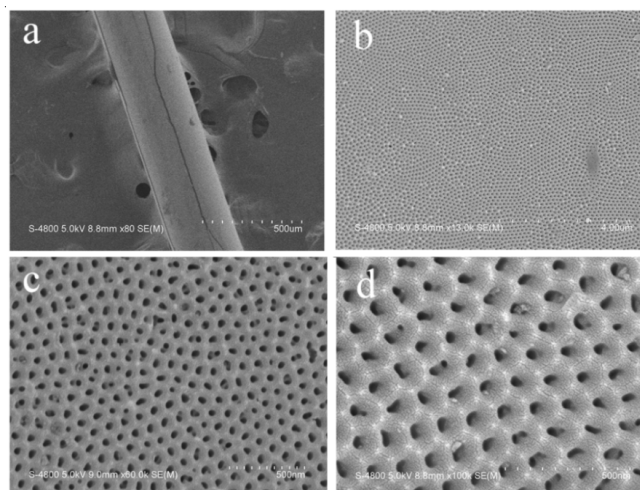


Fig. 1. SEM images of PAA fibers with different magnification

**Optimization of experimental conditions:** To achieve the best extraction efficiency, which is reflected by the chromatogram peak area, the optimum extraction conditions, including extraction temperature, extraction time, salt concentration and desorption time were investigated.

The effect of extraction temperatures on extraction efficiency ranging from 30 to 70 °C were investigated and the results were shown in Fig. 2. The peak areas were increased when the extraction temperature was increased from 30 to 60 °C and then decreased when the temperature continually rised excepted for PBB155. The reason could be that increasing temperature can generally increase the mass transfer of analytes from sample to headspace and further to the fiber. However, further increase of temperature may decrease the partition coefficient of analytes absorbed to the fiber coating, leading to a loss of analytes. PBB155 has a higher boiling point than other PBB congeners and it needs a higher temperature to

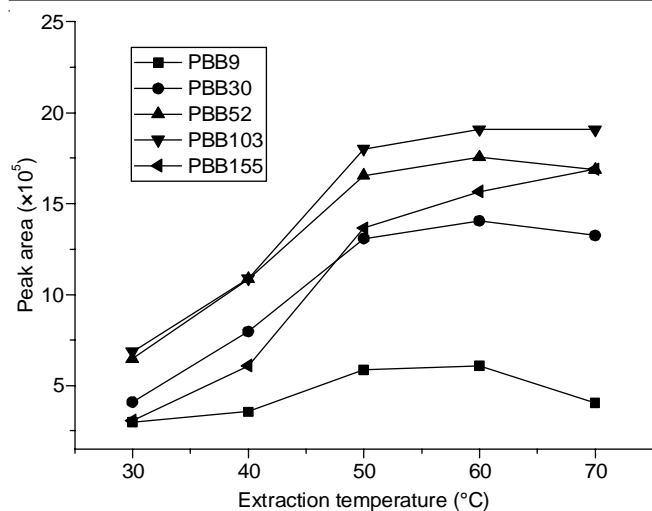


Fig. 2. Effect of extraction temperature on the extraction efficiency of PBBs

reach dynamic equilibrium. Thus, the extraction temperature at 60 °C was chosen in further experiments.

With the increase of extraction time, higher amounts of analytes can be adsorbed to the SPME coating until a steady-state is reached. In this work, the effect of extraction time was evaluated by examining five different extraction times (10, 20, 30, 40 and 60 min). Fig. 3 displays that when the extraction time rise from 10 to 30 min, the peak areas significantly increased. An extraction time longer than 30 min resulted in a decrease in the extraction efficiencies for the majority of PBBs, a likely result of analyte adsorption to the sample vial surface and PAA gave much slower extraction kinetics to PBB155. To achieve the best extraction efficiency for these PBBs, 30 min was chosen as the extraction time.

