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Micropore-free surface-activated carbon for the analysis of polychlorinated dibenzo-*p*-dioxins–dibenzofurans and non-*ortho*-substituted polychlorinated biphenyls in environmental samples

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Abstract

2,3,7,8-Substituted polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDD/Fs) and non-*ortho*-substituted polychlorinated biphenyls (PCBs) account for almost all of the total toxic equivalents (TEQ) in environmental samples. Activated carbon columns are used to fractionate the samples for GC–MS analysis or bioassay. Micropore-free surface-activated carbon is highly selective for PCDD/Fs and non-*ortho*-PCBs and can improve the conventional activated carbon column clean-up. Along with sulfuric acid-coated diatomaceous earth columns, micropore-free surface-activated carbon provides a rapid, robust, and high-throughput sample preparation method for PCDD/Fs and non-*ortho*-PCBs analysis.
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1. Introduction

Polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDD/Fs) are of great concern because of their toxicity and persistence in the environment [1–3]. Some of the 209 polychlorinated biphenyls (PCBs) congeners, such as non-*ortho*-substituted PCBs like 33′44′-tetrachlorobiphenyl (IUPAC#77), 33′44′5-pentachlorobiphenyl (IUPAC#126), or 33′44′55′-hexachlorobiphenyl (IUPAC#169), are considered ''dioxin-like'' compounds because their toxicological properties are similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [3]. Thus, these dioxin-like PCBs need to be monitored along with PCDD/Fs. The World Health Organization (1998) assigned toxicity equivalency factors (TEF) to 12 of the 209 PCB congeners, including four non-*ortho*-substituted congeners and eight mono-*ortho*-substituted congeners [4]. Chen et al. (2000) recently reported, however, that three non-*ortho*-substituted isomers (PCB#77, #126, and #169) account for most of the PCBs' TEQ [5]. Thus, to evaluate toxicity based on TEQ, the present study focused solely on non-*ortho*-substituted congeners of dioxin-like PCBs.

In general, analysis of PCDD/Fs and dioxin-like PCBs is expensive and time consuming because the sample preparation procedures are labor intensive.

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Several studies have described the efficiency of sample preparation with activated carbon columns. Various types of activated carbon column supports and extraction apparatus have been tested in an effort to improve sample preparation efficiency [6–9]. None of them, however, have optimized the pore size on the surface or adsorption mode of activated carbon.

The aim of the present study was to establish a rapid, robust, and high-throughput sample preparation method for the analysis of PCDD/Fs and dioxin-like PCBs. Our goals were as follows: (1) completion of clean-up within 1 h, including column preparation time, (2) simultaneous clean-up of multiple samples, and (3) establish a simple method that does not require special equipment such as high-pressure liquid chromatography (HPLC). We selected micropore-free surface-activated carbon because of its excellent selectivity for PCDD/Fs and non-*ortho*-PCBs. Preliminary studies indicated that direct loading of crude sediment extract onto the activated carbon column led to unstable fractionation. Thus, sulfuric acid-coated diatomaceous earth column was placed just above the activated carbon column to improve the reproducibility.

