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Screening of polychlorinated biphenyls in insulating oil using a microfluidic based pretreatment and immunoassay

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ABSTRACT

Polychlorinated biphenyls (PCBs) are persistent organic pollutants in insulating oil of a large number of transformers. A rapid and economical analytical method to detect PCB contamination is still required. To address this issue, we propose here the first microfluidic screening method for PCB contamination in insulating oil. The insulating oil was pretreated using a multilayer capillary column and a microfluidic liquid–liquid partitioning. PCBs in the pretreated oil were measured using a microfluidic kinetic exclusion assay. In order to detect PCBs with high sensitivity, conditions of the microfluidic kinetic exclusion assay were optimized. Measurements were rapidly completed (within 43 min). The measurement range was estimated to be 0.26–3.3 mg/kg defined as the relative absorbance from 20% to 80%. The screening performance (false positive and false negative rates) was tested on fifty real oil samples; results about these tests were discussed in detail, especially suitable cutoff by comparing with the data analyzed using high-resolution-gas-chromatography/high-resolution-mass-spectrometry. Finally, the screening performance was confirmed using our proposed stochastic screening model. A cutoff of 0.3 to judge as positive is suitable considering the risk of the PCB release into the environment.

1. Introduction

Since polychlorinated biphenyls (PCBs) have a high thermal stability, chemical resistance, and electrical insulating properties, they have been widely used in various applications, such as dielectric fluids in transformers and capacitors, and heat transfer fluids [1]. Complex mixtures of PCBs (Aroclor, Kanechlor, etc.) were commercially produced from 1929 to the early 1970s. Concern about their environmental persistence began in the late 1960s when Swedish researchers reported the presence of PCBs in environmental samples [2]. The first human poisoning due to their toxicity occurred in Western Japan in 1968, and a second similar poisoning occurred in Taiwan in 1979 [3]. The toxicity of PCBs was increasingly recognized in the wake of these incidents. The production and use of PCBs were therefore prohibited in the USA and Japan in the 1970s. However, PCB contamination has been detected in transformers produced after these prohibition laws were passed. Because of the concern of possible PCB contamination in a large number of transformers, PCB contamination in every transformer should be tested. However, typical analytical methods using gas chromatography (GC) coupled with either mass spectrometry (MS) or electron capture detection [4–7] are time consuming and expensive. In the field of medicine, screening with immunoassays is often used, because of its cost- and time-efficiency. Using a similar approach for PCBs may lead to the development of a rapid and economical test method.

We have previously reported immunoassays that employ specific antibodies for PCBs to measure PCBs in insulating oils [8,9]. The previous method needed 3 h for each oil sample. In order to achieve more rapid, simple, and low-cost procedures, the pretreatment method has been improved by using a micro total analysis system [10,11]. Micro total analysis systems are recognized as powerful tools for high-speed, functional, and compact instrumentation used for analytical, synthetic, and biological chemistries [12–14]. Previous methods include batch processes that require an evaporator for concentrating PCBs and a centrifugation device for phase separation. The improved pretreatment enables a continuous process that replaces the evaporator and centrifuge steps by using a multilayer capillary column, allowing for less-diluted samples, interfaced with microfluidic concentrated liquid–liquid partitioning. The multilayer column was miniaturized using a capillary, which reduced the amount of reagent to less than 1/12 of that required in previous columns. Pretreatment was completed within

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20 min. Furthermore, a microfluidic kinetic exclusion assay that can be applied to small molecules with high sensitivity has been reported [15,16]. A combination of microfluidic pretreatment and kinetic exclusion assay results in significantly improved screening procedures.

In this study, we report the screening of PCBs in transformer oil using a multilayer capillary column, microfluidic liquid–liquid partitioning, and microfluidic kinetic exclusion assay. Several measurement parameters for the microfluidic kinetic exclusion assay were examined to achieve high sensitivity. Sensitivity was evaluated from a standard curve generated using microfluidically pretreated oil samples. Fifty real used transformer oil samples were examined by high-resolution-GC/high-resolution-MS (HRGC/HRMS); these were also tested using the developed screening method. Screening performance was evaluated by comparing the false positive and false negative rates of the assays. The evaluation of the screening performance was stochastically confirmed.

2. Materials and methods

2.1. Screening PCBs in oil

Fig. 1 shows the design for the screening of the PCBs in oil, which makes use of a multilayer silica-gel column, microfluidic liquid–liquid partitioning device, and microfluidic kinetic exclusion assay device. The interfering substances in the PCB-contaminated oil are sulfonated and separated in the column. Any remaining interfering substances were cleaned up by microfluidic liquid–liquid partitioning, which permits the extraction of PCBs in the eluate into dimethyl sulfoxide confined in the microrecesses. After the antigen–antibody reaction occurred, PCBs were measured by microfluidic kinetic exclusion assay. Free antibodies labeled with gold nanoparticles accumulated onto the surface of the antigen-immobilized beads. The absorbance of gold nanoparticles on the surface of the beads was measured with a light-emitting diode (LED) and photodiode.

