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New techniques for analysis of organic pollutants in drinking water

by

Larry Dean Kissinger

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INTRODUCTION

Only in the past few years have drinking waters been analyzed for trace levels of organic pollutants. In the past, the inorganic constituents and the biological organisms present in potable waters have been well-characterized, while little was known about the organic matter present in the waters. In an effort to ensure the quality of drinking waters, reliable methods of analysis for trace concentrations of organic compounds in water must be developed (1,2).

Early methods of analysis for organic matter present in waters were developed to determine the total-organic carbon (TOC) in samples (3,4). More recently, methods have been developed to concentrate and determine the organic compounds present in drinking waters (5,6,7). Gas chromatography has been used to separate organic pollutants and mass spectrometry has been used to identify organic pollutants present in drinking waters. More than 400 compounds have been identified in drinking waters by gas chromatography/mass spectrometry (8).

The purpose of this research effort was to improve on the methods available for determining organic pollutants in drinking waters. For the purpose of organization, the thesis is divided into four sections. A technique to abstract amines from gas-chromatographic streams is

discussed in the first section. In the second section, an application of the amine abstractor to a gas-chromatographic method of determining haloforms in drinking waters is described. A high-resolution system for gas chromatography with capillary columns is described in the third section. Finally, fractionation of organic compounds from drinking waters is discussed. Organic compounds from selected drinking waters were fractionated on Florisil columns, chromatographed on capillary columns, and assayed for mutagens by the Ames Test.

ABSTRACTION OF AMINES FROM GAS-CHROMATOGRAPHIC STREAMS

Review of Related Work

Columns packed with a material that completely removes certain types of compounds from gas-chromatographic streams have sometimes been used. These columns are called *subtractors* or *abstractors*. Abstractor columns are usually used to obtain information about the identities of sample components. Innes *et al*. used columns containing silver nitrate and sulfuric acid to abstract unsaturated hydrocarbons (9). A short column of boric acid can be used to remove alcohols selectively from complex mixtures prior to separation of sample components on a gas-chromatographic column (10). Other abstractors have been reported for removal of dienes (11), phenols (12), and acids (13). Fryka and Pospisil used phosphoric acid on Chromosorb W AW/DMCS as an abstractor packing for nitrogen bases (14). A book by Leathard and Shurlock has a chapter discussing the use of abstractors in gas chromatography for compound identification (15).

Various techniques have been developed to obtain chromatograms free of solvent peaks. Chang used thermal desorption from columns of Amberlite XAD-2 and Tenax GC to introduce organic compounds from water samples to a gas chromatograph without using a solvent (16). Bellar and

Lichtenberg developed a "purge and trap" technique that allows for the analysis of volatile compounds in water without using a solvent (17). This technique uses a Tenax GC column to trap compounds purged from water by a gas flow, then thermally desorbs compounds from the Tenax GC column into a gas chromatograph. A capsule sampler has been marketed by Perkin-Elmer Corporation (18). After evaporating the solvent from a sample in a capsule, the capsule sampler can be used to introduce the capsule into a gas chromatograph. The capsule is ruptured, and a solventless chromatogram is obtained.

Although abstractors have been developed for a number of compound classes, most abstractors have only been used to remove small amounts of material from gas-chromatographic streams. Removal of a solvent from a gas-chromatographic stream could be used when a large solvent peak interferes with peaks for compounds of interest. If an abstractor of high capacity was to be used to remove a solvent, a number of solventless chromatograms could be obtained without changing the abstractor.

In this work, Colin D. Chriswell and myself investigated the use of metal-amine complexes to obtain chromatograms free of a solvent peak (19). Various means of subtracting amines from gas streams were investigated before selecting a packing of 25% copper(II) chloride coated on

Chromosorb G AW/DMCS as the abstractor packing. Interaction between nonamine compounds and the abstractor packing was minimized by using an inorganic abstractor packing.

Experimental

Apparatus and reagents

Gas chromatograph A Hewlett-Packard model 5711A gas chromatograph and a Tracor model 550 gas chromatograph were used for this work. Both instruments were equipped with a linear temperature programmer and a dual flame ionization detector. Chromatograms were recorded using a Houston Instruments Series 5000 strip chart recorder.

Abstractor columns For the Hewlett-Packard 5711A, a 12-in x 1/8-in o.d. stainless-steel tube filled with abstractor packing was connected to the injection port and to the analytical column of the gas chromatograph. A segment of stainless-steel tubing, 7-in x 1/4-in o.d., with a female 1/8-in Swagelok (trademark of Crawford Fitting Company, Solon, Ohio) fitting was used as the abstractor column with the Tracor 550. The tubing was filled with abstractor packing, inserted into the injection port of the Tracor 550, sealed to the inlet with a brass ferrule, and connected to the analytical column of the gas chromatograph.

Before use with an analytical column, the abstractor columns were conditioned with a gas flow at 220°C.

Analytical columns   Chromatograms were obtained using either a 6-ft x 1/8-in o.d. stainless-steel column packed with 10% SE-30 on 100/120-mesh Chromosorb W AW/DMCS or a 2-ft x 1/8-in o.d. stainless-steel column packed with 80/100-mesh Amberlite XAD-2.

Reagents   Pyridine, distilled-in-glass grade and stored under nitrogen, was obtained from Burdick and Jackson Laboratories, Inc., Muskegon, Michigan.

All grades of Chromosorb (products of Johns-Mansville) were obtained from Applied Science Laboratories, Inc., State College, Pennsylvania. Chromosorbs labeled with AW have been washed with acid. The Chromosorbs which carry the DMCS label have been treated with dimethyldichlorosilane to reduce the activity of their surfaces.

Amberlite XAD-2 (a styrene-divinylbenzene copolymer), Amberlite IRC-50 (a methylacrylic acid-divinylbenzene copolymer), and Amberlyst A-15 (a sulfonated styrene-divinylbenzene copolymer) were obtained from Rohm and Haas, Philadelphia, Pennsylvania.

Other materials and chemicals were the grade available within the laboratory.

Techniques and procedures

Preparation of abstractor packing   Chromosorb G AW/DMCS (80/100 mesh) was coated by evaporation of an aqueous solution containing 2.5 g of copper(II) chloride to near dryness onto 7.5 g of Chromosorb using a rotary evaporator. The Chromosorb was wet with acetone and re-evaporated in a rotary evaporator to dryness. The material was then dried at 115°C for 1 hr, sieved, and the 80/100-mesh fraction collected.

Solutions   Solutions for analysis by abstraction gas chromatography were made with distilled-in-glass grade pyridine. Stability of pyridine solutions was increased by storage in a freezer at -17°C.

Gas-chromatographic technique   Pyridine samples were analyzed by introducing a few microliters of the solution into a gas chromatograph with a Hamilton 701N syringe. The analytical column was subjected to temperature programming to affect separations. The abstractor columns were operated at a temperature below 180°C. Samples containing polar organic compounds were separated using the XAD-2 analytical column, and samples containing only nonpolar organic compounds were separated using the SE-30 analytical column.

Results and Discussion

Development of abstractor packing

Cation-exchange resins in various metal-ion forms were examined for use as abstractor packings. The following ion-exchange resins were investigated: Amberlite IRC-50, Amberlyst A-15, and a low-capacity sulfonated Amberlite XAD-2 (20). Nickel(II) and copper(II) forms of the 80/100-mesh fraction of these resins were packed into stainless-steel columns and installed in the gas chromatograph. Neither form of IRC-50 was effective for removal of amines; however, both nickel(II) and copper(II) forms of A-15 and sulfonated XAD-2 retained amines at elevated temperatures. The nickel(II) forms of A-15 and sulfonated XAD-2 caused peaks for most nonamine compounds to be significantly tailed and some alcohols and alkenes were retained. If the area under a peak after the maximum is greater than the area before the maximum, the peak is tailed. Because tailing reduces the maximum of a peak, sensitivity for a compound is reduced if the peak is tailed. Resolution of chromatographic peaks is reduced by tailing, because tailing broadens peaks. Pyridine and diethylamine were retained by the copper(II) forms of A-15 and sulfonated XAD-2 at temperatures up to 275°C; however, retention times were increased and peaks for many compounds were tailed.

The effectiveness of columns packed with Chromosorb G AW/DMCS coated with other salts was evaluated. Copper(II) sulfate coated on Chromosorb G AW/DMCS was effective in retaining amines and did not affect the chromatographic behavior of nonamine compounds. Copper(II) sulfate is unstable at temperatures greater than 200°C (21); thus, copper(II) chloride was preferred for making the abstractor packing. Chromosorb G AW/DMCS coated with nickel(II) chloride abstracted amines; however, peaks for alkenes and some other compounds were tailed. When temperature programming columns containing Chromosorb G AW/DMCS coated with zinc(II) chloride, the background current from the flame ionization detector increased. The increased background current indicates that pyridine was not completely retained by the column.

Copper(II) chloride coated on Chromosorb G AW (not treated with dimethyldichlorosilane) retained amines; however, peaks for many compounds were tailed. Because of interactions between polar compounds and active hydrogens on the surface of Chromosorb, peaks for polar compounds are often tailed. Treatment of Chromosorb with dimethyldichlorosilane replaces the active hydrogens and reduces tailing of polar compounds (22).

Chromosorb W AW/DMCS coated with 10% concentrated phosphoric acid (85%) has been used to abstract amines from

gas-chromatographic streams (14). Because many compounds interact with strong acids at high temperatures, peaks for alcohols, ketones, and other compounds were tailed when Chromosorb coated with phosphoric acid was used to abstract amines. The capacity of a packed column of Chromosorb coated with phosphoric acid was much less than a packed column of Chromosorb coated with copper(II) chloride. Columns packed with the phosphoric-acid packing retain less than a fifth the amount of pyridine retained by the copper(II) chloride packing.

Amines were removed from the gas-chromatographic stream by the abstractor because nonvolatile complexes were formed with copper. The thermal stability of the copper(II)-amine complexes varies with the amine. Aniline, butylamine, diethylamine, triethylamine, and cyclohexylamine were retained at temperatures in excess of 140°C. Pyridine was retained up to a temperature of 180°C, while dibutylamine was retained only at temperatures below 120°C.