2. Experimental

2.1. Materials

NK-LCS-AD PCDD/Fs standard mixture and MBP-MXS PCB standard mixture (Wellington Laboratories, Ontario, Canada) were used for quantification and quality control. Appropriate amount of each standard was added to a 10-ml Kuderna-Danish (K-D) concentrator tube and diluted to 1 ml with toluene prior to use. Carboxen 1016 (C-1016; Supelco, Bellefonte, PA, USA) was used as the micropore-free surface-activated carbon column (has only mesopores). Carboxen 1000 (C-1000; Supelco) was used as an intermediate activated carbon column (has both micropores and mesopores). And AC-2 (Waters, Milford, MA, USA) was used as the conventional activated carbon (has only micropores) column for comparison. BET surface area and pore size distributions of the carbons were determined with a Omnisorp 360 (Beckman-Coulter, Fullerton, CA, USA) coulter counter. CE-1010 diatomaceous earth column (Varian, Harbor City, CA, USA) was used for the sulfuric acid treatment. Sulfuric acid (96+% grade) was purchased from Wako Pure Chemical (Osaka, Japan). Sediment samples containing 150 pg TEQ/g (dry matter) of PCDD/Fs were used as the reference clean-up material. Ignition loss of the sediment was 8.6% (600 °C, 2 h). In order to guarantee the concentration, the sediment was analyzed twice, in our laboratory and in an independent laboratory (Shimadzu Techno-Research, Kyoko, Japan) separately. Then the sediment (600 g as dry matter) was air-dried firstly, ground to powder and put into an oven at 45 °C overnight before extraction. The sample was divided into 20 portions and each portion was placed in a stainless steel cell. PCDD/Fs and PCBs were extracted with toluene using an ASE 300 (Dionex, Salt Lake City, UT, USA) accelerated solvent extractor according to a method described earlier [10]. Each crude extract was recombined, homogenized thoroughly, and concentrated using a rotary evaporator prior to the following sample preparation.

2.2. Sulfuric acid treatment with the diatomaceous earth column

Two to 10 ml of sulfuric acid was applied to the top of the CE-1010 column containing 7.2 g of diatomaceous earth. The acid was naturally dispersed in the column and coated the diatomaceous earth surface. Silica gel was then put on top of the acid-coated CE-1010. ^{13}C-labeled standards (1 ng each; 2 ng for OCDD/F) were added to the sediment extract (2 ml; equivalent to 30 g of the sediment), then the extract was put through the column. The efficiency of the sulfuric acid treatment was evaluated by measuring the color of the solution. As the efficiency of the acid treatment increased, the color degree of the solution decreased. In this study, the basic color of the solution was yellow and color degree was scored according to the Japan Industrial Standard method K 0101-10.1 [11]. (the method was only applicable when the basic color of the solution was yellow). A color degree of ‘‘1’’ indicates the lightest yellow, ‘‘20’’ indicates the strongest yellow, and ‘‘0’’ indicates clear. Concentration of the PCDD/Fs and PCBs in the treated extract was determined

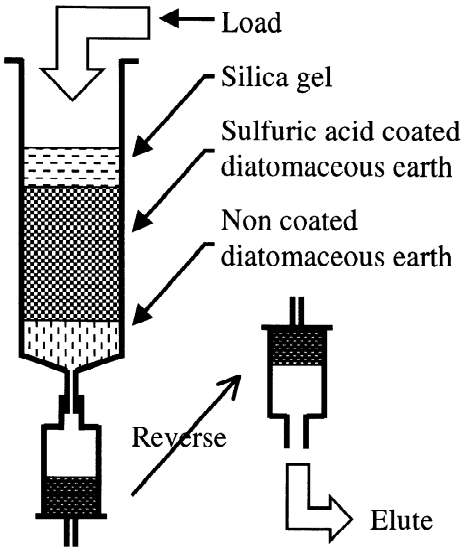

Fig. 1. Column layers and combination for clean-up.

by a Polaris ion trap mass spectrometer (ThermoQuest, Austin, TX, USA).

2.3. Activated carbon column fractionation

Prior to the fractionation study, 50 each of all three commercial activated carbon columns were unpacked, and the carbon was mixed, respectively. Then, BET surface area and pore size distributions of C-1016, C-1000 and AC-2 were determined based on a published method [12]. All of C-1016, C-1000 and AC-2 were pre-washed with 30 ml of heated (45–50 °C) toluene for the conditioning. The activated carbon column was placed just under the CE-1010 column (Fig. 1). ^{13}C-labeled internal standards (1 ng each; 2 ng for OCDD/F) were added to the sediment extract (2 ml; equivalent to 30 g of sediment), then the extract was loaded onto the silica gel. As for AC-2, the sediment extract was not used and only PCDD/Fs and PCBs standards were applied. The column was washed with 30 ml of *n*-hexane, followed by 30 ml of a 3:1 *n*-hexane–dichloromethane mixture. The activated carbon column was then detached, reversed, and PCDD/Fs and PCBs were eluted with heated (45–50 °C) toluene. The toluene was supplied using a standard HPLC pump (Waters) and six parallel SUS tubes in an isothermal oven (Yamato, Tokyo, Japan). The temperature of the toluene for the elution was 45–50 °C. Concentrations of PCDD/Fs and PCBs in the treated extract were determined with the Polaris ion trap mass spectrometer. The detection limits were 0.33 pg/g for T4CDD/Fs, P5CDD/Fs and PCBs, 0.67 pg/g for H6CDD/Fs, H7CDD/Fs, and 1.7 pg/g for O8CDD/Fs, respectively.

