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Separation of polychlorinated terphenyls from lipoidal material by preparative gel permeation chromatography and gas chromatography

ROBERT C. HALE*, ELIZABETH BUSH, KATHRYN GALLAGHER, JENNIFER L. GUNDERSEN and ROBERT F. MOTHERSHEAD, II

Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062 (U.S.A.)

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ABSTRACT

The potential of preparative gel permeation chromatography to separate polychlorinated terphenyls (PCTs) from lipoidal material derived from fish was investigated. When applied to styrene–divinylbenzene gel permeation chromatography columns, PCTs eluted prior to commonly examined environmental contaminants, such as polychlorinated biphenyls (PCBs). Coelution of PCTs with lipoidal material was observed with some columns tested, attributable to the large molecular size of the chlorinated terphenyls. Capillary gas chromatography coupled with electrolytic conductivity detection provided adequate characterization of two PCT formulations, Aroclor 5432 and 5460. Some lowly chlorinated PCTs eluted in the same retention window as highly chlorinated PCBs.

INTRODUCTION

Polychlorinated terphenyls (PCTs) are structurally similar to the comparatively well-studied polychlorinated biphenyls (PCBs). Substantial volumes of the former compounds were produced, amounting to approximately 15% that of PCBs [1]. PCTs were synthesized as complex mixtures, the components of which possessed varying degrees of chlorination. Two common formulations marketed by Monsanto were Aroclor 5432 and 5460, which possessed 32% and 60% chlorine by weight, respectively. A third formulation, Aroclor 5442, has been observed to consist of a mixture of the two previously described products [1]. Manufacture of PCTs apparently ceased prior to 1980 [2]. Chemical and physical properties of PCTs and PCBs are similar. As a consequence, the two groups of chemicals were employed commercially for related purposes. This similarity also raises concerns about their environmental distribution, fate and toxicity.

Reports of the analysis of PCTs are rare in the literature, particularly when compared with those concerning PCBs. The majority of papers were published in the 1970s and utilized gas chromatography (GC) [3–6]. The packed-column techniques used in those investigations provided extremely poor resolution of the constituents of

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these complex mixtures. The presence of a third phenyl ring in PCTs not only allows additional positions for chlorination, but also permits the terphenyl molecule to assume *ortho, meta* and *para* orientations. This greatly increases the number of possible PCT congeners, compared with PCBs, posing a complex task for the chromatographer.

Discovery of significant concentrations of PCTs in sediment and shellfish samples during an exploratory monitoring program in the Commonwealth of Virginia, U.S.A. [7], prompted an examination of analytical methods for the accurate determination of these compounds. Gel permeation chromatography (GPC) has received increasing interest for the separation of organic pollutants from naturally occurring material [8,9]. This latter material typically interferes with subsequent GC analyses, which are ultimately used for identification and quantification purposes. The behavior of PCTs on several preparative GPC columns and with two solvent systems is described. In addition, the application of capillary GC with electrolytic conductivity detection (ELCD) for the analysis of PCTs is introduced.

EXPERIMENTAL

Chemicals and test materials

High-purity-grade dichloromethane (DCM) and cyclohexane (CCH) were obtained from Burdick and Jackson (Muskegon, MI, U.S.A.). Aroclor standards and 2,2',4,4',5,5'-hexabromobiphenyl were obtained from Chem Service (West Chester, PA, U.S.A.). Decachlorobiphenyl was purchased from Aldrich (Milwaukee, WI, U.S.A.).

Non-polar material, operationally defined as lipid, was extracted from catfish (*Ictalurus catus*). The organisms were collected from the James River, VA, U.S.A. The entire organism was initially homogenized in a tissue grinder and the resulting material dried in an FTS Systems lyophilizer for 24–48 h. Approximately 20 g of lyophilized tissue were then extracted with DCM for 48 h in Soxhlet apparatuses. The resulting lipid extracts were reduced in volume with a rotary evaporator.

Gel permeation chromatography

An automated gel permeation chromatograph (ABC Labs., Autoprep 1002A) was used to separate the PCT formulations, Aroclor 5432 and 5460, from lipoidal material. GPC columns (60 cm × 2.5 cm) equipped with PTFE fittings were obtained from the same source. One column was prepacked with 60 g of 200–400 mesh Bio-Beads S-X3 resin by the manufacturer. Additional columns were slurry packed in the laboratory with 70 g S-X3 or 60, 80 or 100 g of 200–400 mesh S-X8 (Bio-Rad Labs.). This resin was sieved over a 250-mesh screen to remove fines and allowed to swell for 24 h in the packing solvent prior to use. The 60-g S-X3 column was packed in a mixture of DCM–CCH (1:1, v/v). The remaining columns were packed in 100% DCM. S-X3 and S-X8 resins have manufacturer-specified molecular-size-exclusion limits of approximately 2000 and 1000 dalton, respectively. The flow-rate through the columns was 5.0 ml min^{-1}. Extracts, representing less than 1 g of total lipid, and solutions containing Aroclor 5432, Aroclor 5460 and decachlorobiphenyl standards were injected onto the columns. Decachlorobiphenyl was proposed as a surrogate standard for the analysis of PCTs in environmental matrices. Fractions, 5 or 10 ml in