The capacity of the abstractor packing was measured by injecting 2-µl aliquots of pyridine into the chromatograph until break-through was observed. A 12-in x 1/8-in column of the abstractor packing retained 30 µl of pyridine at 150°C. The internal volume of the column was 0.75 ml, which implies that 1 ml of the abstractor packing can retain 40 µl of pyridine at 150°C. The capacity of an abstractor

column can be increased by decreasing the operating temperature.

Thermal regeneration of the abstractor columns was investigated. Partial regeneration of the capacity was obtained by heating columns that had been saturated with pyridine. By passing nitrogen through the abstractor columns at 220°C for 1 hr, one-third of the initial capacity was regained. Rather than regenerate the abstractor columns thermally, the abstractor packing was replaced periodically.

The abstractor packing has no affinity for most organic compounds; thus, retention times for most compounds were not significantly affected by a short pre-column of the abstractor packing. The retention times of the compounds listed in Table 1 were not affected by the abstractor column. Of the compounds listed in Table 1, significant reduction of peak height was only observed with methanol. Reduction of the peak height for methanol indicates that some interaction occurs between methanol and the abstractor packing. Some reduction of peak height was observed for all compounds when using the abstractor column, because of peak broadening that occurs during the added column length.

<u>Applications of amine-abstractor columns</u>

Figures 1-4 demonstrate the usefulness of the abstractor columns for the analysis of nonamine compounds

Table 1. Compounds not affected by abstractor column

n-pentane	benzene
n-hexane	toluene
n-heptane	ethylbenzene
n-octane	isopropylbenzene
n-decane	*n*-butylbenzene
n-undecane	naphthalene
n-tridecane	hexene
methanol[a]	heptene
ethanol	octene
1-propanol	acetonitrile
1-butanol	acetaldehyde
1-pentanol	benzaldehyde
acetone	nitrobenzene
butanone	phenol
2-pentanone	*o*-cresol
3-methyl-2-pentanone	1,4-dioxane
dichloromethane	benzyl alcohol
chloroform	isopropyl ether
carbon tetrachloride	ethyl benzoate
1-bromobutane	diethyl ether
1,3-dichlorobenzene	ethyl acetate

[a]Retention time not affected, peak height decreased.

in an amine solvent. In each figure, one chromatogram is without the abstractor column and has a large peak for pyridine. The other chromatogram in each figure is with the amine abstractor column and has no peak for pyridine. By removing pyridine from the gas-chromatographic stream, peaks for components of the sample are not lost under a large solvent peak. Pyridine was selected as the solvent for samples, because it is commercially available in high purity from Burdick and Jackson Laboratories, Inc. In each figure, the peaks that are well-resolved from the solvent peak appear the same in both chromatograms, indicating that the nonamine compounds are not retained by the abstractor column.

Because only amines are removed from gas streams by the abstractor packing, use of the abstractor column makes it possible to analyze amines by gas chromatography for trace levels of nonamine impurities. Analysis of several brands of pyridine revealed that most had significant concentrations of nonamine compounds. Based upon retention times, benzene and toluene were identified in one brand of pyridine at concentrations of 50 and 160 ppm (mg/l), respectively.

Volatile components were extracted from coal with pyridine and analyzed by gas chromatography. Ground coal (100/120 mesh) was extracted with pyridine in a Soxhlet extractor for 12 hrs. Chromatograms for injections of an

Figure 1. Chromatograms with (a) and without (b) abstractor column for a separation of normal alkanes on a SE-30 column. Sample components are: (A) pentane, (B) hexane, (C) heptane, (D) octane, (E) nonane, and (F) dodecane. Temperature: initial temperature of 70°C, programmed to 90°C at 5°C/min, initial hold of 2 min and final hold of 2 min.

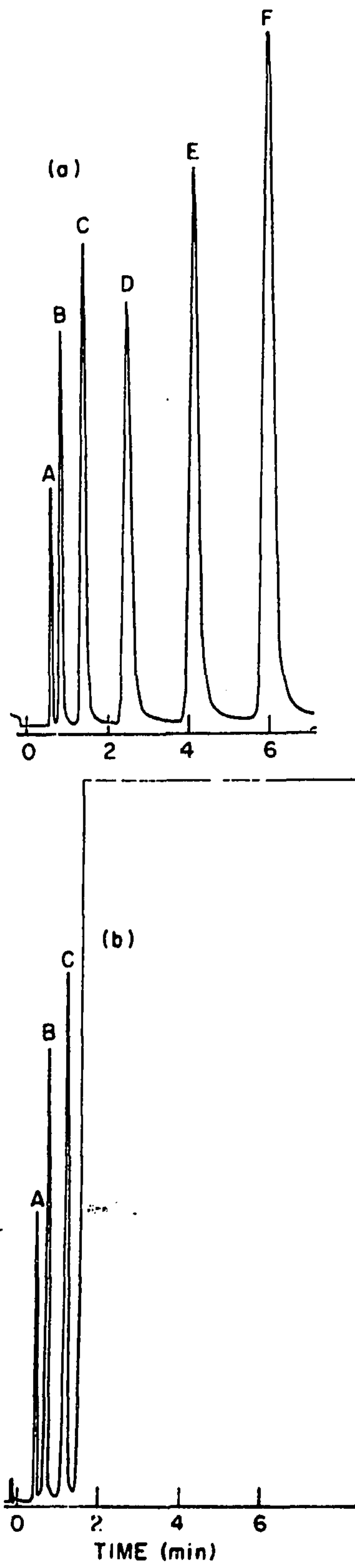
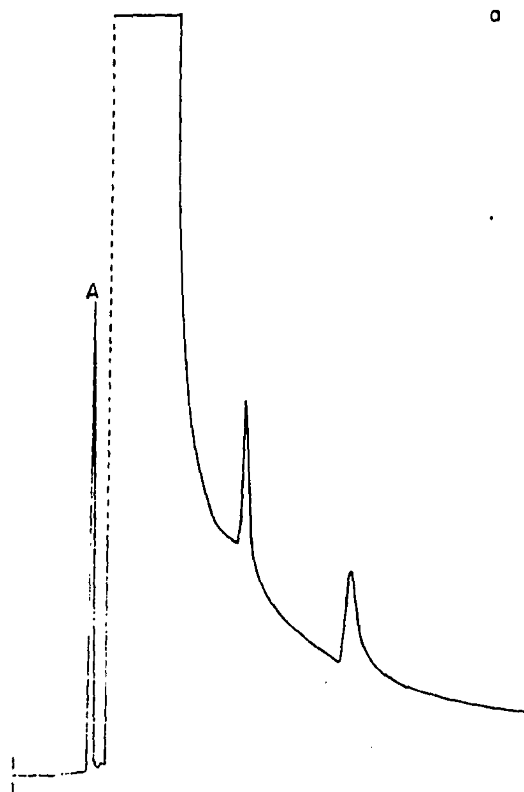

(a)
A
B
C
D
E
F
0
2
4
6
(b)
A
B
C
0
2
4
6
TIME (min)

Figure 2. Chromatograms with (b) and without (a) abstractor column for a separation of aromatic compounds on a SE-30 column. Sample components are: (A) benzene, (B) toluene, (C) ethylbenzene, (D) isopropylbenzene, (E) butylbenzene, and (F) naphthalene. Temperature: initial temperature of 120°C, programmed to 160°C at 16°C/min.


a
A

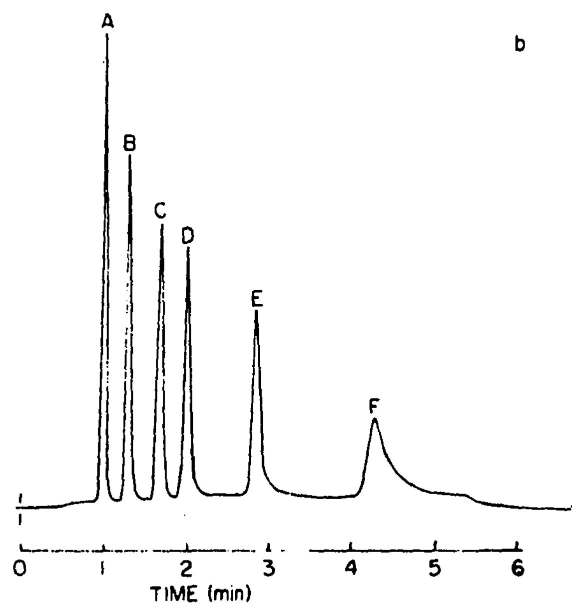
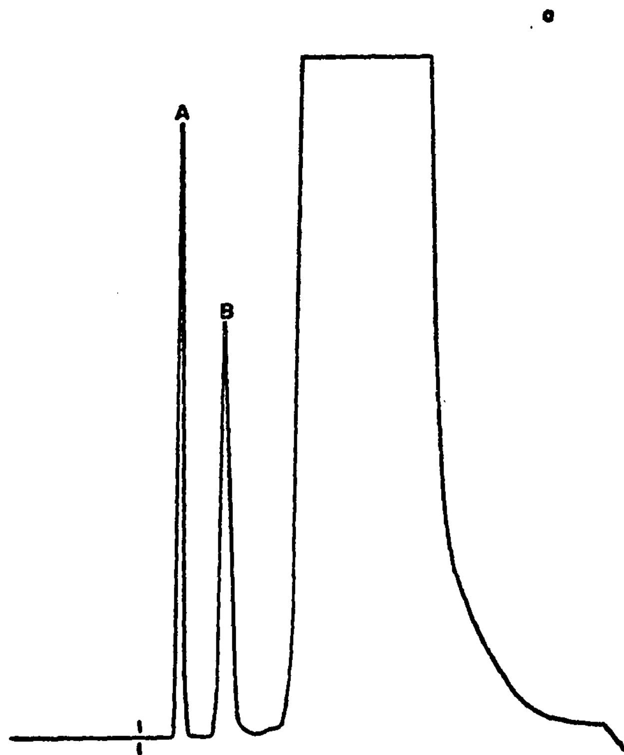

b
A
B
C
D
E
F
0
1
2
3
4
5
6
TIME (min)

