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# Fractionation of technical octabromodiphenyl ether by countercurrent chromatography combined with gas chromatography/mass spectrometry and offline and online $^1$H nuclear magnetic resonance spectroscopy☆

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ABSTRACT

Countercurrent chromatography (CCC) is a technique, which uses two immiscible liquid phases for a separation process in a long and hollow tube. The technique allows the separation of high amounts of sample (50 mg to several grams) with a low consumption of solvents. In this study, we fractionated 50 mg technical octabromodiphenyl ether (DE-79) and analyzed the fractions by gas chromatography with mass spectrometry (GC/MS) and proton nuclear magnetic resonance ($^1$H NMR) spectroscopy. CCC separations were performed with *n*-hexane/acetonitrile as solvent system in tail-to-head (i.e. the upper phase is mobile) mode. Twelve CCC fractions were studied for the PBDE composition. CCC elution of PBDE congeners was dependent both on the degree of bromination and substitution pattern. Higher brominated congeners eluted faster than lower brominated congeners and isomers with vicinal hydrogen atoms eluted last. In addition to several known PBDE congeners in DE-79, we were able to unequivocally identify BDE 195 in DE-79 and we could verify the presence of BDE 184. Finally, we also established the online hyphenation of CCC with $^1$H NMR. The use of deuterated solvents could be avoided by using *n*-hexane/acetonitrile as two-phase system. By online CCC-$^1$H NMR in stop-flow mode we were able to detect eight PBDE congeners in the mixture.



## 1. Introduction

Countercurrent chromatography (CCC) is a separation technique, in which two immiscible liquids are used as stationary and mobile phase [1,2]. The separation is carried out in a tube coiled around two or three bobbins, while one of the liquid phases is kept stationary and the other is continuously pumped through the system [1]. Rotation both around the coil axis and a central axis generates a changing gravitational field which leads to mixing and settling of the two phases [1,3]. CCC can be performed with high sample loads (~50 mg to several grams, depending on the column volume) and is therefore mostly used as a (semi)-preparative technique [3,4]. Accordingly, it was successfully employed for the separation of natural products [5,6], crude products of organic syntheses and complex technical mixtures [7,8].

In this research, we focused on the detailed analysis of technical octabromodiphenyl ether (DE-79) after CCC fractionation. Polybrominated diphenyl ethers (PBDEs) have been heavily used as flame retardants, which resulted in environmental contamination of concern [9]. Technical octabromodiphenyl ether (DE-79) is a mixture of several major and minor compounds, some of which are not structurally known [9–12]. For a detailed study, CCC fractions were subsequently analyzed by gas chromatography coupled with mass spectrometry (GC/MS) as well as proton magnetic resonance ($^1$H NMR) spectroscopy. The combination of CCC with other chromatographic methods has been successfully used for thorough analysis of other complex mixtures of polyhalogenated compounds such as toxaphene [8] and for the enrichment of minor compounds from synthesized methoxy-PBDEs [7].

Components leaving the CCC system are typically monitored with UV/Vis or evaporative light scattering detectors (ELSD) which do not provide detailed structural information. For online monitoring purposes, CCC has already been hyphenated with mass

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spectrometry [6]. In addition, Spraul et al. [13] used the online hyphenation of centrifugal partition chromatography (also called hydrostatic CCC) with NMR to monitor the separation of three *N*-2,4-dinitrophenyl substituted amino acids. This hyphenation was a transfer of the established online coupling of high performance liquid chromatography (HPLC) with NMR, which enabled to acquire NMR spectra of several compounds within one HPLC run without isolating every single compound beforehand [14,15]. During the recording of NMR spectra, the flow of the mobile phase is usually stopped (stop-flow mode) for longer acquisition times and thus better sensitivity [16,17]. Also, solvent suppression techniques such as the WET technique, a special pulse sequence, have been developed [18,19].

As a further goal we aimed to establish an online CCC-$^1$H NMR setup to monitor the separation and gather spectral information during the separation of DE-79 in stop-flow mode. Since $^1$H NMR signals of the aromatic PBDE analytes are >6 ppm, we aimed to use a solvent system for CCC-$^1$H NMR analysis which did not interfere with these signals thus avoiding the necessity of expensive deuterated solvents.

## 2. Materials and methods

### 2.1. Chemicals and standards

Acetonitrile (ACN, >99.9%) and *n*-hexane (HPLC grade) were from Th. Geyer (Renningen, Germany) and *iso*-octane was from Fluka (Steinheim, Germany). Ferulic acid was from Sigma Aldrich (Steinheim/Germany) while 2,2′,4,4′-tetrabromodiphenyl ether (BDE 47), 6′-methoxy-2,3′,4,4′-tetrabromodiphenyl ether (6′-MeO-BDE 66) and perdeuterated α-hexachlorocyclohexane (α-PDHCH) were synthesized in our laboratory [7,20,21]. Deuterated chloroform ($CDCl_3$, 99.8%) was from Deutero (Kastellaun, Germany) and deuterated dichloromethane ($CD_2Cl_2$, 99.6%) was from Euriso-Top (Saarbrücken, Germany), helium 5.0 and nitrogen 5.0 were from Westfalengas (Münster, Germany). Technical octabromodiphenyl ether (DE-79) was from Great Lakes Chemical (Indianapolis, IN, USA). An analytical reference standard with 40 PBDE congeners (EO-4980) was ordered from Cambridge Isotope Laboratories (Andover, MA, USA).

