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Separation of Polychlorinated Biphenyls from Toxaphene by Silicic Acid Column Chromatography

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The presence of multicomponent constituents such as the polychlorinated biphenyls (PCBs) or toxaphene in residues being analyzed for organochlorine pesticides by electron-capture gas chromatography typically necessitates prior analytical fractionation to eliminate or decrease interferences. Silicic acid column chromatography, developed by HOLDEN & MARSDEN (1969), and ARMOUR & BURKE (1970), has been employed by BIDLEMAN et al. (1978) to separate a mixture of PCBs, chlordane, DDT and analogs from toxaphene. The procedure employed 100-mesh silicic acid deactivated with 3.3% water. While toxaphene interference was reduced in the fractions containing PCBs and pesticides, complete class separation was not achieved. About 40% of the toxaphene appeared in the fractions eluted with petroleum ether; the remaining toxaphene (60%) was eluted with methylene chloride.

There is a need for a procedure for preanalytical separation of essentially intractable mixtures of PCBs and toxaphene. We have investigated use of silicic acid to separate these two multicomponent mixtures and found that small particle-size silicic acid (325 mesh) is capable of separating PCBs from toxaphene. This report describes our initial results on separation and recovery of test mixtures of toxaphene and PCBs.

EXPERIMENTAL

REAGENTS

(a) **Silicic Acid** – 325 mesh, certified, Lot No. 735-400, Fisher Scientific Co., Pittsburg, Pa.

(b) **Eluting Solvents** – hexane, methylene chloride (both pesticide grade), Fisher Scientific Co., Pittsburgh, Pa.

(c) **Toxaphene and Polychlorinated Biphenyls, Aroclors 1242, 1248, 1254, 1260** – Supelco Inc., Bellefonte, Pa.

APPARATUS

(a) **Chromatographic Columns** – Chromaflex column, pressure, 200-mm x 10 mm i.d., 50-ml capacity solvent reservoir (K-420520, size 23, Kontes, Evanston, Il).

(b) **Kuderna-Danish (K-D) Concentrators** – Size 125-ml (K-570000, Kontes, Evanston, IL), or K-D Micro-Concentrator (6-4688, Supelco, Inc., Bellefonte, Pa).

(c) **Gas Chromatograph** – Hewlett Packard Model 5880A, Ni-63/Electron Capture Detector, 184-cm x 0.4 cm i.d. coiled glass column containing 3% OV-1 on 100/

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200 mesh Suplecoport or 1.5% SP-2250/1.95% SP-2401 on 100/200 mesh Suplecoport. Operating conditions: column and oven temp. 210°C, isothermal; injector temp. 265°C; carrier gas Argon-5% methane at flow rate of 60 ml/min.

COLUMN CHROMATOGRAPHY

(a) Preparation of Silicic Acid

Silicic acid, 325 mesh, was heated at 130°C for at least 48 hours and cooled to room temperature in a desiccator. Weight loss was 15% for the material used.

Screw-capped Erlenmeyer flasks and 100μl-syringes were found to be convenient for the following steps: to a 125-ml flask, 160 μl (0.16 g) of water was added and spread as evenly as possible, and 16 g of the dried silicic acid was added. The flask was tightly capped and shaken on a mechanical shaker for one hour. One gram of the material was accurately weighed, dried at 130°C for 8 hours and weighed again. The weight loss was 1.5/ to 2.0%. Since only 1% water was introduced, it appeared that additional moisture was absorbed by the fine powder during manipulation. The amount thus prepared was sufficient for three columns.

(b) Preparation of Column B

A plug of prewashed glass wool was placed on the bottom of the Chromaflex column. Portions of 5 g of silicic acid were slurried in about 30 ml of methylene chloride in a beaker and poured into the column with the stopcock open. The column was gently tapped and swirled to settle the powder evenly and remove air bubbles. Pressure of about 15 psi from a nitrogen gas cylinder was applied. After the powder had settled in the column, predried sodium sulfate was added to a height of about 1 cm. The column was washed once with 20 ml of methylene chloride and twice with 20 ml of hexane.