Figure 3. Chromatograms with (b) and without (a) abstractor column for a separation of ketones on a XAD-2 column. Sample components are: (A) acetone, (B) butanone, (C) 2-pentanone, and (D) 4-methyl-2-pentanone. Temperature: Isothermal at 150°C.


a
A
B

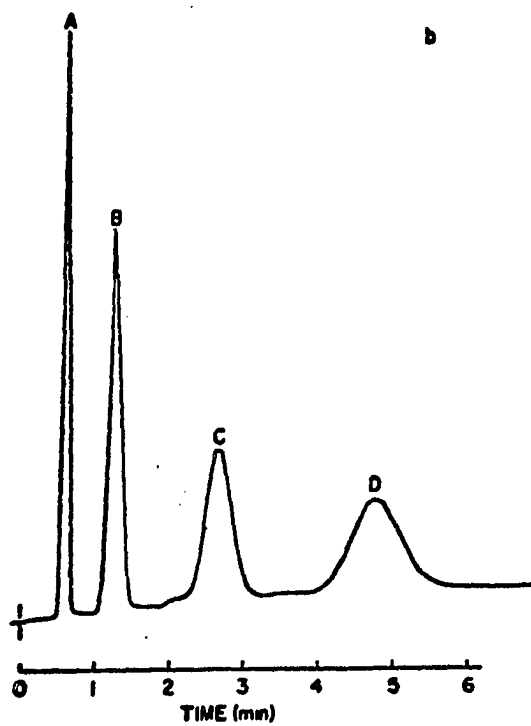
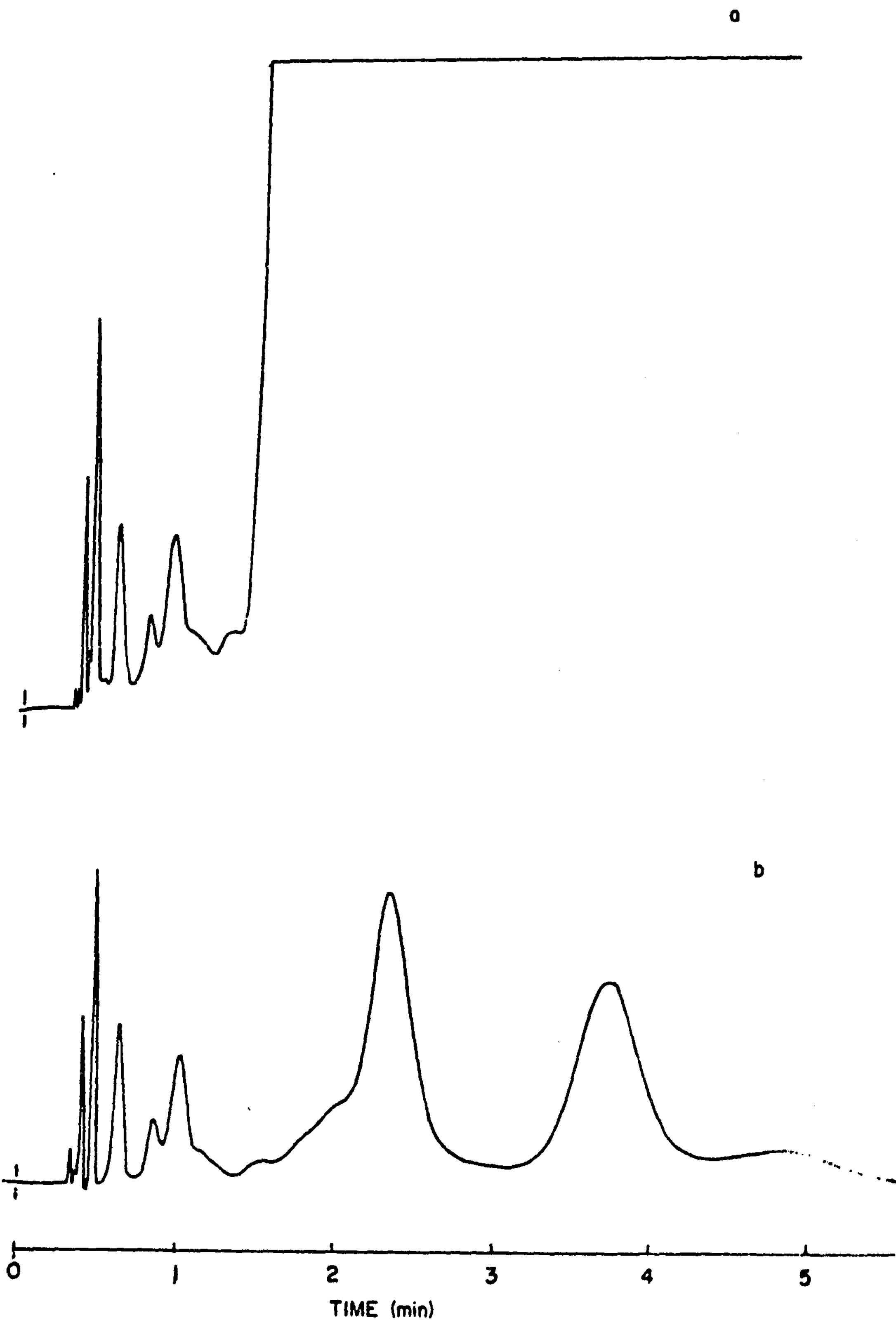

b
A
B
C
D
0
1
2
3
4
5
6
TIME (min)

Figure 4. Chromatograms with (b) and without (a) abstractor column for a separation of volatile components extracted from Iowa coal with pyridine on a SE-30 column. Temperature: Initial temperature of 120°C, programmed to 160°C at 32°C/min.


a
b
0
1
2
3
4
5
TIME (min)

Iowa-coal extract are in Figure 4. This application demonstrates the usefulness of the abstractor column when interferences caused by a solvent peak are to be avoided.

Organic compounds in synthetic-water samples were concentrated on Amberlite XAD-2 and analyzed by gas chromatography. A solution containing 100 ppb (µg/l) of benzene, toluene, ethylbenzene, isopropylbenzene, butylbenzene, and naphthalene in triple-distilled water was analyzed. A 100-ml aliquot of the synthetic-water sample was passed through a 15-cm x 6-mm o.d. (4-mm i.d.) column of XAD-2 by gas pressure. After removing excess water by passing gas through the resin bed, organic compounds were eluted with 2 ml of pyridine. A 10-µl aliquot of the pyridine extract was injected into a gas chromatograph containing a SE-30 column in series with an abstractor column. All compounds were quantitatively recovered from the synthetic-water sample. Small amounts of water in the pyridine did not interfere with the chromatographic determination. Slow elution of water from the abstractor column and the low response of the flame ionization detector for water are factors that caused water present in the pyridine sample to have little effect on the gas chromatograms. Organic compounds can be eluted from XAD-2 with a smaller volume of pyridine than diethyl ether. Because diethyl ether solutions can be reduced in volume by distillation without significant

losses of sample components, more sensitivity may be obtained when using diethyl ether to elute XAD-2 columns than when using pyridine.

Conclusions

Columns packed with copper(II) chloride coated on Chromosorb G AW/DMCS have been shown to be effective when used as a pre-column to remove amine solvents from gas-chromatographic streams. Chromatographic interferences caused by a solvent peak are eliminated by using the abstractor column and pyridine as the solvent for samples.

The abstractor columns could be used to gain information about the components of a sample or to simplify the chromatograms from complex mixtures. Techniques have been developed that use the selective abstraction of classes of compounds as a means of identifying sample components (15). Comparison of chromatograms with and without the amine abstractor would indicate which chromatographic peaks were caused by amines. It may be feasible to simplify the analysis of nonamine compounds in samples that have a large amount of amines. A possible application may be to screen biological fluids for a potentially harmful substance such as benzene.

The ability of the abstractor packing to remove amines quantitatively from a gas stream indicates that the

amine-abstractor column may be useful for concentrating amines. Chriswell used the abstractor packing to concentrate amines from water samples (23). Amines were purged from water samples with a flow of inert gas, then the amines were sorbed from the gas stream on the abstractor packing. Two techniques were used to quantify the amines sorbed on the amine packing. One technique of quantification was to measure the length of the band formed by the copper(II)-amine complex. Alternatively, amines were eluted from the abstractor column with an aqueous potassium-hydroxide solution and analyzed by gas chromatography.

DETERMINATION OF HALOFORMS IN WATER

Review of Related Work

In 1974 the presence of chloroform and other volatile halogenated hydrocarbons in drinking-water samples was reported (24). In 1975 a United States Environmental Protection Agency (USEPA) study of 79 cities indicated that chloroform and related halogenated hydrocarbons occur in virtually all chlorinated-drinking waters (25). The concentrations of halogenated hydrocarbons in drinking waters ranged from less than 1 ppb (µg/l) to greater than 300 ppb. The amount of haloforms in drinking water is a matter of concern because recent information indicates that chloroform may be carcinogenic (26).

Rook suggested that chloroform and other volatile halogenated hydrocarbons are produced in drinking water by the reaction of chlorine with humic matter (27,28). For purposes of this discussion, chlorine will refer to all oxidizing forms of chlorine present in water, including: chlorine, hypochlorous acid, hypochlorite ion, and chloramines. Rook investigated the production of haloforms by chlorinating humic matter from various sources. Experiments were also performed on chlorinating model compounds. The results of the experiments suggest chlorine reacts with the polyhydroxybenzene building blocks of humic matter to

yield chloroform. Bromide present in chlorinated-water samples was correlated with the presence of brominated haloforms.

Analysis of water for volatile halogenated hydrocarbons has most frequently been done by "purge and trap" techniques (17,29,30). Essentially, volatile compounds are purged from water samples with a gas stream, then sorbed from the gas stream onto a solid sorbent such as Tenax GC. By passing nitrogen, the heated sorbent compounds are eluted from the sorbent and introduced into a gas chromatograph. The gas-chromatographic separation is usually monitored with a special type of conductivity detector. The Hall detector is an electrolytic-conductivity detector that is quite specific for halogenated compounds (31).

