

ANALYSIS OF POLYCHLORINATED BIPHENYLS IN OIL USING MICROFLUIDIC BASED PRETREATMENT METHOD AND IMMUNOASSAY

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ABSTRACT

Polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs), which are worldwide problem. Analysis of PCBs in oil is generally difficult because there are 209 congeners and similar chemical substances in oil. The general analysis methods, which primarily include GC/MS or GC/ECD, are time-consuming and costly. We developed a pretreatment method using a multilayer capillary column and a microfluidic dimethyl sulfoxide partitioning for the simple and rapid immunoassay of PCBs in oil. The microfluidic process time of the column and the DMSO partitioning without evaporation, which require in the previous method, was evaluated to be 19 min. Our method could measure 0.2 and 0.4 mg/kg PCBs in oil.

KEYWORDS

Immunoassay, Solvent extraction. Microfluidics, Polychlorinated biphenyls, Two phase

INTRODUCTION

PCBs are a worldwide problem on account of their toxicity and environmental persistence. PCBs are contaminated into soils, rivers, biological cells, and oils, and so on. Analysis of PCBs generally uses instruments, such as gas chromatography-mass spectrometry or gas chromatography-electron capture detection because PCBs have 209 congeners. Furthermore, there are many interfering substances, whose structures are similar to those of PCBs in insulating oil. Therefore, complex pretreatment for the analysis of PCBs in oil is required. Although these conventional analysis methods are highly sensitive and accurate, they are also time-consuming, expensive, and require complex laboratory equipments. We have previously reported the analysis method using a large-scale multilayer column, an evaporator, a centrifuge separator, dimethyl sulfoxide (DMSO) partitioning, and an immunoassay.[1] Microfluidic technologies may make it faster and more economical by reducing the reagent amounts and simplifying the chemical processes. Although we have demonstrated a microfluidic DMSO partitioning as a component technology,[2] highly efficient column and highly sensitive immunoassay should be developed and the microfluidic device should be improved. In this paper, we report preliminary experiments of a capillary multilayer column, an improved microfluidic DMSO partitioning, and highly sensitive immunoassay for achieving of our concept shown in Figure 1. Oil components other than PCBs are decomposed and separated in the column. Remaining components are cleaned up by the extraction of PCBs into DMSO in the microchip. The PCBs in the eluate from the column are directly extracted into DMSO in the microrecesses without the evaporation.[3]

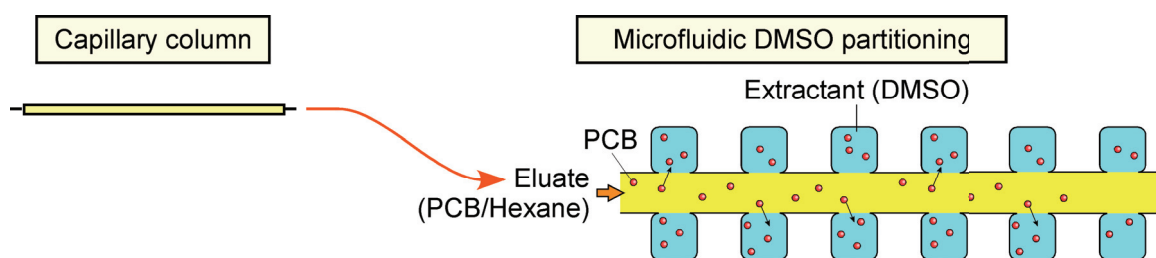


Figure 1. Concept of the pretreatment using a column and a microchip.

EXPERIMENT

Figure 2 shows a photograph of the multilayer silicagel capillary column, which could reduce the amount of reagent required to 1/5 of that required in the previous method. Anhydrous sodium sulfate, oleum impregnated silica gel, and aminopropyl silica gel were packed in a Teflon tube, whose length and diameter were 330 mm and 1.6 mm. A 25 μ L-aliquot of PCB-contaminated oil was injected and the PCBs were eluted by hexane flowed by microsyringe pump. The microfluidic device for the extraction of PCBs into DMSO was designed and fabricated as shown in Figure 3. 1,212 rectangle microrecesses having a length and a width of 520 μ m, and a depth of 50 μ m were fabricated aligned along the main microchannel having a length of 610 mm, a width of 260 μ m, and a depth of 50 μ m. PCB-contaminated oil was prepared by adding the mixture of PCBs (Kanechlor 300, 400, 500, 600, 1:1:1:1 mixture) to pure insulating oil (Barrel Trans M, Matsumura Oil Co. Ltd.). PCB immunoassays were performed on the KinExA 3000 supplied by Sapidne Instruments Inc. as described in our previous paper.[2,3] For the PCB immunoassay, two kinds of blocking reagents, bovine serum albumin (BSA) and N101, were used and a monoclonal

anti-PCB antibody (K2A) was used.