(c) Elution

When the last hexane wash was about 1 cm above the Na_2SO_4 layer, sample solution (0.5 to 1.0 ml hexane solution) was introduced with a pipette, and a receiving flask was placed under the column. After the liquid reached the surface of the Na_2SO_4 layer, about 3 ml of the eluting solvent was introduced as a rinse, and eluted to about 1 cm above the surface. Eluting solvent was then added to the column (the capacity of the column reservoir was about 50 ml) and eluted under pressure at 15 psi. The flow rate was about 20 ml/hour.

For determinations of elution patterns, consecutive 20 ml fractions were collected. For recoveries, measured amounts of eluting solvents were used, and mixtures of PCBs (7.6 to 10.4 μg) and toxaphene (10.2 μg) were eluted by 60 ml of hexane followed by 50 ml of methylene chloride.

The fractions were concentrated to about 1.5 ml in a K-D concentrator, then evaporated to 1.0 ml under a gentle stream of nitrogen. The methylene chloride fractions were transferred to hexane solution by further concentrating twice with 25 ml hexane in a K-D concentrator. Five to 10 μl aliquots of the solutions were analyzed by electron-capture gas chromatography.

RESULTS AND DISCUSSION

The aim of the present work was to develop a column material capable of separating complex mixtures of PCBs and toxaphene, with sufficient procedural simplicity for routine analytical applications. Therefore, we chose single solvents, rather than mixed ones. Under the experimental conditions described here, PCBs were completely eluted by 60 ml of hexane. PCB elution began in the first 20 ml fraction, continued in the second 20 ml (about 80% total PCB), and was completed in fraction 3 (the third 20 ml).

Toxaphene did not elute in the first 3 fractions (total 60 ml of hexane). Trace amount began to appear in fraction 4. As observed by BIDLEMAN et al. (1978), small amounts of the toxaphene components were eluted by hexane in each of the subsequent fractions. We estimated that not more than 10% of the total toxaphene was removed up to the 10th fraction (total of 200 ml of hexane). There appeared to be some intramixture fractionation of toxaphene because gas chromatograms of these latter fractions showed gradual increases and decreases of certain peaks. Both PCBs and toxaphene were rapidly eluted by the relatively polar solvent methylene chloride.

Water content affected elution of both PCBs and toxaphene by hexane. For the activated material (i.e., dried at 130°C without aqueous deactivation), toxaphene was virtually totally retained, while PCBs required at least 8 hexane fractions (160 ml) for complete elution. Deactivation with 4% water gives overlapping elution of PCBs and toxaphene in fraction 3 (60 ml), and the remaining toxaphene was diffusely eluted in subsequent fractions without completion.

These elution patterns indicated that a complete separation of PCBs and toxaphene was possible using hexane followed by methylene chloride on the 2% water-deactivated 325-mesh silicic acid. This conclusion was tested using mixtures of PCBs and toxaphene, with elution by 60 ml hexane followed by 50 ml methylene chloride. Results of recoveries are summarized in Table 1.

Table 1. Percent Recoveries of Added PCBs and Toxaphene Using 325-Mesh Silicic Acid Column.*

		Hexane Fraction (PCB)		Ch_2Cl_2 Fraction (Toxaphene)	
Mixtures	Added (μg)	Range (%)	Avg. (%)	Range (%)	Avg. (%)
Aroclor 1242/Toxaphene	10.4/10.2	80-96	88	77-101	91
Aroclor 1248/Toxaphene	7.6/10.2	83-94	89	84-86	85
Aroclor 1254/Toxaphene	7.7/10.2	84-102	91	89-108	97
Aroclor 1260/Toxaphene	7.6/10.2	88-94	91	81-103	96

*Each experiment performed in triplicate.

As can be seen, separation of PCBs containing 42%, 48%, 54%, and 60% chlorine content from toxaphene was complete using the simple, two-solvent elution system described above. Thus, it now appears possible to analyze mixtures of these two materials. While these results have indicated the feasibility of separation, additional experimentation will be required to completely characterize the utility of this

methodology for analysis of environmental samples. For example, the small particle size of the silicic acid employed, which apparently is required for separation, gives low flow rates with attendant long elution times. We are investigating use of moderate to high pressure to increase the flow rate. In addition, silicic acid is known to show variations among lots, as well as manufacturers. Each lot or even each bottle of the material should be individually tested for adjusting the chromatographic procedure.

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