Sometimes a water sample is heated to speed up stripping of volatile compounds. Kopfler _et al_. showed that heating to 95°C increased substantially the amount of haloforms obtained from chlorinated-water samples (29). The increased temperature increases the reaction rate of residual chlorine with organic matter. Using direct injections of water samples, Nickolson and Meresz observed similar results (32). By directly injecting chlorinated-water samples into the hot injection port of a gas chromatograph, higher haloform concentrations were obtained than by the "purge and trap" technique.

Rook partitioned haloforms between the aqueous phase and a gas phase, then analyzed an aliquot of the gas phase to determine haloforms in water samples (27,28,33). Mieure (34) and Richard and Junk (35) have used solvent extraction to analyze water samples for haloforms. Although haloforms are not extracted from water quantitatively into a gas or organic phase, the efficiency of extraction can be determined and greater sensitivity obtained than by direct aqueous injection.

This research effort had two objectives. One was the development of a new method for measuring the concentrations of haloforms in drinking waters. The second objective was to investigate the reactions that produce haloforms in chlorinated-drinking waters. Once the parameters affecting production of haloforms are understood, methods of handling samples can be implemented which will ensure that correct values are obtained for haloform concentrations.

Experimental

Apparatus and reagents

Gas chromatograph   A Tracor model 550 gas chromatograph equipped with a linear temperature programmer, heated injection ports, and an electron capture detector (ECD) was used to determine haloforms in water extracts. The ECD was equipped with an electronic linearizer and nickel-63 as the

ionization source. Chromatograms were recorded on a Houston Instruments series 5000 strip chart recorder.

Water-sampling apparatus The apparatus that was fabricated to pass water samples through small resin beds is shown in Figure 5. Connection of the sorption column was accomplished with glass connectors from Altex Scientific, Inc., Berkeley, California. The apparatus was pressure tested at 45 psig.

Sorption columns Glass columns, 10-cm x 4-mm i.d. (2-mm i.d.), with Altex glass connectors on one end, were packed with acetyl XAD-2 (100/120 mesh). Before using the columns, the columns were conditioned by passing 20 ml of pyridine through the column with a syringe.

Gas-chromatographic column A 6-ft x 1/4-in o.d. glass column was used in the gas chromatograph to separate samples containing haloforms. The 7-in portion of the column that fit into the injection port of the Tracor model 550 gas chromatograph was packed with 25% copper(II) chloride coated on 100/120-mesh Chromosorb G AW/DMCS (19) and the remainder of the column was packed with 10% FFAP on 80/100-mesh Chromosorb W AW. Chromosorb G AW/DMCS and the FFAP coated phase were obtained from Applied Science Laboratories, Inc., State College, Pennsylvania.

Water Volatile organic compounds were purged from laboratory-distilled water with helium. A helium flow of

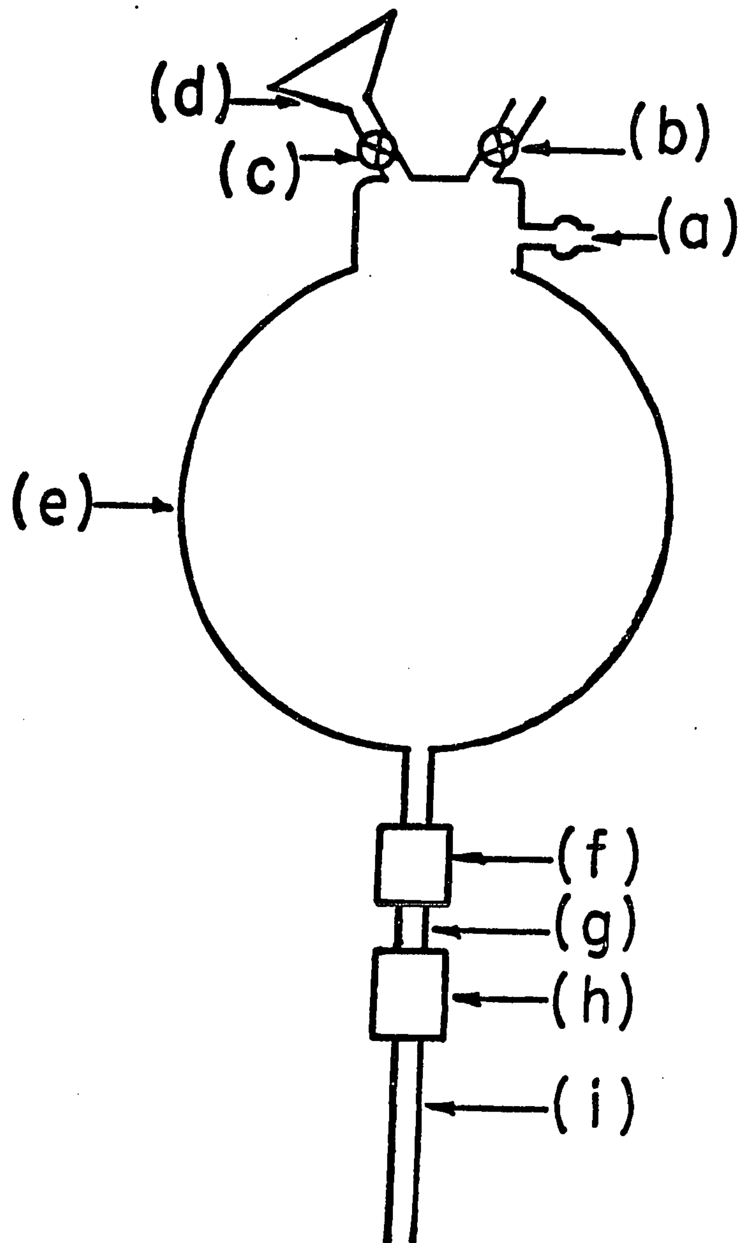


Figure 5. Apparatus for water sampling: (a) inlet for helium; (b) valve for gas venting; (c) valve for filling; (d) glass funnel; (e) 1-l glass reservoir; (f) Altex glass connector; (g) Kel-F connector; (h) Altex glass connector; (i) 2-mm i.d. glass column.

90 ml/min was passed through 2 ℓ of water with a gas dispersion tip. Fresh water was prepared daily to ensure a low blank.

Reagents   Pyridine, distilled-in-glass grade and stored under nitrogen, was obtained from Burdick and Jackson Laboratories, Muskegon, Michigan. The pyridine was stored until used in an automatic filling buret with a positive pressure of nitrogen.

L-Ascorbic acid and chloroform were obtained from Fisher Scientific Company, Fair Lawn, New Jersey. Bromodichloromethane and technical-grade humic acid were purchased from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. Dibromochloromethane was obtained from Columbia Organic Chemicals Company, Inc., Columbia, South Carolina and bromoform was purchased from Eastman Organic Chemicals, Rochester, New York.

Chlorine gas from Matheson Gas Products, East Rutherford, New Jersey, was used to make aqueous chlorine solutions.

Amberlite XAD-2 was obtained from Rohm and Haas, Philadelphia, Pennsylvania.

Other materials and chemicals were the grade available within the laboratory.

Techniques and procedures

Preparation of acetyl resin Amberlite XAD-2 was cleaned by Soxhlet extraction with methanol for 24 hrs. The cleaned resin was dried, ground, and sieved. The 100/120-mesh fraction was collected by dry sieving. Acetylation of the resin was performed under mild conditions using a method similar to that used by Moyers (36). To 4 g of XAD-2, 30 g of anhydrous aluminum trichloride, and 30 ml of petroleum ether, 5 ml of acetic anhydride was added slowly with stirring. The reaction mixture was heated to 50-60°C and stirred for 3 hrs. After allowing the reaction mixture to cool, the mixture was poured into a beaker containing hydrochloric acid and ice. The resin was filtered off and washed several times with concentrated hydrochloric acid and ammonium hydroxide.

Sample collection Glass bottles, 300 ml, with standard-tapered necks were filled to overflowing with as little aeration as possible. Standard-tapered stoppers were slowly inserted into the filled bottles to ensure that no air was trapped in the bottles. For chlorinated-water samples, 2 mg of L-ascorbic acid was placed in the bottles before taking the samples.

Analytical procedure for determining haloforms in water Water samples, usually 100 ml, were passed through the sorption column using the sampling apparatus shown in Figure

5. Helium pressure was adjusted to give a flow rate of 5 to 10 ml/min. After passing the water through the resin bed, approximately two liters of helium was passed through the column to remove excess water.

After removing the column from the sampling apparatus and connecting a luer adaptor to it, haloforms were eluted from the column with 1 ml of pyridine. A few microliters of the pyridine solution were analyzed for haloforms by gas chromatography with electron capture detection. While maintaining the injection-port temperature at 140°C and the column-oven temperature at 90°C, haloforms were separated on a column with FFAP as the stationary phase. A flow of 10% methane in argon was maintained at approximately 60 ml/min to affect the separation. Haloforms from water samples were quantified by comparison of peak heights. Peak heights for haloforms from water samples were compared to peak heights for haloforms from standard samples.

<u>Chlorine solution</u> Chlorine gas was slowly bubbled through triple-distilled water for 5 min. The chlorine solution was neutralized by adding sodium hydroxide. After allowing the chlorine solution to stand for 24 hrs, the chlorine in the solution was measured colorimetrically with N,N-diethyl-p-phenylenediamine (DPD). Chlorine quantitatively reacts with DPD to produce a color that can be

measured at 515 nm (37,38). Bromine solutions were also analyzed using DPD.

Results and Discussion

Development of analytical technique

Copper(II) chloride coated on Chromosorb G AW/DMCS was packed into the first portion of the gas-chromatographic column to remove pyridine from the gas stream (19). Because haloforms are volatile and elute quickly from most gas-chromatographic columns, removal of pyridine from the gas-chromatographic stream eliminates interferences caused by large solvent peaks. When the abstractor packing is used to remove pyridine, resolution of the solvent from the components of a sample is not important. After the pyridine is removed from the gas-chromatographic stream, any gas-chromatographic column that separates the haloforms from each other will provide the desired separation.