volume, were then collected, evaporated to dryness at room temperature and the lipid residue determined gravimetrically. PCTs were determined as described below. The PCT solutions were augmented with PCBs consisting of Aroclor 1242, 1254 and 1260 for tests conducted on the 100-g S-X8, and the 60- and 70-g S-X3 columns to examine the relative elution characteristics of these compounds.

Gas chromatography

The presence of PCTs, as well as PCBs, in the various GPC fractions was determined by capillary GC, employing a Varian Model 3300 gas chromatograph. The instrument was equipped with an OI Analytical Model 4420 ELCD system. The detector was operated in the halogen-specific mode with *n*-propanol as the conductivity solvent. Helium at a linear velocity of 30 cm s^{-1} was used as the carrier gas. The injector was maintained at 300°C and the reactor tube of the ELCD system at 950°C. The chromatograph was equipped with a DB-5 fused-silica column (J & W Scientific, 30 m long × 0.32 mm I.D., 0.25 μm film thickness). Following exchange of the original GPC solvent to hexane, injections onto the chromatograph were made in the splitless mode. The column effluent was purged for 3 min after injection, to eliminate the bulk of the solvent prior to its entrance into the detector. Column temperature was initially held at 90°C for 2 min. It was then programmed at 4°C min^{-1} to 300°C. Final column temperature was held at 300°C for 10 min.

RESULTS AND DISCUSSION

The major constituents of Aroclor 5460 contain seven to ten chlorines [1]. Its presence in the environment has been reported sporadically in the literature, generally at low concentrations. However, Aroclor 5460 was detected recently in sediments collected near the mouth of the James River at concentrations as high as 26 mg kg^{-1} [7].

Aroclor 5432 is composed of terphenyls with a lower degree of chlorination than 5460, 2–5 chlorines per molecule [1]. Our laboratory has identified Aroclor 5432 in sediments and shellfish from a tributary of the Chesapeake Bay at concentrations as high as 250 and 35 mg kg^{-1}, respectively [10]. The only other report uncovered, detailing the environmental occurrence of this formulation was published in 1978 [11]. Those authors separated Aroclor 5432 from 5460 by thin-layer chromatography. The resulting residue was then perchlorinated and analyzed by packed-column GC with electron-capture detection.

In an attempt to improve the methodology for the detection of PCTs, the ability of various GPC packing and solvent systems to resolve PCTs from lipoidal material was examined. The technique with S-X8 and 100% DCM as the eluting solvent has been employed in this laboratory to purify biota and sediment extracts since 1978. It was found to be effective in separating lipids from a range of anthropogenic compounds, such as PCBs, polynuclear aromatic hydrocarbons and various heterocyclic xenobiotics [12,13]. However, difficulties were initially encountered when PCTs were detected in the Chesapeake Bay samples mentioned above. Some of the terphenyls, particularly the highly chlorinated components of Aroclor 5460, eluted in the early lipid fraction. This was attributed to the relatively large molecular size of the PCTs. As a consequence, a more detailed examination of the separation characteristics of various GPC columns was conducted.

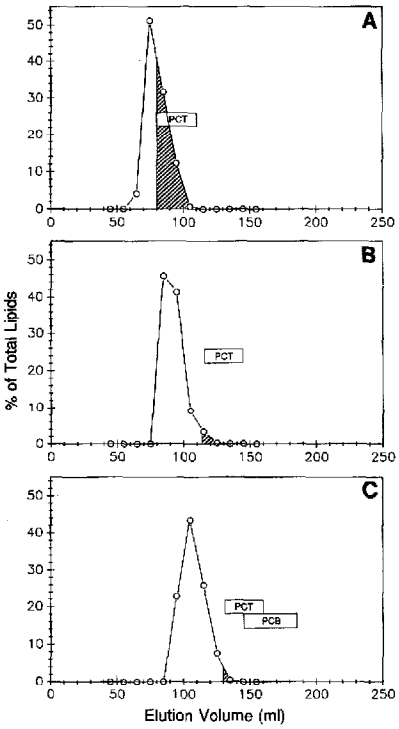

Fig. 1. GPC elution profiles of lipoidal material and windows for PCTs on columns packed with: (A) 60 g S-X8; (B) 80 g S-X8; and (C) 100 g S-X8. The elution window for PCBs on the 100-g column is also shown. All columns were eluted with 100% DCM. Shaded areas of the lipid curves denote overlap with PCTs.