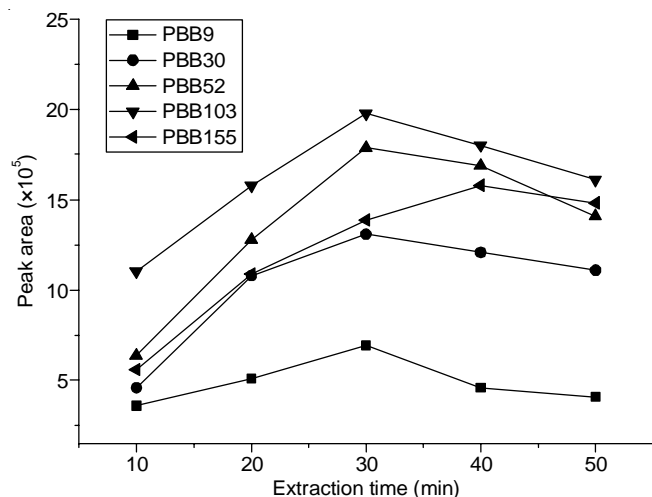


Fig. 3. Effect of extraction time on the extraction efficiency of PBBs

It is well-known that the addition of salt to the aqueous solution can decrease the solubility of the analytes and further increase the amounts of these compounds to the headspace. In this work, solutions with different concentrations of NaCl [0, 10, 20 and 30 % (w/v)] were investigated. However, there are no significant increase of peak areas was observed. A similar study reported by Shu and co-workers saturated a PCB aqueous sample with sodium chloride and observed a decrease

of extraction efficiency while using a 100  $\mu\text{m}$  PDMS fiber<sup>23</sup>. Therefore, the “salting-out” effect did not work in every studied sample and the sodium chloride is not added in later experiments.

A proper desorption time is helpful to effective desorption of the analytes from extraction fiber and avoid carry-over effects. In this work, investigation of desorption time was tested at 280 °C for 2, 3, 4 and 5 min. There was no significant increase of the chromatogram peak area was observed until desorption for 4 min. Thus, desorption time of 4 min was chosen.

In conclusion, the HS-SPME conditions were optimized by extraction at 60 °C for 30 min and desorption at 280 °C for 4 min in PBBs solutions without sodium chloride.

**Extraction capability:** In order to show the performance of PAA for the SPME of PBBs, the fiber was compared with commercial 85  $\mu\text{m}$  polyacrylate and 75  $\mu\text{m}$  CAR/PDMS coatings. As shown in Fig. 4, extraction efficiency of PAA is obviously higher than CAR/PDMS for all PBB congeners. Compared with polyacrylate, PAA gave better extraction capability to PBB52, PBB103 and PBB155. It may be due to the higher polarity of PBB9 and PBB30, polyacrylate shows better adsorbability to them as a polar coating.

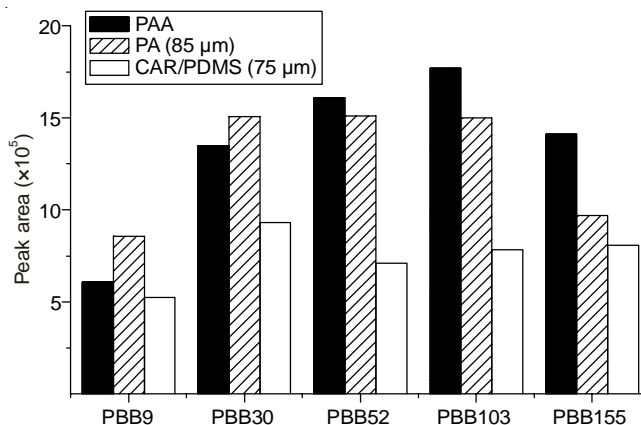


Fig. 4. Comparison of the PAA fiber with the commercial fibers for the SPME of the PBBs

In order to investigate the adsorption mechanism of the PAA fiber coatings to PBBs, a compared experiment was carried out. Fig. 5a and 5b are chromatograms of PBBs for SPME using aluminum wire anodized at one time and twice, respectively. A significant increase of the chromatogram peak area was observed when SPME using the fiber fabricated by two-step anodizing procedure. It has been reported that two-step anodizing procedure offered more regular nanopores and higher pore density than simple one oxidization<sup>24</sup>. Therefore, the extraction capacity of the PAA coating for PBBs could be attributable to the highly regular nanoporous structure, which lead to larger specific surface area and more adsorption sites. On the other hand, hydroxyl groups present in the surface of the alumina due to dissociative chemisorption of water molecules<sup>29</sup>. These hydroxyl groups may have hydrophilic interaction with the bromine atoms of PBBs, which is similar to hydrogen bonding. In addition, induced interaction of the charge exists in  $\text{Al}_2\text{O}_3$  and delocalized  $\pi$  electron-rich molecules of PBBs. This may strengthen the affinity of  $\text{Al}_2\text{O}_3$  and PBBs further.