Pyridine is an effective solvent for eluting haloforms from the resin bed. Haloforms were completely eluted from the sorption columns with 0.5 ml of pyridine. Because haloforms were eluted from the resin bed with a small volume of pyridine, the concentrations of haloforms in the pyridine solution was much greater than the concentrations present in the water sample. To ensure that recovery of the haloforms

from the sorption columns was complete, 1.0 ml of pyridine was used to elute the sorption columns.

When the injection port was maintained at 140°C, the abstractor packing would retain more than 60 µl of pyridine. Before the capacity of the abstractor packing was exceeded, the first section of the gas-chromatographic column was replaced with fresh abstractor packing.

Although ordinary XAD-2 resin could be used to recover haloforms from water samples, better recoveries were obtained using the acetyl resin. Low recoveries for haloforms using XAD-2 were confirmed by Chang (16). Using water samples containing 0.5 ppb of each haloform, Chang observed 56, 68, 80, and 98% recoveries from chloroform, bromodichloromethane, dibromochloromethane, and bromoform, respectively. A potassium-bromide pellet was made of the acetyl resin, then the pellet was analyzed by infrared spectroscopy. Only a small band for a carbonyl group was present at 1685 cm^{-1} on the infrared spectrum of the pellet which indicates only a few acetyl groups were introduced to the resin. The low yield of acetyl groups and the lack of an apparent mechanism for haloforms to interact with the acetyl groups indicate that the increased recoveries were a result of changes in the surface or pores of the resin. Incomplete recoveries of the haloforms were also observed with XAD-4, XAD-7, XAD-8. amd XAD-11 resins.

Stock solutions of haloforms were made with methanol as the solvent. A few microliters of the stock solutions were added to triple-distilled water to produce dilute aqueous solutions of the haloforms. At concentrations between 100 and 0.1 ppb, haloforms were essentially quantitatively recovered from 100 ml of water. Ultimate detection limits for haloforms can be reduced to below 0.1 ppb by altering the gas-chromatographic conditions or using a larger water sample. Using a column-oven temperature of 90°C, the limit of detection for chloroform was imposed by the presence of an impurity peak from the pyridine.

The amounts of haloforms found in water samples from various central-Iowa cities are shown in Table 2. The data in Table 2 indicate that concentrations of haloforms in various water supplies are very different. A typical chromatogram for an extract of a water sample is shown in Figure 6. Water from a tap in the laboratory was collected and analyzed on several days in January 1976. The data in Table 3 demonstrate the daily variations of haloform concentrations in water samples from a single source. Water source and sampling time are two variables that affect the haloform concentrations. In general, the range of haloform concentrations in water samples from a single source was small compared to range of haloform concentrations from water samples from various sources.

Table 2. Concentration of haloforms (in ppb) in central-Iowa cities on January 15, 1976

City	$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
Huxley	28.3	7.1	11.0	1.0
Slater	16.0	4.8	4.2	---
Ankeny	9.7	1.2	3.2	---
Clive	6.8	0.1	---	---
Des Moines	22.5	---	---	---
Altoona	10.9	---	---	---
Marshalltown	7.0	---	---	---
Nevada	13.8	---	---	---

Table 3. Concentration of haloforms (in ppb) in laboratory tap water

Date	$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
1/6/76	19.6	1.2	1.7	---
1/7/76	13.1	4.0	7.1	1.4
1/8/76	12.3	5.5	8.6	2.3
1/12/76	20.3	5.9	9.1	1.4
1/13/76	13.8	4.8	8.6	1.4

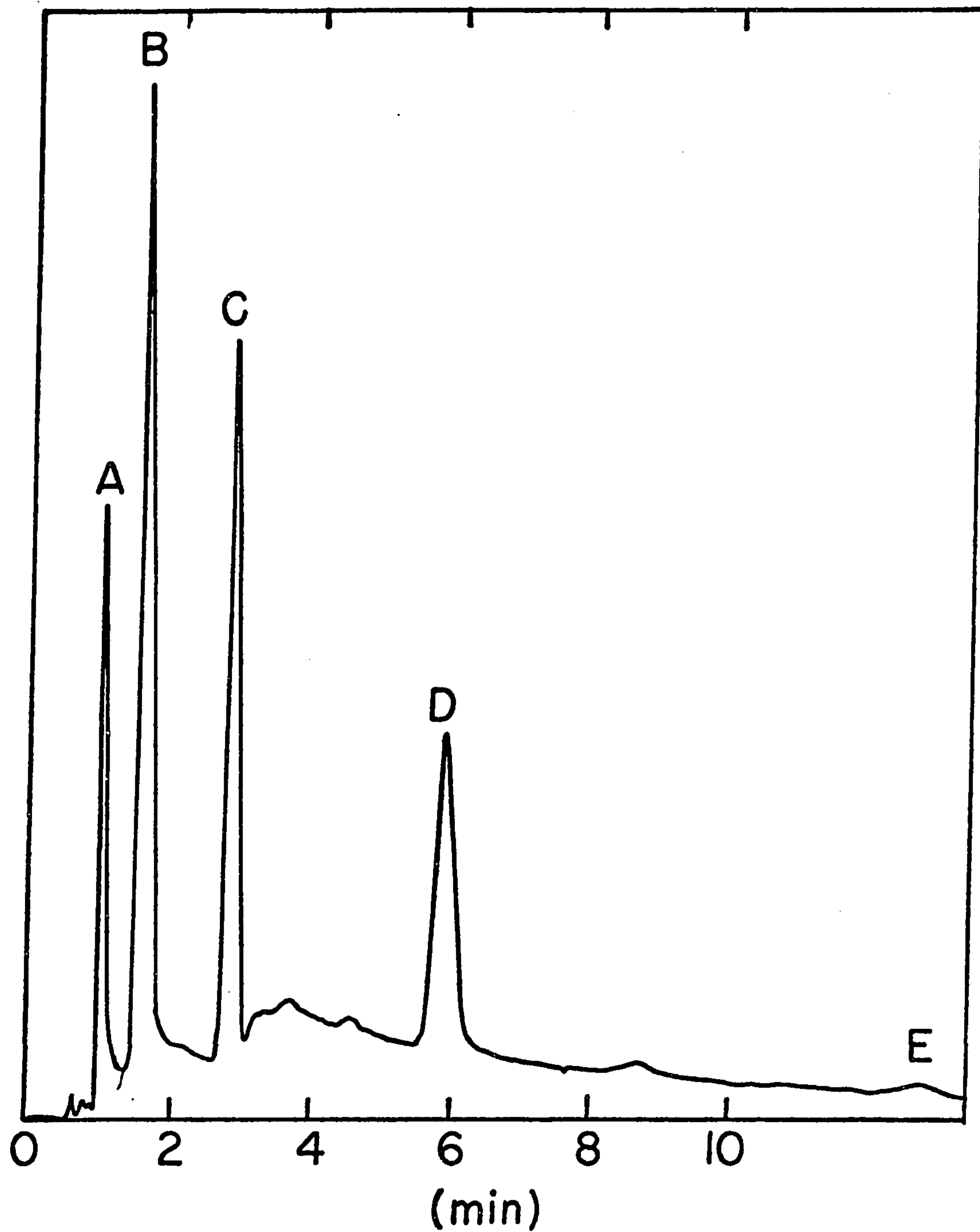


Figure 6. Chromatogram of haloforms from drinking water distributed in Huxley, Iowa. Sample components are: (A) solvent impurity; (B) chloroform (21 ppb); (C) bromodichloromethane (7 ppb); (D) dibromochloromethane (11 ppb); and (E) bromoform (∿1 ppb).

Storage of water samples

Because the haloforms are only slightly soluble in water and are quite volatile, haloforms in water samples can be lost to the gas phase. A study by the USEPA in 1975 considered this point and noted some losses for water samples stored 20 to 30 days (29). Results in Table 4 show a substantial increase in haloform concentrations when chlorinated-water samples were stored. Because the concentrations of haloforms in water samples may change during storage, concentrations of haloforms in stored samples may not reflect the concentrations of haloforms in the samples at the time the samples were collected.

Addition of a small amount of ascorbic acid to a water sample reduces all chlorine present in a water sample. Data in Tables 4 and 5 show that the concentrations of haloforms in water samples can be retained for long periods of time. Removing residual chlorine from a water sample ensures that no haloforms are produced in the sample during storage. Storage of water samples in bottles without a head space and with ascorbic acid ensures that the haloform concentrations are preserved. Changes in haloform concentrations resulting from volatilization and production are eliminated when proper conditions for storage of water samples are used.

Table 4. Concentrations of haloforms in laboratory tap water during storage

Compound	24 hr	60 hr	24 hr ascorbic	60 hr ascorbic
	% recovery (0 hr = 100%)			
$CHCl_3$	111	133	95	95
$CHBrCl_2$	148	156	96	93
$CHBr_2Cl$	160	170	100	90
$CHBr_3$	Present	Present	Absent	Absent

Table 5. Concentrations of haloforms in selected drinking waters during storage with ascorbic acid

Source	Days stored	% Recovery (0 hr = 100%)			
		$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
Slater, Ia	2	94	100	100	100
	4	94	100	100	100
	6	92	90	100	75
Huxley, Ia	2	100	99	94	100
	4	97	100	94	100
	6	95	99	94	100
ISU	2	100	97	100	100
	4	91	92	100	100

A detailed study was performed in which water samples from an Iowa State University tap were analyzed for each of the four haloforms. The samples were carefully stored without head space and in the dark. Results of daily analysis of the water samples are in Figure 7. The chloroform concentration reached a maximum at three days, then the chloroform concentration began to decrease. The concentrations of the two bromo-chloro compounds increased and gradually leveled off, while the concentration of bromoform increased substantially over the entire storage time. Figure 8 shows that the total-molar concentration of the haloforms initially increased, then gradually decreased. Water samples from two other central-Iowa cities showed somewhat similar changes, but it should not be concluded that all drinking waters will behave similarly.