3. Results and discussion

3.1. Sulfuric acid treatment

The optimal amount of sulfuric acid for treating a 30-g sample of sediment was 8 ml (Table 1). As the amount of the sulfuric acid was increased from 2 to 8 ml, the color degree of the solution decreased. When 10 ml were added, the color degree increased slightly compared to that obtained with 8 ml, due to the composition of the column layer. The sulfuric acid capacity of the CE-1010 was 10 ml. Thus, when 10 ml of sulfuric acid were added to the column, there was no space remaining for the uncoated diatomaceous earth layer. The uncoated layer after the acid treatment, therefore, had an important role in the sample clean-up procedure.

Table 1
Sulfuric acid treatment efficiency of a CE-1000 diatomaceous earth column

	Sulfuric acid (ml)							
	0	2	4	6		8		10
Silica gel (g)	0	0	0	0	0	2	4	0
Flow rate (ml/min)	N/A	N/A	N/A	N/A	7.4	2.4	2.2	N/A
Color degree	>20[a]	20	20	10	5	3	2	7
Recovery rate (%)	100[b]	108	102	95	83	112	112	81

[a] The color was dark brown, thus the color degree could not be scored properly.
[b] The recovery rate of the column without addition of sulfuric acid was set at 100% as the reference.

There was no difference between the 2- and 4-ml samples in terms of the color degree, likely due to the reaction time. Both samples produced a relatively thin sulfuric acid layer, and the flow-rate of the sediment extract through the column was rather high (7.4 ml/min). Thus, there was only a limited time for the sediment extract to contact the sulfuric acid, suggesting that the reaction time is important.

As the amount of sulfuric acid was increased, the recovery rate decreased; the worst recovery rate was with 10 ml sulfuric acid. The increase in the sulfuric acid coating seemed to increase channeling through the column. Silica gel was placed on top of the acid-coated CE-1010 column to slow the flow-rate. Therefore, channeling was suppressed and the recovery rate was increased while the flow-rate was regulated to approximately 2 ml/min (Table 1).

The advantage of diatomaceous earth is its affinity for sulfuric acid. Unlike silica gel, diatomaceous earth is easily coated by sulfuric acid. The procedure requires only the addition of 8 ml sulfuric acid on top of a CE-1010 column and a 15-min waiting period. The surface of the diatomaceous earth in the column is easily covered with the acid. Diatomaceous earth thus considerably improves the rapidity and throughput of the sulfuric acid treatment. With previous methods, one analyst needed more than 1 h just to prepare 10 multi-layer sulfuric acid-coated columns using silica gel. With diatomaceous earth, however, one analyst can complete both preparation of 10 columns and acid treatment/elution of 10 samples within 1 h.

We developed a simple and effective sulfuric acid treatment method as follows:

(1) add 8 ml of sulfuric acid onto a CE-1010 diatomaceous earth column and wait for 15 min;

(2) place 2–4 g of silica gel on the CE-1010;

(3) add sample extract;

(4) add 10 ml *n*-hexane followed by 30 ml *n*-hexane–dichloromethane (3:1).

Flow-rate does not need to be regulated by an automation apparatus or stopcock. The sulfuric acid treatment productivity is significantly improved by using diatomaceous earth.