Elution profiles of lipoidal material from tissue extracts on 60-, 80- and 100-g S-X8 columns are provided in Fig. 1; column bed lengths were 30, 40 and 50 cm, respectively. The elution volumes of the PCTs, in relation to extracted fish lipids, are also presented. Considerable overlap between PCTs and lipids occurred, particularly with the two shorter columns. Approximately 45 and 3.7% of the total lipid injected coeluted with the PCTs in the case of the 60- and 80-g columns, respectively. Coelution of more than 0.5% of the lipids with PCTs was observed on the 100-g S-X8 column. The majority of the PCTs eluted from 131 to 160 ml on this column, with the higher-molecular-weight Aroclor 5460 components eluting first. PCBs eluted from 146 to 185 ml. The inability of a 50-g S-X8 column, eluted with DCM, toluene or CCH, to adequately resolve fish lipids and organochlorine pesticides has been described previously [14].

The 60-g S-X3 column employed a solvent regime of CCH–DCM (1:1). This column had a bed length of 45 cm. The PCTs were well resolved from the bulk of the lipids, although the latter peak tailed (Fig. 2A). This suggests the presence of modest amounts of relatively low-molecular-size lipoidal material. No such tail was observed for the S-X8 columns examined. PCTs began to elute at 151 ml and continued to 200 ml. PCBs eluted from 171 to 210 ml.

A column containing 70 g of S-X3, using 100% DCM as the solvent, is recommended by the U.S. Environmental Protection Agency for the elimination of

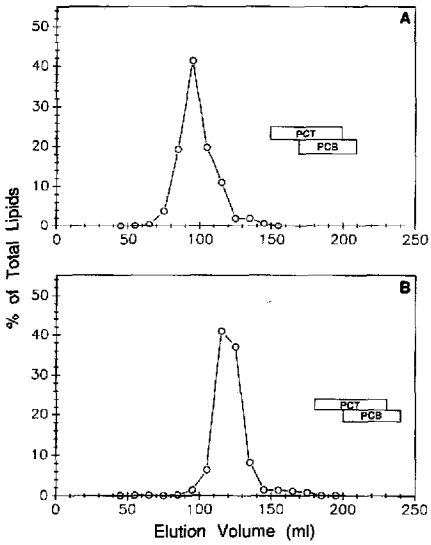

Fig. 2. GPC elution profiles of lipoidal material and windows for PCTs and PCBs on: (A) the 60-g S-X3 column, eluted with DCM–CCH (1:1); and (B) the 70-g S-X3 column, eluted with 100% DCM.

interferents in solid waste extracts [9]. The column tested possessed a bed length of 52 cm. PCTs eluted from 181 to 230 ml. PCBs eluted from 201 to 240 ml. Lipids and PCTs were resolved from each other, although the lipid peak again tailed (Fig. 2B). The observation that tailing occurred with both solvent regimes, with S-X3 but not with SX-8, suggests a simple molecular-size effect, rather than a polarity-based interaction with the resin.

A chromatogram obtained with the GC–ELCD system for Aroclor 5460 and 5432 standards is provided in Fig. 3A. Although the electron-capture detector is widely used to determine the concentration of organochlorine pollutants, ELCD provides greater specificity and response is less sensitive to the structure of the compounds examined [15]. The electron-capture detector does exhibit somewhat higher sensitivity. Aroclor 5460 elutes from relatively non-polar GC columns after decachlorobiphenyl. As many investigators discontinue their chromatographic analyses prior to the emergence of this compound, they may fail to detect the presence of this late-eluting formulation. Decachlorobiphenyl elutes from the chromatographic column between Aroclor 5432 and 5460 (Fig. 3A), demonstrating its potential as a standard in the GC analysis of PCTs. This compound has been used in some investment casting applications [2] and is a minor constituent of Aroclor 1260 [16]. Nonetheless, it typically represents less than 1% of the total PCB burden in environmental samples [17,18]. As shown in Fig. 3A, 2,2′,4,4′,5,5′-hexabromobiphenyl also elutes between the two PCT formulations examined.

Some difficulties have been encountered in distinguishing the components of Aroclor 5432 from highly chlorinated biphenyls, even with high-resolution GC columns. This is apparent when a chromatogram of Aroclor 5432 and that of a mixture of PCB formulations are compared (Fig. 3A and B). Confirmation of the identities of individual chromatographic peaks requires mass spectrometric analysis [10].