The increase of total-haloform concentration with time suggests that the water samples contained organic matter that reacted with residual chlorine or bromine to produce additional haloforms. The decrease in chloroform concentration after 3 or 4 days together with the increase in concentration of chloro-bromo compounds and bromoform suggests that an exchange reaction may be taking place in which chloroform is converted to brominated haloforms.

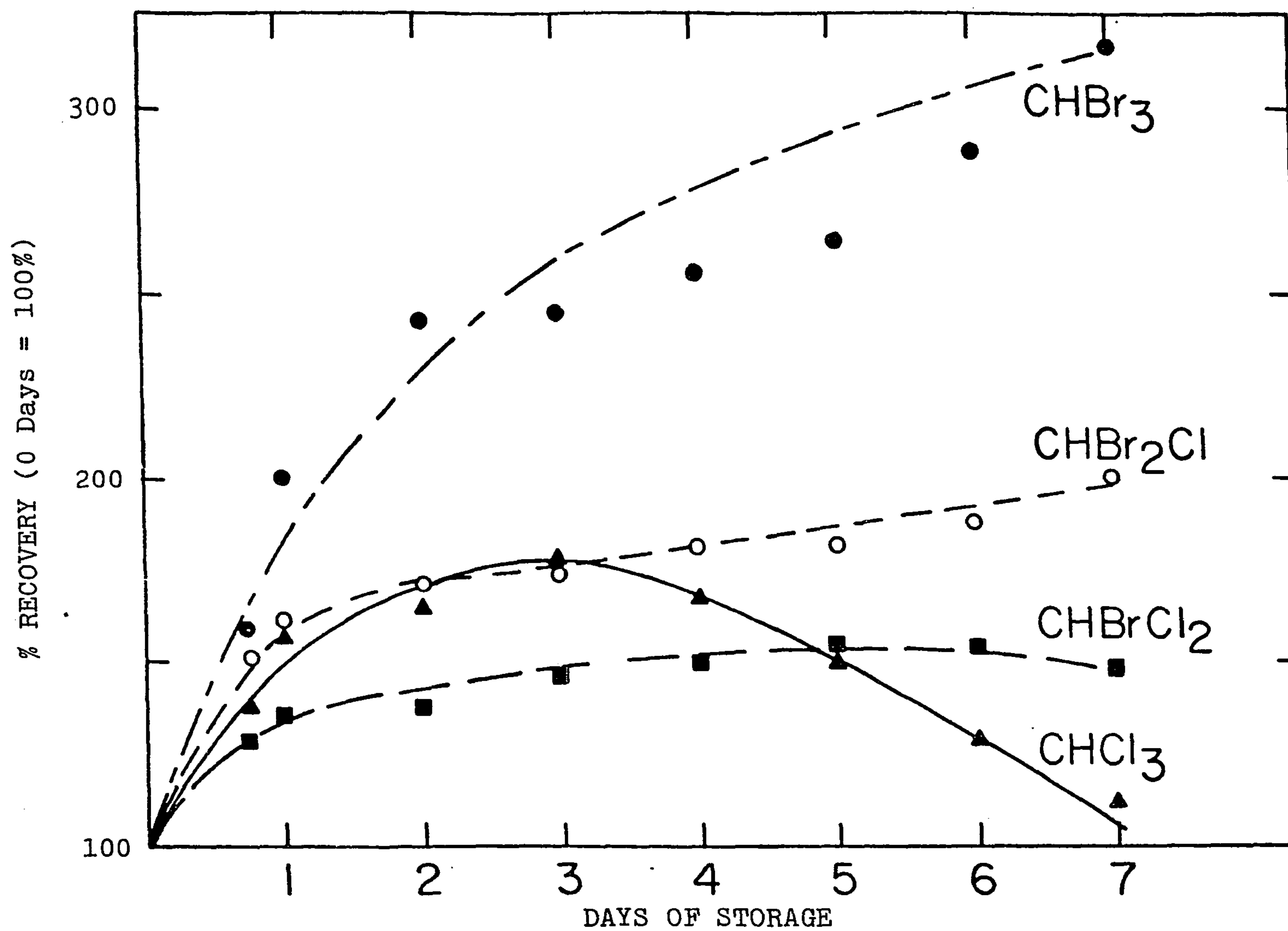


Figure 7. Changes in haloform concentrations on storage

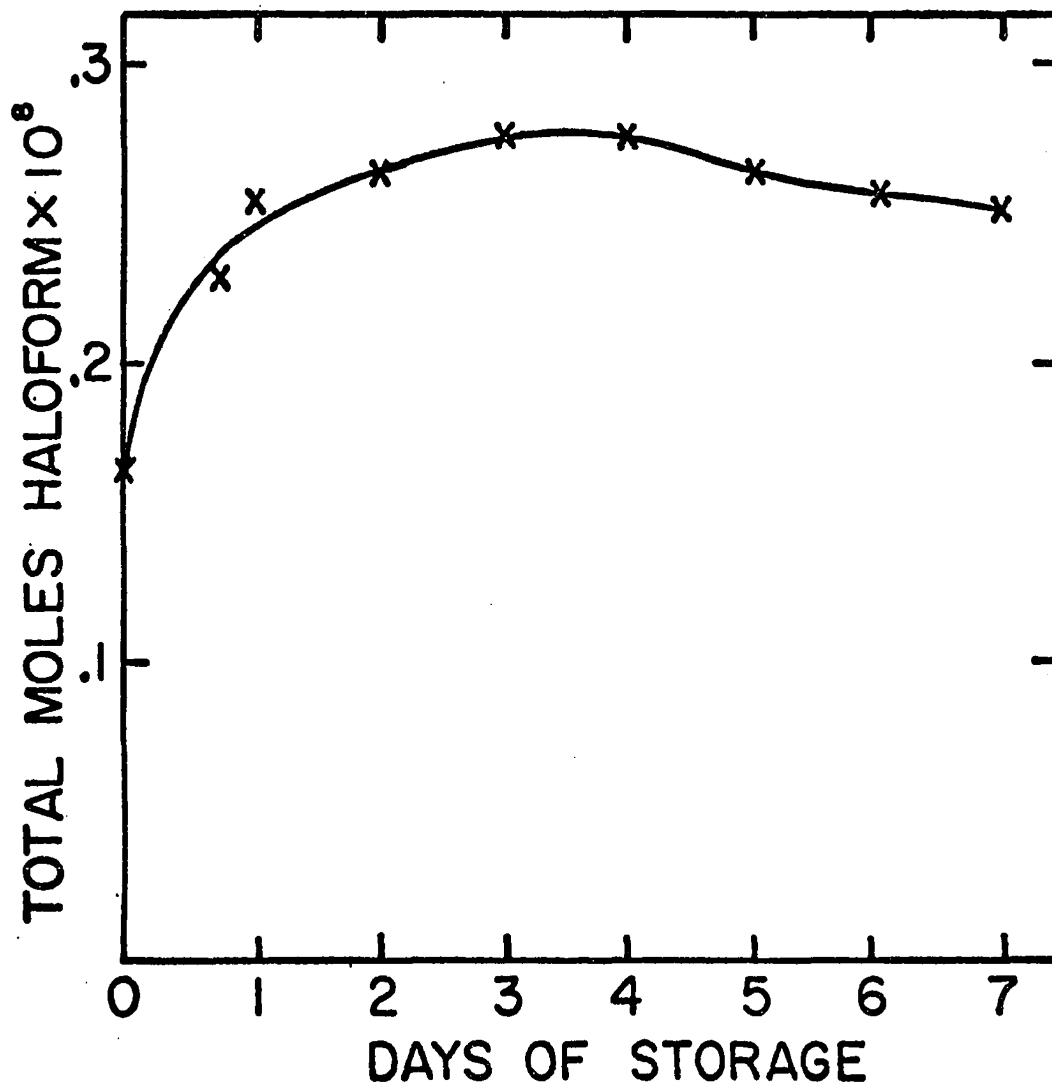


Figure 8. Change in total moles of haloforms per liter on storage

Experiments on haloform production and exchange reactions

Several experiments were performed in which triple-distilled water containing chloride, bromide, and/or humic acid approximating levels found in drinking waters were treated with chlorine or bromine. The results of these experiments are shown in Table 6. Water containing humic acid and bromide reacted with chlorine to produce all four haloforms. Addition of bromine to water samples containing humic acid and chloride resulted in the production of only bromoform. Since it is more difficult to oxidize chloride than bromide, these results indicate that the oxidized form of the halogen is involved in the haloform production reaction.

Halogen-exchange reactions are indicated by experiments 4 and 8 of Table 6. Although exchange of chlorine for bromine was observed in the presence of chlorine, experiments 5 and 9 demonstrate that production of brominated haloforms by an exchange reaction does not occur if only bromine is present. Experiment 6 shows that bromide ion alone is not capable of exchanging with the chlorine atoms of chloroform.

Table 6. Experiments on haloform production and exchange reactions

Exp. No.	Reactions[a]	$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
1	HA, Br^-, Cl_2	+	+	+	+
2	HA, Br_2				+
3	HA, Br_2, Cl^-				+
4	$CHCl_3$, Br^-, Cl_2	+	+	+	+
5	$CHCl_3$, Br_2	+			
6	$CHCl_3$, Br^-	+			
7	$CHCl_3$, Cl_2	+			
8	$CHBrCl_2$, Br^-, Cl_2		+	+	+
9	$CHBrCl_2$, Br_2		+		

[a]Concentration of reactants: Cl_2, Br_2, and HA (humic acid) - 5 ppm; Br^- - 2 ppm; Cl^- - 50 ppm; $CHCl_3$ and $CHBrCl_2$ - 5 ppb. Reaction time - 4 days.

Conclusions

Trace concentrations of haloforms can be quantitatively removed from water and determined by the described method. Addition of a reducing agent, such as ascorbic acid, to water samples stops the production of haloforms, allowing the instantaneous concentrations of haloforms in water samples to be measured accurately. Results obtained from analysis of water samples that have not been properly stored may be in error.