3.2. Micropore-free surface-activated carbon fractionation

The differences of pore size distributions of C-1016 (Lot #SP30700), C-1000 (Lot #SP100201) and AC-2 were clearly indicated in Fig. 2 and Table 2. C-1016 is a mesopore-oriented activated carbon, AC-2 is a micropore-oriented activated carbon, and C-1000 is an intermediate activated carbon that has both mesopores and micropores. It is interesting that according to the technical data sheet from the carbon column supplier (Table 3), C-1000 is supposed to have more micropores and less mesopores. It is not always easy to reproduce a pore size distribution because of the diversity of raw material and the uncertainty in activation process [12,13]. The differences could be within the supplier's tolerance. The pore size distribution of C-1016 was exactly the same as shown in the supplier's technical data sheet (Fig. 2 and Table 3).

Table 2
Physical properties of AC-2, C-1000 and C-1016

	BET surface area (m^2/g)	Micropores (ml/g)	Mesopores (ml/g)
Carboxen-1016	69	0.01	0.32
Carboxen-1000	890	0.03	0.30
AC-2	1100	0.05	0.05

Fractionation of PCDD/Fs and PCBs with conventional activated carbon, AC-2, is shown in Fig. 3. AC-2 has been proven to have strong adsorption power against PCDD/Fs and PCBs because there

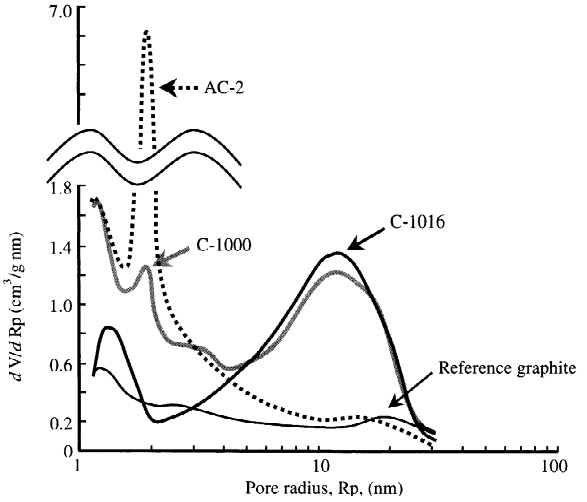

Fig. 2. Pore size distributions of AC-2, C-1000, and C-1016.

Table 3
Technical data from Supelco

	BET surface area (m^2/g)	Density (g/ml)	Micropores (ml/g)	Mesopores (ml/g)	Macropores (cc/g)	Micropores diameter (nm)
Carboxen-1000	1200	0.48	0.44	0.16	0.25	1.0–1.2
Carboxen-1016	75	0.52	None	0.34	None	N/A

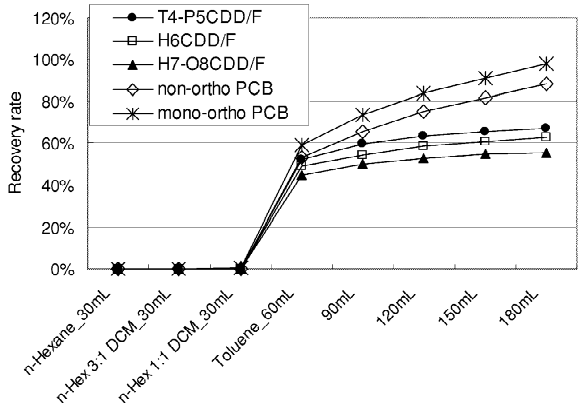

Fig. 3. Fractionation of PCDD/Fs and PCBs with AC-2.

was no leakage in the washing solvent. But the adsorption power was too strong so that PCDD/Fs could not be eluted sufficiently.

Fractionation with intermediate carbon, C-1000, is shown in Fig. 4. Mono-*ortho*-PCBs began to elute with 3:1 *n*-hexane–dichloromethane fraction. Thus, C-1000 has less adsorption power than AC-2. However, it was still too strong that more than 300 ml of toluene was required to elute 99% of the PCDD/Fs. The recovery rates of three carbon columns are compared in Table 4. The flow-rate of the elution was 1 ml/min, thus 2 h were required to recover more than 80% of the standards for all the PCDD/Fs isomers. The amount of toluene was too large and the elution time was too long, therefore the conventional activated carbon column C-1000 was not suitable for rapid PCDD/Fs sample preparation.