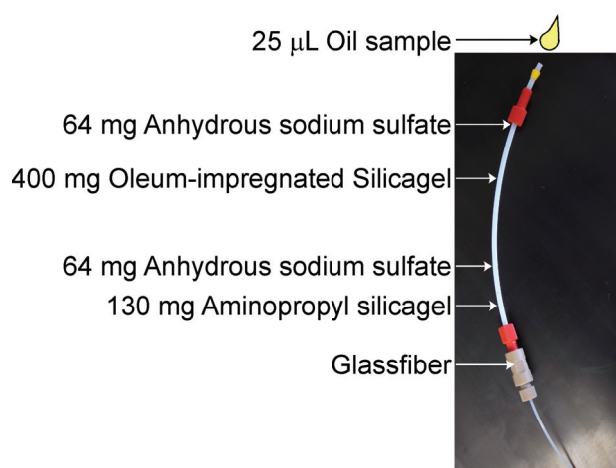


Figure 2. Photograph of multilayer capillary column.

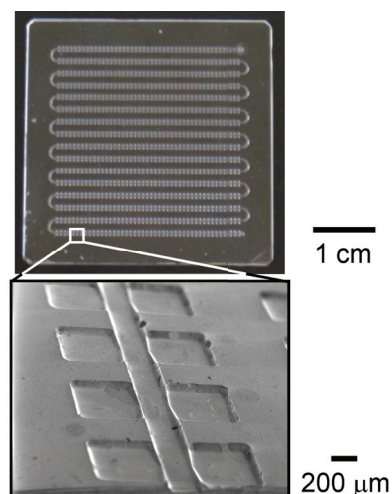


Figure 3. Photograph and SEM image of the microchip

RESULTS AND DISCUSSIONS

Table 1 shows recovery rate dependence on the flow volume of hexane as an eluate at 500 $\mu\text{L}/\text{min}$. The column process with a recovery rate of nearly 100% could be completed within 2 min, while 6 min is required in the previous method. The previous column utilizes partial oleum-impregnated silicagel, while the present column could utilize almost all by miniaturizing column size. Further, the highly efficient separation of the interfering substances was achieved by miniaturizing column size. Therefore, rapid decomposition of interfering substances was achieved. The microflow control was demonstrated in the microfluidic device having microrecesses (Figure 4). The eluate could be flowed only in the main microchannel, while the DMSO was kept in the microrecesses. Vortex flow was found in the microrecesses by the microscopic observation of fluorescent microparticles. The vortex can rapidly mix the transported PCBs in the microrecesses as described in our previous paper.[2] After the DMSO partitioning, PCBs in the DMSO could be eluted by dissolving DMSO in 1 mL of the buffer solution flow. Samples for the immunoassays were prepared by adding phosphate buffered saline, blocking reagent, DMSO, and the antibody to obtain total volume of 1.5 mL. The antibody concentration was 250 pM and the measurement solutions were incubated at room temperature for a minimum of 30 min. Since the total volume of DMSO confined in the microrecesses was estimated to be 15 μL , the dilution rate is estimated to be 100.

Table 1. Recovery rates on flow volume of hexane.

Flow volume (mL)	Recovery rate (%)
0.6	67 \pm 7
0.8	90 \pm 5
1.0	97 \pm 11
1.2	101 \pm 18

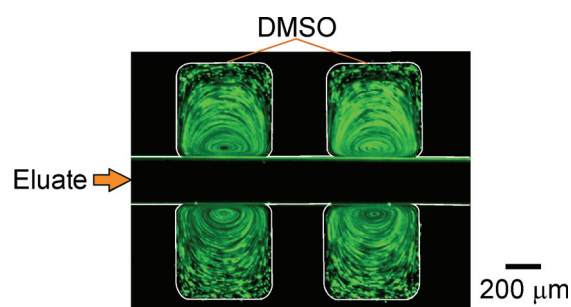


Figure 4. Fluid motions in the microfluidic device with microrecesses. PCBs were extracted into the DMSO confined in the microrecesses, which generated a vortex flow.