Because the reactants are present in water distributed by a water utility, water used by consumers may have greater concentrations of haloforms than the water leaving the utility. Daily and seasonal variations of haloform concentrations in a water supply are expected, because of changes in water temperature, humic content, chlorine dose, and chlorine-contact time. Because consumption of haloforms could be harmful to human health, more must be learned about the patterns of concentration changes that occur from the time chlorinated water enters the distribution system until it is used by the consumer. The harmful effects of haloforms must be evaluated versus the beneficial effects of chlorination. Chlorination of drinking water has been extremely effective for control of water-borne diseases. Investigations of water treatment practices should provide

for treatment techniques that produce a disinfected water with lower concentrations of haloforms.

CAPILLARY-COLUMN SYSTEM FOR GAS CHROMATOGRAPHY

Review of Related Work

The use of wall-coated open tubular (WCOT) columns for gas chromatography was first reported by M. J. E. Golay in 1957 (39). Golay developed the method of preparing small-bore (0.01 to 0.03 inch) columns with the inner surface coated with a stationary phase. Although WCOT columns were developed many years ago, only recently has the use of capillary columns become widespread. The following are reasons for the slow acceptance of capillary-column gas chromatography: poor quality of commercially available columns, difficulty of preparing WCOT columns, the small sample capacity of capillary columns, and problems related to connection of small columns to inlets and exits of common gas chromatographs (40). Because many of the problems related to capillary-column gas chromatography have been overcome and the high efficiency offered by capillary columns, many laboratories have turned to capillary columns to analyze complex mixtures.

Since the work of Golay, many researchers have worked in the area of preparing stable, efficient, and inert capillary columns for gas chromatography. Many researchers have worked in the area of preparing WCOT columns from small-bore metal tubing (41,42). Because

metal tubing has good mechanical strength and can easily be bent, many researchers felt that metal tubing was ideal for preparation of capillary columns. However, interactions between sample components and the hot metal surfaces of the column caused peaks for some compounds to be tailed. Although small-bore glass tubing has much less mechanical strength then metal tubing, most WCOT columns are presently prepared from glass tubing.

In order to prepare satisfactory WCOT columns from glass tubing, methods of treating the inner surface of the tubing were developed. Hydrochloric acid is usually used to etch the inner surface of glass tubing to increase its surface area (43,44). Because the amount of liquid phase in a WCOT column is small, methods to increase the surface area of the glass tubing have been investigated. Sodium-chloride crystals have been deposited (45,46) and silica whiskers have been formed (47,48) on the inner surface of the glass tubing to increase the surface areas of glass columns. To improve the capacity of capillary columns, some researchers have produced porous-layer open tubular (PLOT) and support-coated open tubular (SCOT) columns. The inner surface of a PLOT column is very porous and is coated with a film of stationary phase (49). A SCOT column is produced by coating the walls of a column with a slurry of finely divided packing material from a solution of the stationary phase (50).

Because the surface areas of SCOT and PLOT columns are greater than WCOT columns, SCOT and PLOT columns have greater sample capacities than WCOT columns. In general, PLOT and SCOT columns have fewer theoretical plates than WCOT columns.

The problems caused by reactivity of glass surfaces and poor adhesion of stationary phases to glass have been approached in numerous ways. Hydroxyl sites on the surfaces of glass columns cause peaks for many compounds to be tailed. Silanization of the glass with trimethylchlorosilane, dimethyldichlorosilane, or hexamethyldisilazane will remove the hydroxyl sites from the surface of glass (22). An added advantage of silanization is that the polar character of the glass surface is reduced, which increases the adhesion of most stationary phases.

The small capacity of a capillary column was considered to be a problem when applying capillary columns to trace analysis. In order to keep the amount of material injected into a capillary column small enough to avoid overloading the column, splitters were designed to vent most of the sample to the atmosphere. When large split ratios are used, the detection limit with capillary columns is not as low as can be obtained with packed columns. Grob developed a technique that overcomes the problems associated with the application of capillary columns to trace analysis. The

technique is commonly referred to as Grob splitless or splitless injection. Injection of samples onto a capillary column is done through an injection port without venting any of the carrier-gas flow (51,52). After a short interval of time, the inlet is backflushed with a flow of carrier gas to remove the last traces of the injected sample.

Grob showed that by proper selection of the solvent for injection of samples onto a column, the efficiency of a chromatographic system will be greater with a splitless injection than with a split injection (52). The apparent efficiency of a capillary column may be greater when splitless injection is used, because sample components are concentrated on the column in a plug of solvent. The solvent plug forms over the first few plates of the column. Sample components start to move through the column after the solvent plug is totally volatilized. To eliminate broadening of peaks because of nonplug injection, the injection port is backflushed with carrier gas before the solvent plug evaporates. Although the column capacity is exceeded by the solvent, the sample components are retained in the solvent plug until the overload condition no longer exists. The technique cannot be used if the amount of any sample component will exceed the capacity of the column. Grob has further developed the technique of splitless injection for on-column sample introduction (53). By introducing a sample

directly onto a column, problems caused by volatilization of the sample are avoided.

The specificity and the sensitivity of detectors used for gas chromatography are different; thus, chromatograms with more than one detector are often obtained for a sample. Rather than perform a separation twice, various types of exit splitters have been developed. The objective of an exit splitter is to simultaneously introduce a fraction of the column effluent to two or more detectors. In order to retain the separation that has been achieved on a capillary column, the void volume of the exit splitter must be kept to a minimum and the linear-flow rate in all parts of the exit splitter must be greater than in the chromatographic column. Because of the mechanical strength of metal, exit splitters are often fabricated from metal. Oak *et al*. described an exit splitter fabricated from various lengths of capillary-bore, 1/16-in o.d., stainless-steel tubing connected to a column with a tee (54). By changing the lengths of tubing used for connecting the column to the detectors, the ratio of the amount of column effluent going to each detector was varied. Etweiler and Nauner-Jahle used an exit splitter fabricated from platinum capillaries to split the effluent from a capillary column to an electron capture detector (ECD) and a flame ionization detector (FID) (55). Secondary pressures of carrier gas were applied to each platinum

capillary to vary the exit-split ratio. A major disadvantage of using metal for an exit splitter is that the metal surfaces of the exit splitter may cause tailing or loss of some compounds eluted from the column.

Experimental

Apparatus

Gas chromatograph   A Tracor model 550 gas chromatograph equipped with a dual flame ionization detector, linearized electron capture detector, heated injection ports, and temperature programmer was modified for operation with capillary columns. Figure 9 shows a flow diagram of the system used with the gas chromatograph to allow the use of capillary columns.

Columns   WCOT columns made from glass were purchased from J & W Scientific Company, Inc., Orangevale, California. The columns had an internal diameter of about 0.25 mm, an external diameter between 0.8 and 1.1 mm, and were purchased in 30-m lengths. The columns were guaranteed to have in excess of 50,000 effective theoretical plates (N_{EFF}) as calculated from the equation below.

$$N_{EFF} = 5.54\left(\frac{t'_R}{PWHH}\right)^2$$

t'_R = Adjusted Retention Time = (Retention Time) - (Retention Time for Non-Retained Substance)

PWHH = Peak Width at Half Height

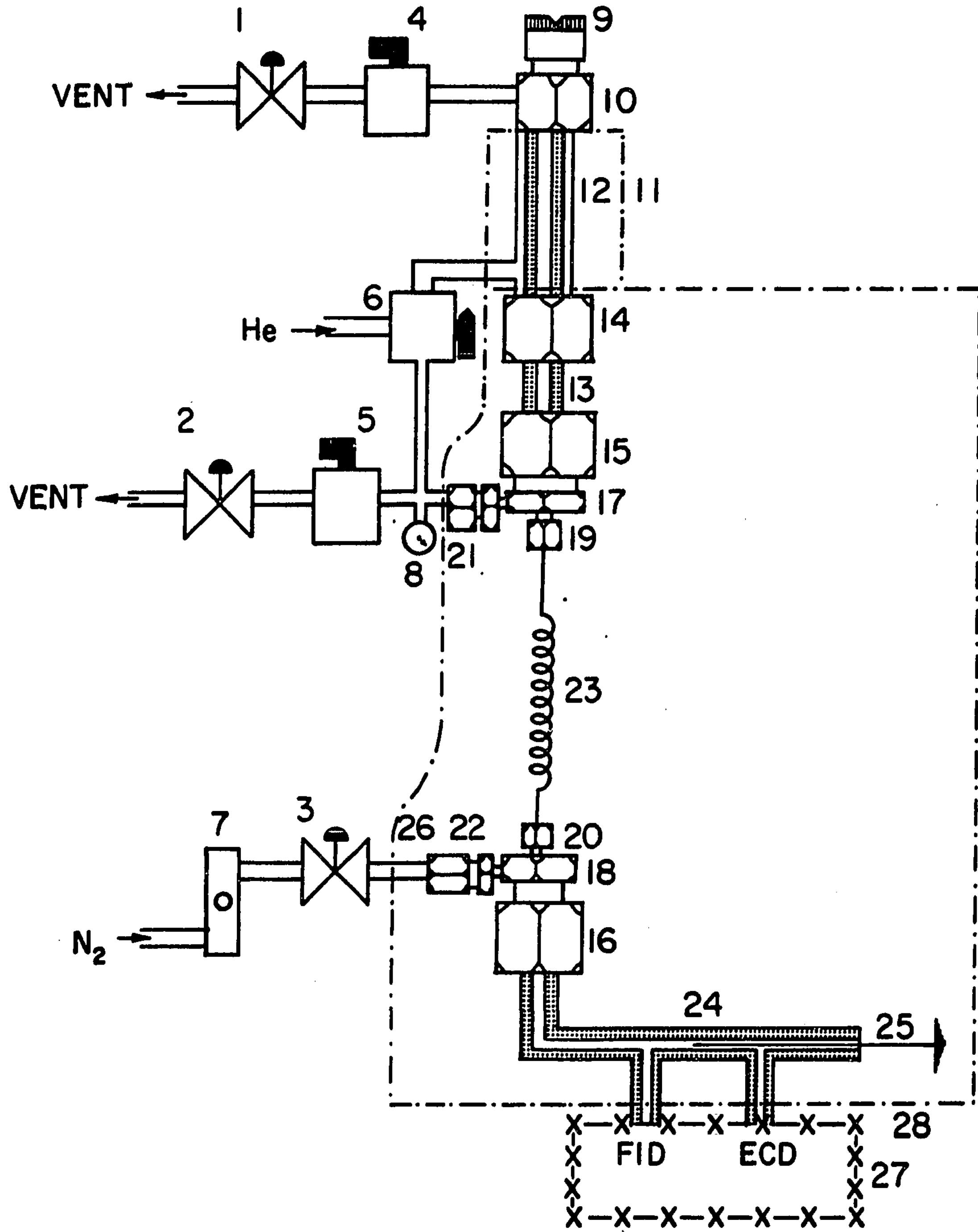
The columns were supported in the chromatographic oven by a 1/8-in stainless-steel rod. Brackets were placed by the injection port and detector ports to secure the rod.