Fractionation of PCDD/Fs and PCBs with micropore-free surface-activated carbon, C-1016, is shown in Fig. 5. Almost all of the mono-*ortho*-PCBs

Table 4
PCDD/Fs and PCBs recovery in the first toluene fraction

	C-1016 ($n=6$) (mean±SD)	C-1000 ($n=6$) (mean±SD)	AC-2 ($n=3$) (mean±SD)
T4-P5CDD/F	93±2.0	81±3.1	52±4.4
H6CDD/F	94±3.0	73±4.5	49±7.3
H7-O8CDD/F	93±3.5	69±4.8	45±3.6
Non-*ortho*-PCB	83±4.2	84±2.8	53±3.6
Mono-*ortho*-PCB	0±0.0	70±5.1	59±5.5

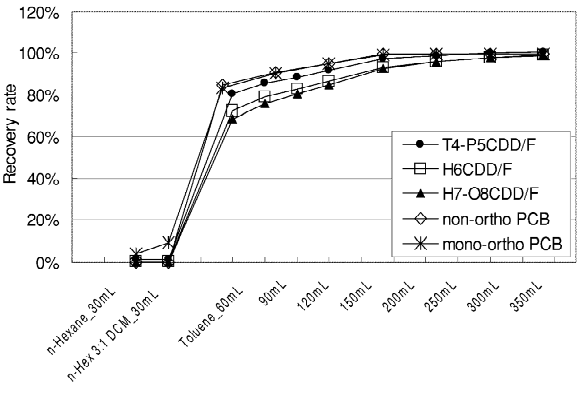

Fig. 4. Fractionation of PCDD/Fs and PCBs with Carboxen 1000.

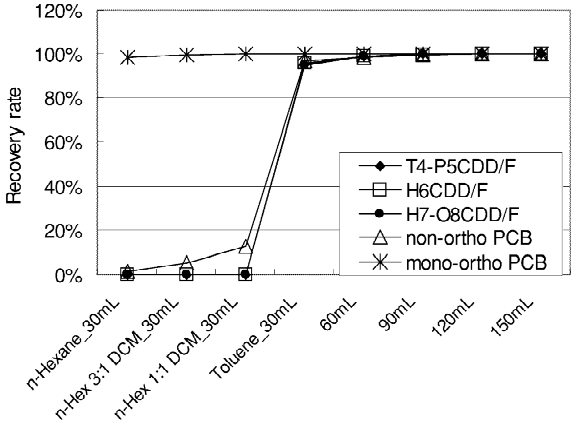

Fig. 5. Fractionation of PCDD/Fs and PCBs with Carboxen 1016.

were eluted in the first *n*-hexane fraction. Thus, C-1016 could easily separate PCDD/Fs and non-*ortho*-PCBs from other *ortho*-PCBs. C-1016 has a sufficient adsorption power for PCDD/Fs. There was no PCDD/Fs elution in the 3:1 *n*-hexane–dichloromethane fraction, indicating that C-1016 had the necessary adsorption power. In contrast, more than 90% of PCDD/Fs and non-*ortho*-PCBs were eluted in the first 30 ml of the toluene fraction, indicating that the adsorption power was not as strong as with C-1000. The flow-rate was 1.5 ml/min and required only 20 min for the elution. The amount of toluene was 30 ml and relatively small for an activated carbon clean-up procedure. C-1016 is suitable for PCDD/Fs sample preparation in terms of the rapidity and the amount of solvent required.