For highly sensitive immunoassay, a flow-based kinetic exclusion assay [4] was applied and blocking reagents, BSA and N101, were examined. Figure 5 shows standard curves for two kinds of the blocking reagents. The results were fitted with four-parameter logistic equation. The IC₅₀% values for BSA and N101 were evaluated to be 1.0 $\mu\text{g}/\text{kg}$ and 0.3 $\mu\text{g}/\text{kg}$, respectively. The detection limit and dynamic range defined as 30% relative standard deviation (RSD) and the region with <10% RSD [5] were evaluated. Figure 6 shows the precision profiles of the standard curves in Figure 5. The limits of detection of PCBs in aqueous solution for BSA and N101 were evaluated to be 89 ng/kg and 31 ng/kg, respectively. The dynamic ranges for BSA and N101 were evaluated to be 0.38–14 $\mu\text{g}/\text{kg}$ and 0.07–1.5 $\mu\text{g}/\text{kg}$, respectively. Therefore, higher sensitive could be obtained by using N101. BSA has

been used for preparation of the PCB-conjugate as antigen to obtain anti-PCB antibody, K2A. Free antibody concentration may be varied due to the reaction of BSA with antibody in the sample solution. Therefore, N101 was used for the following experiment.

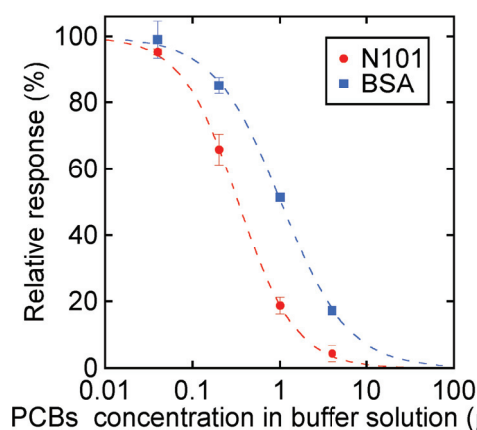


Figure 5. Relative response on PCBs concentration in buffer solution.

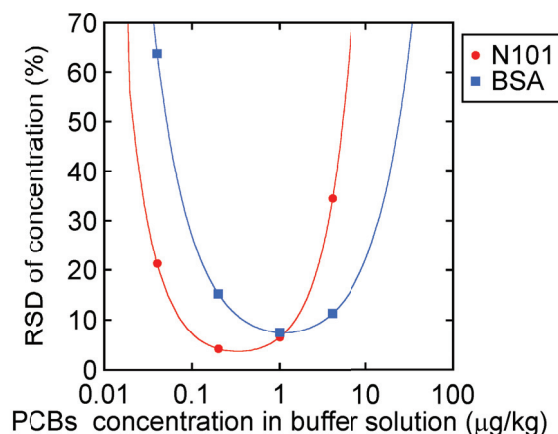


Figure 6. Precision profile of the standard Curves in Figure 5.

The capillary column and the microfluidic device were interfaced and PCBs in the eluate were directly extracted into the DMSO without evaporation (Figure 7), which is required in the previous method. Flow rates for DMSO partitioning and DMSO elution from the microrecesses by buffer solution were optimized, respectively [3]. 76% and 61% of relative responses were obtained at 0.2 mg/kg and 0.4 mg/kg PCBs in oil, which satisfied Japanese regulation value of 0.5 mg/kg. Our method can be useful for realizing a simple, rapid and low-cost analysis system for PCBs in oil.

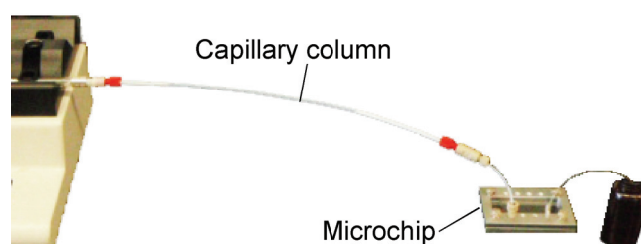


Figure 7. Photograph of the interface between the capillary column and the microchip.

CONCLUSIONS

We realized simple and rapid analysis method for PCBs in oil by utilizing the multilayer capillary column, the microfluidic DMSO partitioning, and the kinetic exclusion assay. The microfluidic process time of the column and the DMSO partitioning without evaporation, which require in the previous method, was evaluated to be 19 min. PCB immunoassay by the use of N101 was achieved with high sensitivity, whose limit of detection was 31 ng/kg PCBs in the aqueous solution. Our method could measure 0.2 mg/kg and 0.4 mg/kg, which satisfied Japanese regulation value. When the flow-based kinetic exclusion assay system is miniaturized, our method can be useful for on-site PCBs analysis.

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