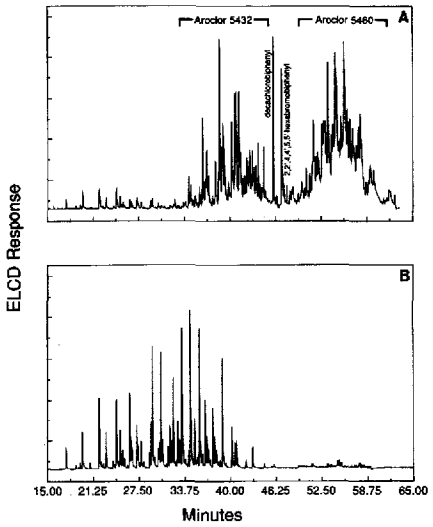

Fig. 3. GC–ELCD of: (A) Aroclors 5432 and 5460; (B) PCB formulation Aroclor 1260. GC retentions of decachlorobiphenyl and 2,2′,4,4′,5,5′-hexabromobiphenyl are also provided.

Alteration or removal of selected components of PCB formulations via processes such as microbial or physical degradation have been reported [19,20]. The structural similarity between PCTs and PCBs suggests that both groups of compounds may be acted upon in analogous manners. To date, residues of Aroclor 5432 detected in the environment have closely resembled the parent formulation [10]. However, some depletion of the lowly chlorinated components of Aroclor 5432 from sediments, at sites several kilometers from the original source of the PCTs, have been observed (Fig. 4). At present, insufficient data are available to establish the mechanism of the alterations.

In summary, the S-X8 columns tested in this study did not adequately separate extracted lipoidal material from PCTs. However, resolution from PCBs was ac-

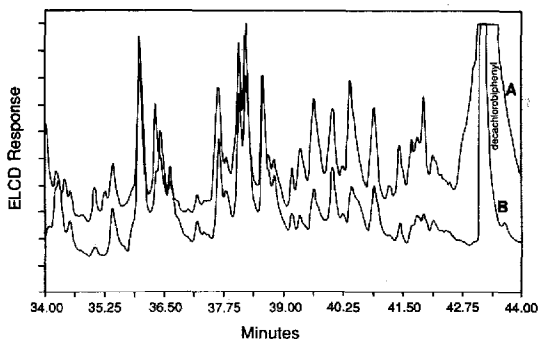

Fig. 4. (A) GC–ELCD of an extract of sediment containing Aroclor 5432. The sample was collected 5 km from the suspected source of the contamination. (B) Chromatogram of an Aroclor 5432 standard. Note the diminution of the lowly chlorinated PCT constituents in the sediment extract, compared with the standard.

ceptable with the 100-g column. Retention times and attendant solvent consumption were lower than with the S-X3 resin. Both S-X3 systems provided satisfactory resolution. The progression in elution from large to small molecular-size compounds, for complex organochlorine mixtures, with GPC is demonstrated in Fig. 5A–C. In the first chromatogram presented (Fig. 5A), representing the 181–190-ml fraction, Aroclor 5460 predominates. This formulation is succeeded by Aroclor 5432 (Fig. 5B) in the 191–200-ml aliquot and finally the PCB mixture in the 201–210-ml fraction (Fig. 5C). Thus, the potential for coelution of lipoidal material with PCTs was most severe for Aroclor 5460. The use of the 100% DCM solvent regime has the advantage of simplicity and is not vulnerable to compositional changes in the eluting solvent over time, as is the binary solvent system. Utilization of DCM as the sole solvent also allows more gentle conditions to be applied during subsequent concentration steps, with attendant minimization of losses of the more volatile sample constituents. The GPC and GC–ELCD methods described are currently being applied to the analysis of PCTs in a variety of aquatic biota and sediments in this laboratory.

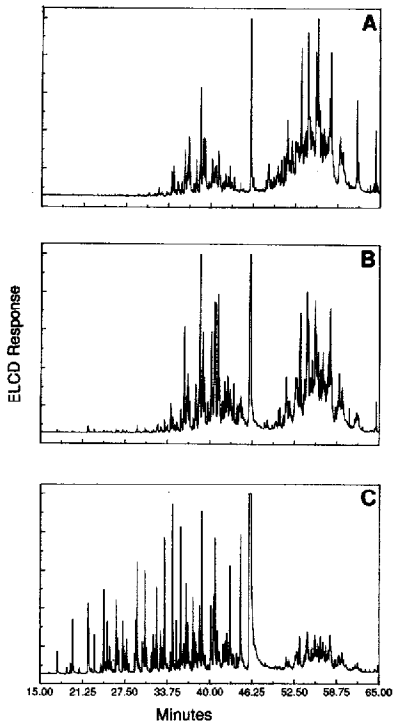

Fig. 5. GC–ELCD of fractions obtained from the 70-g S-X3 column. Fractions analyzed were: (A) 181–190 ml; (B) 191–200 ml; and (C) 201–210 ml. The succession in elution from predominantly highly chlorinated PCTs to lowly chlorinated PCTs and finally PCBs is shown.

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