There are significant differences among the elution profiles of AC-2, C-1000 and C-1016, as shown in Figs. 3–5. It is due to the differences of their physical properties (Fig. 2 and Table 2). Fig. 3 indicates that AC-2 has strong adsorption power against PCDD/Fs and PCBs. In the meantime, Fig. 2 and Table 2 suggest that the dominant adsorption sites on the AC-2 surface are micropores (around 2 nm). So, it is very likely that the micropores have strong adsorption power against PCDD/Fs and PCBs. In contrast, the C-1016 elution profile indicated that practically all the PCDD/Fs eluted with the first toluene fraction, suggesting that there was only one type of adsorption sites on the C-1016 carbon surface with adequate adsorption power. Fig. 2 and Table 2 indicate that the dominant adsorption sites on the C-1016 surface are mesopores (4–30 nm). Hence, it is certainly that mesopores have adequate adsorption power against PCDD/Fs. Fig. 5 also suggests that the adsorption power of mesopores against mono-*ortho*-PCBs is very weak.

Fig. 2 and Table 2 show that C-1000 has both mesopores and micropores. In the meantime, the elution profile of C-1000 indicates that more than half of the PCDD/Fs was eluted with the first toluene fraction. The remaining PCDD/Fs, however, eluted very gradually, suggesting that there were two different types of adsorption sites on the C-1000 surface. One type has adequate adsorption power so that PCDD/Fs can be eluted with a relatively small amount of toluene. The other type has a very strong adsorption power so that a large amount of toluene was needed to elute the remaining PCDD/Fs. As judged from the AC-2 and C-1016 fractionation results, the former type of adsorption took place in mesopores, and the latter type of adsorption happened in micropores. The relation between pore size and adsorption power is shown in Table 5.

With conventional activated carbon, most adsorption occurs between two graphite-like walls in the micropores. However the distance between two graphite-like walls in a mesopore is very great considering PCDD/Fs molecular size, and the adsorption occurs between single graphite-like wall and a dioxin molecular. Suzuki suggested that oxygen complexes had an important role on the carbon surface [13]. These complexes on the surface add a polar nature to activated carbon, e.g., hydrophilicity, acidity, and negative ζ-potential. In addition, it is very likely that a planar molecular like PCDD/Fs interacts more with planar graphite wall than non-planar molecular. The fractionation results indicate that the interference between a single graphite wall and mono-*ortho*-PCBs is weak. In contrast, PCDD/Fs and non-*ortho*-PCBs interact sufficiently with a single graphite wall. This finding suggests that PCDD/Fs and non-*ortho*-PCBs are more polar nature than mono-*ortho*-PCBs. Conventional alumina column clean-up (EPA Method 1613) also indicated that PCDD/Fs and non-*ortho*-PCBs are the most polar dioxin-related congeners because they remained in the alumina column until the last fraction [14]. Many reports have pointed out the difficulty in using acumina as column fractionation material [7–9]. Unlike in the alumina column, a graphite wall on the carbon surface is affected little by humidity. Hence, the fractionation of PCDD/Fs and non-*ortho*-PCBs is more stable than that of the alumina column. C-1016 has excellent properties as column packing material for the clean-up of PCDD/Fs and non-*ortho*-PCBs in terms of stability, cost, speed, and ease of use.

Table 5
Pore size and adsorption power

	Micropores	Mesopores
Mono-*ortho*-PCBs	Adequate	Very weak
Non-*ortho*-PCBs	Strong	Adequate
PCDD/Fs	Very strong	Adequate

4. Conclusion

We developed a clean-up method for PCDD/Fs and non-*ortho*-PCBs by combining diatomaceous earth and micropore-free surface-activated carbon column. Diatomaceous earth reduced the sample preparation time for acid treatment and improved acid treatment efficiency due to its excellent affinity for sulfuric acid. Micropore-free surface-activated carbon had superb selectivity to PCDD/Fs and non-*ortho*-PCBs. With this method, an analyst can complete the clean-up for dioxin analysis in approximately 1 h including column preparation time. There is also no need for special equipment such as HPLC or automated apparatus to simultaneously analyze six samples. The combination of CE-1010 and C-1016 provides a rapid, robust and high-throughput sample preparation method for PCDD/Fs and non-*ortho*-PCBs analysis.

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