2.2. Reagents

Commercial mixtures of PCBs (Kanechlor 300, 400, 500, and 600; 1:1:1:1 mixture) were purchased from GL Sciences Inc. (Tokyo, Japan). PCB-free insulating oil for electric transformers was purchased from Matsumura Oil Co., Ltd. (Barrel Trans M; Osaka, Japan). Anhydrous sodium sulfate, oleum-impregnated silica gel, and aminopropyl silica gel were purchased from Sumika Chemical Analysis Service, Ltd. (Osaka, Japan). DMSO (Catalog No. 346-03615) was purchased from Dojindo Laboratories (Kumamoto, Japan). Acetone and hexane were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). PBS (consisting of 137 mM sodium chloride, 3 mM potassium chloride, 20 mM disodium hydrogen phosphate, 1.5 mM potassium dihydrogen phosphate, and 1.5 mM sodium azide; pH 7.4) was prepared in-house. A blocking reagent, N101, was purchased from NOF Corporation (Tokyo, Japan). BSA (bovine serum albumin) was purchased from Sigma Aldrich (Tokyo, Japan). A monoclonal anti-PCB antibody (K2A) was purchased from Kyoto Electrics Manufacturing Co., Ltd. (Kyoto, Japan). The PCB contamination in used insulating oil is of commercial PCB mixtures origins (Kanechlor in Japan, and Aroclor in USA, etc.). Since K2A showed equal response towards the Kanechlor (KC-300, 400, 500, and 600) [17], K2A can be applied to samples composed of various PCB congeners.

2.3. Oil samples

Commercial mixtures of PCBs (Kanechlor 300, 400, 500, and 600; 1:1:1:1 mixture) were added to pure insulating oil used in electric transformers to prepare the standard oil samples. Real PCB-contaminated oil samples for the evaluation of the screening performance were obtained from fifty used transformers. The main component of the real oil samples was mineral oil.

2.4. Pretreatment of insulating oil

Oil samples were pretreated with a microfluidic device for liquid–liquid partitioning interfaced with a multilayer capillary column [11]. A 190 mm long 1/16″ × 1/8″ Teflon tube was used for the multilayer capillary column. The multilayer capillary column consisted of 64 mg of anhydrous sodium sulfate, 170 mg of oleum-impregnated silica-gel, 64 mg of anhydrous sodium sulfate, and 50 mg of aminopropyl silica-gel, from top to bottom. Quartz wool was packed in the lowest layer of the capillary to prevent leakage of the fillers. A 50 μL aliquot of oil sample was injected at a distance of 10 mm from the fillers with a micropipette to prevent broadening of the oil by capillary force in the fillers; this was followed by elution with hexane at a 50 μL/min flow rate, which was lower than that reported in our previous study (the flow rate was 100 μL/min) [11]. A portion of interfering substances was separated in the column. The remaining interfering substances were separated using a microfluidic liquid–liquid partitioning. A photolithographic wet-etching method was used to fabricate microchannels on a glass substrate, as described in our previous study [11]. The fabricated microchannels were thermally bonded to another substrate with 0.5 mm inlet and outlet holes. Fig. 2 shows a photograph of the microfluidic device and an illustration of the microchannel structure, which consisted of a main microchannel (L 610 mm × W 260 μm × D 50 μm) and 1212 rectangular microrecesses (L 520 μm × W 520 μm × D 50 μm). The volume of the main microchannel was estimated to be 7.3 μL. Considering the rounded structures of the microrecesses, which result from the fabrication procedure carried out with a wet-etching method, the volume of

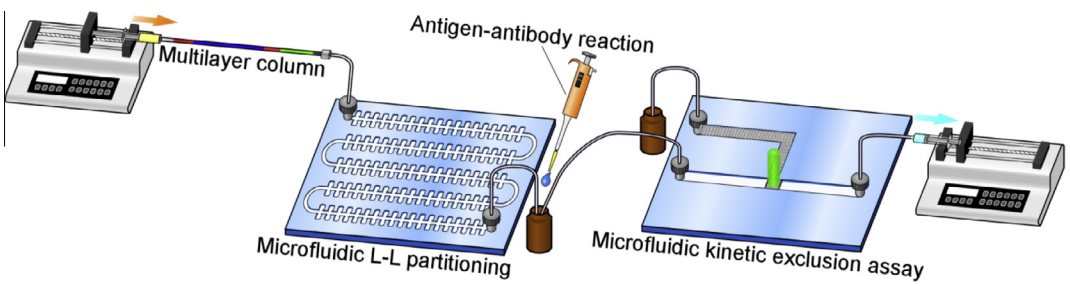

Fig. 1. Schematic view of the screening of PCBs in oil using multilayer silica-gel capillary column separation, microfluidic liquid–liquid partitioning, and microfluidic kinetic exclusion assay.