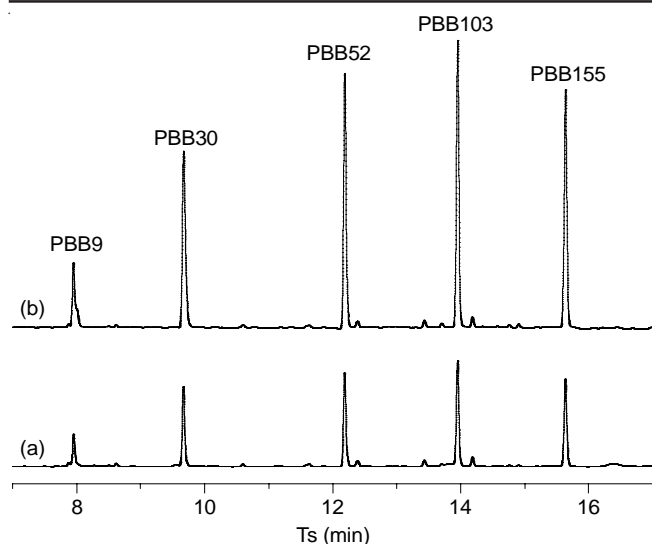


Fig. 5. Chromatograms of PBBs for SPME using two types of fiber; (a) anodized at one time (b) anodized twice

**Analytical figures of merit:** Experiments were performed under the optimized conditions to evaluate the proposed method for the determination of PBBs in different levels (0.02, 0.05, 0.1, 0.5, 1, 2, 3  $\mu\text{g/L}$ ). Linearity, limits of detection (LOD) and precision are listed in Table-1. As seen from the table, the linearity concentration range was from 0.05 to 3  $\mu\text{g/L}$  for all PBBs, with correlation coefficients greater than 0.9961. The LODs were between 0.006 and 0.013  $\mu\text{g/L}$  for the tested PBBs. The relative standard deviation (RSD) for three replicate extractions of PBBs ranged from 2.6 % to 8.2 %. The RSDs for fiber-to-fiber were less than 11.3 % using three different fibers.

Analytes	Linear range ( $\mu\text{g/L}$ )	$r^2$	LOD ( $\mu\text{g/L}$ )	RSD (% , n = 3)	
				Single fiber	Fiber-to-fiber
PBB9	0.05-3	0.9975	0.010	7.9	8.5
PBB30	0.05-3	0.9968	0.009	6.4	7.5
PBB52	0.05-3	0.9972	0.006	8.2	9.7
PBB103	0.05-3	0.9961	0.013	4.2	11.3
PBB155	0.05-3	0.9984	0.007	2.6	3.7

**Application to real samples:** The optimized final methodology was applied to determine the target compounds in real water sample collected from Yangtze river of China. The results showed that no detectable concentrations of PBBs exist in the samples. Thus, recoveries of the method were determined to evaluate the performance of the method. As a result, the recoveries of the five PBBs from the samples (spiked with 0.2 and 2  $\mu\text{g/L}$ ) are in the range from 85 to 115 % with the RSDs less than 11.7 % (Table-2), which demonstrates the accuracy and precision of the present method.

## Conclusion

A method of HS-SPME combined with GC-ECD has been presented in this study as a viable approach for the

TABLE-2  
ANALYTICAL RESULTS FOR THE DETERMINATION  
OF PBBs IN REAL SAMPLES

Analytes	Found ( $\mu\text{g/L}$ )			Recovery $\pm$ RSD (% , n = 3)	
	No spiking	Spiked (0.2 $\mu\text{g/L}$ )	Spiked (2 $\mu\text{g/L}$ )	Spiked (0.2 $\mu\text{g/L}$ )	Spiked (2 $\mu\text{g/L}$ )
PBB9	nd.	0.18	2.34	90 $\pm$ 7.6	117 $\pm$ 9.6
PBB30	nd.	0.19	1.83	95 $\pm$ 9.4	92 $\pm$ 10.3
PBB52	nd.	0.23	2.21	115 $\pm$ 10.2	111 $\pm$ 8.4
PBB103	nd.	0.17	2.17	85 $\pm$ 11.7	109 $\pm$ 8.7
PBB155	nd.	0.20	1.97	100 $\pm$ 10.3	99 $\pm$ 5.9

nd = Not detected.

determination of PBBs in environmental samples. The developed method doesn't require previous treatment of the samples, implying a significant reduction of working time and organic solvent consumption. Owing to the multiple extraction mechanisms, PAA fiber has the potential for meeting challenges in complex real sample analysis. Besides, it is expected that the selectivity of the fiber would be further improved if some functional groups are modified on the wall of the PAA membrane.

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