### 2.2. Sample preparation

For the standard mixture, 5 mg ferulic acid, 6 mg 6′-MeO-BDE 66 and 10 mg BDE 47 were dissolved in 5 mL of both upper and lower phases. For the analysis of the technical octabromo diphenyl ether mixture, 50 mg DE-79 was dissolved in 4.5 mL of both upper and lower phases. Then, 1 mL of deuterated chloroform was added to fully dissolve the analytes. Both sample solutions were used for CCC separations and injected completely.

### 2.3. Determination of partition coefficients $K_{U/L}$ by shake flask experiments and gas chromatography with electron capture detection (GC/ECD) evaluation

Partition coefficients were determined by means of shake flask experiments [1]. ACN and *n*-hexane were mixed 1:1 (v/v) and stored for 24 h. Then the upper phase (mostly *n*-hexane) and the lower phase (mostly ACN) were separated. About 9 μg DE-79 as well as about 1 μg of BDE 47 were given into individual 1-mL screw cap vials, 0.5 mL of both the upper and lower phases were added and the vials were vigorously shaken. After phase separation, 100 μL of upper and lower phases were transferred into separate vials, the solvent was removed and the residue was re-dissolved with 1 mL *iso*-octane. After addition of the syringe standard α-PDHCH, the sample solutions were analyzed by GC/ECD using a 5890 series II plus gas chromatograph (Hewlett-Packard/Agilent, Waldbronn, Germany) equipped with a GC PAL autosampler (CTC Analytics, Zwingen, Switzerland). One microliter was injected in splitless mode. A DB-5 column (30 m, 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) was used in combination with the following oven program: After 3 min at 50 °C, the temperature was raised at 10 °C min$^{-1}$ to 280 °C and then held for 24 min. Nitrogen was used as the carrier gas with a constant flow rate of 1.2 mL min$^{-1}$. The injector temperature was set to 250 °C and the detector temperature to 300 °C, nitrogen was also used as the makeup gas with a flow rate of 60 mL min$^{-1}$. The partition coefficient (i.e. the quotient between the concentration of the analyte in the upper and the lower phases, $K_{U/L}$) was calculated from the peak areas in the GC/ECD chromatograms. The $K_{U/L}$ of 6′-MeO-BDE 66 of 0.51 was known from earlier work [7]. Ferulic acid ($K_{U/L}$ < 0.1) was almost insoluble in *n*-hexane and was therefore expected to elute with the solvent front.

### 2.4. Countercurrent chromatography (CCC)

CCC separations were performed with a PTR-1000 CCC apparatus (PharmaTech Research, Baltimore, MD, USA) equipped with a 68 mL coil volume and a 10 mL sample loop as recently described by Englert and Vetter [22]. Solvents were pumped with a Varian 9012 pump and the UV/Vis signal was recorded with a Varian 9050 UV/Vis detector at 305 nm. The outlet of the UV/Vis detector was hyphenated with a PEEK capillary to the NMR (Section 2.7). The rotation speed was set to 950 rpm. The solvent system *n*-hexane/ACN 1:1 (v/v), prepared as shown in Section 2.3, was used with the lower phase as mobile phase (head-to-tail) for the separation of the standard mixture and with the upper phase mobile (tail-to-head) for the separation of DE-79. Displacement of the stationary phase during hydrodynamic equilibrium was 24 mL in both cases.

In a first attempt, only 15 mg of DE-79 could be dissolved in the solvent system. Adding deuterated chloroform (∼10%) to the sample solution enabled us to inject about 50 mg of DE-79 into the CCC system. The CCC fractionation was based on the results of the shake-flask experiments. Due to the $K_{U/L}$ values of the major PBDE congeners in DE-79 we chose to collect one fraction of 35 mL (Fraction 1), 10 fractions of 5 mL (Fractions 2–11) and one final fraction of 10 mL (Fraction 12). For $^1$H NMR measurements, aliquots (∼90%) of the CCC fractions (Section 2.6) were evaporated to dryness and the residue was re-diluted with 1 mL of deuterated chloroform. For subsequent GC/MS analysis (Section 2.5), the solvent was removed from sample aliquots (∼10%) and the residue was re-dissolved in *n*-hexane.

### 2.5. Gas chromatography with mass spectrometry (GC/MS)

Samples and CCC fractions were analyzed on a 6890 GC/5973 MSD system equipped with a cool-on-column inlet (Hewlett-Packard/Agilent, Waldbronn, Germany) fitted with a pre-column (2 m, 0.53 mm i.d., deactivated with 1,3-diphenyl-1,1,3,3-tetramethyldisilazane, BGB Analytics, Boeckten, Switzerland). The pre-column was connected with a press fit to a 15 m, 0.25 mm i.d. capillary column coated with 0.1 μm film thickness dimethyl polysiloxane (ZB-1, Phenomenex, Aschaffenburg, Germany). Injections (1 μL) were made with a 7683 autosampler system (Hewlett-Packard/Agilent, Waldbronn, Germany). Helium was used as carrier gas with a flow rate of 1.0 mL min$^{-1}$. The GC oven temperature was programmed as follows: After 1 min at 60 °C, the oven was heated at 10 °C min$^{-1}$ to 320 °C and this temperature was held for 14 min. The temperatures of the transfer line, ion source and quadrupole were set at 350 °C, 230 °C and 150 °C, respectively. The temperature of the injector port was set to track the oven