Inlet system The inlet system for the Tracor 550 GC used with capillary columns was similar to the inlet system designed by German and Horning (56). Modifications were made in the inlet system to monitor the head pressure on the capillary column and to allow sample introduction by the splitless-injection technique. Details of the inlet system are shown in the top portion of Figure 9. Capillary glass tubing, 6-mm o.d., 1-mm i.d., was used in the injection port to protect the sample from hot-metal surfaces and to reduce the void volume in the inlet. If the capillary column had an external diameter greater than 1 mm, a special piece of glass was used to sleeve the injection port. This sleeve was a 1½-in segment of 2-mm i.d., 6-mm o.d. tubing butted to a 6½-in segment of 1-mm i.d., 6-mm o.d. tubing. The capillary columns were connected to the 2-mm i.d. end of this injection-port sleeve. To remove active sites from the surfaces of the glass sleeves, the sleeves were submerged in five-percent solutions of hexamethyldisilazane in toluene overnight.

The tee that was used to connect the injection-port sleeve, the split-exit line, and the capillary column was fabricated from a 1/16-in to 1/4-in Swagelok Reducing Union and a 1/16-in to 1/8-in Swagelok Reducing Union. The 1/16-in

Figure 9. Capillary-column system

1,2,3	Nupro™ micro-needle valve (stainless-steel)
4,5	Whitey™ shut-off valve, toggle type (brass)
6	Whitey™ 3-way valve (brass)
7	Rotometer (plastic)
8	Pressure gauge (brass)
9	Septa retainer nut (brass)
10	Connector (brass)
11	Heated inlet zone (aluminum)
12	Injection port (stainless-steel)
13	Injection-port sleeve (glass)
14,15,16	1/4-in Swagelok nut (brass)
17,18	1/4-in, 1/8-in, 1/16-in Swagelok tee (stainless-steel)
19,20	1/16-in Swagelok nut (brass)
21,22	1/8-in Swagelok nut (brass)
23	WCOT capillary column (glass)
24	Exit splitter body (glass)
25	1.5-mm Rod (stainless-steel)
26	1/8-in Tubing (stainless-steel)
27	Heated detector zone (stainless-steel)
28	Heated column oven (air)


1
4
9
VENT
10
12
11
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13
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VENT
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23
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3
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22
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18
N_2
16
24
25
28
FID
ECD
27

end of the 1/16-in to 1/8-in Swagelok Reducing Union was turned down to a diameter of 1/8-in, inserted into a hole that was drilled in the side of the 1/16-in to 1/4-in Swaglok Reducing Union, and silver soldered into place. The injection-port sleeve was connected to the 1/4-in Swagelok fitting of the tee, the capillary column was connected to the 1/16-in Swagelok fitting of the tee, and a segment of 1/8-in stainless-steel tubing to carry the split flow was connected to the 1/8-in Swagelok fitting of the tee.

Graphite ferrules were used to seal glass tubing to Swagelok fittings. Brass ferrules were used to seal metal tubing to Swagelok fittings.

Exit interface The exit of the capillary columns was coupled to the detectors of the Tracor 550 GC with a 1/4-in, 1/8-in, 1/16-in Swagelok tee and a 3-in segment of 1-mm capillary glass tubing (6.5-mm o.d.). The Swagelok tee was fabricated in the same manner as the one used on the inlet system. The capillary glass tubing was inserted into the detector port of the GC and connected to the 1/4-in Swagelok fitting of the tee. Prior to use in the GC, the 1-mm capillary glass tubing was submerged in a five-percent solution of hexamethyldisilazane in toluene overnight to remove hydroxyl sites from the glass surface. The exit interface was used to connect the capillary column to either the FID or the ECD. The capillary column was connected to

the 1/16-in Swagelok fitting of the tee and nitrogen was added through the 1/8-in Swagelok fitting of the tee.

Vespal ferrules were used to produce seals between the capillary glass tubing and Swagelok fittings. The capillary column was sealed to the Swagelok tee with a graphite ferrule. Make-up gas was added through a 1/8-in tube connected to the Swagelok tee with the aid of brass ferrules.

Exit splitter Instead of using the exit interface, a glass exit splitter that allowed the split ratio to be varied was used. The ECD and FID were connected to capillary columns with the exit splitter. A model 41-364a exit splitter from Scientific Glass Engineering, Inc., North Melbourne, Australia, was modified to provide a low-volume connection to both detectors. The design of the exit splitter is shown in the lower portion of Figure 9. The design of the exit splitter minimizes contact of the sample with metal surfaces. With the Tracor 550 GC, the sample contacts metal surfaces in the detectors. The body of the exit splitter was 8-mm o.d., 1.5-mm i.d. glass tubing. The glass tubing going to the detectors and capillary column from the exit splitter was 6-mm o.d., 1-mm i.d. The capillary column was connected to the exit splitter and the make-up gas line using a 1/4-in, 1/8-in, 1/16-in Swagelok tee. A 1.5-mm stainless-steel rod fit into the bore of the exit splitter allowing the split ratio to be varied. Pushing the rod into

the splitter increased the fraction of the column effluent going to the FID and pulling the rod from the splitter increased the fraction of the column effluent going to the ECD.

Glass surfaces of the exit splitter were treated by submerging the glass in a five-percent solution of hexamethyldisilazane in toluene overnight. Seals to the detector ports for the FID and ECD and seals to the 1/4-in, 1/8-in, 1/16-in Swagelok tee were made with graphite ferrules.

Techniques and procedures

Installing the WCOT capillary columns In order to use capillary columns from J & W Scientific Company, Inc. with the system for the Tracor 550 GC, it was necessary to straighten the ends of the capillary columns. A 2-in portion on each end of the columns was made perpendicular to the coils of the column. The straightened ends were connected to the inlet system and the exit interface or exit splitter.

The first step in bending the capillary column was to clean the portion of glass to be straightened with a paper tissue that had been thoroughly moistened with methanol. If the glass was not cleaned before bending, the glass became brittle and often broke during installation.

While supporting the column in a horizontal position, a fuel-rich flame from a microburner was used to straighten the lower end of the capillary column. The other end of the

column was connected to a source of helium. A helium flow through the column was maintained during the bending procedure to remove degradation products from the stationary phase. The last 3-in portion of the column was bent perpendicular to the coils of the column by slowly working the cool flame of the microburner from where the bend was to be started to the end of the column. Gravity caused the section of the capillary column to straighten perpendicular to the coils of the column. The column was turned over and this procedure was repeated on the other end of the capillary column.

To ensure the glass surface of the column was covered by stationary phase, the sections of the column that were heated were recoated. The ends of the capillary column were coated by the dynamic coating method (57). A 0.5-percent solution of the stationary phase in methylene chloride was repeatedly introduced and withdrawn from the column ends with a syringe. The syringe was connected to the column ends with heat-shrinkable Teflon tubing.

The column was connected to the 1/16-in Swagelok fittings of the inlet system and the exit interface or exit splitter using graphite ferrules. Graphite ferrules that fit 1/16-in Swagelok fittings and accept 0.8-mm tubing are available from J & W Scientific Company, Inc., Orangevale, California. Both the inlet and exit ends of the capillary columns were inserted

through the Swagelok tees and about 1/2 in into the glass tubing of the inlet system, exit interface, or exit splitter. By inserting the capillary column into the glass tubing, the sample was protected from hot-metal surfaces. The coaxial insertion of the capillary column into the injection-port sleeve produced an annular split when the inlet system was used in the split mode.

Operation of the inlet system in the split mode When the inlet system was used in the split mode, valve 6 in Figure 9 was set to provide helium pressure on the septum side of the injection-port sleeve. Valve 4 was closed and valve 5 was opened. By adjusting needle valve 2, the portion of the carrier gas that was vented before the capillary column was established.

To maximize the efficiency of the capillary-column system, the flow rate of carrier gas through the column was adjusted. By adjusting the helium pressure applied to the column, the maximum number of theoretical plates from the capillary columns were obtained. When needle valve 2 was adjusted, the flow rate of carrier gas through the injection-port sleeve changed. The change in flow through the injection-port sleeve caused the pressure drop across the inlet to change. If the efficiency of the capillary-column system was to be maintained when needle valve 2 was adjusted, the pressure regulator on the carrier gas was adjusted to

re-establish the proper flow of carrier gas through the capillary column.

The minimum split ratio that could be attained, before the column efficiency was reduced, was dependent on the volume of the injection-port sleeve and the flow rate of carrier gas through the capillary column. The efficiency of the capillary column decreased at low split ratios, because the sample was not transferred to the column as a plug. Low flow rates of carrier gas through the injection-port sleeve caused the samples to be slowly introduced to the capillary column. When small split ratios were used, broad peaks for the solvent and early eluted substances were observed. When using an injection-port sleeve of 1-mm i.d., a split ratio of 1 to 10 was attained before the efficiency of the capillary column was significantly reduced.

The capillary column was inserted about 1/2 in into the injection-port sleeve to keep the sample from contacting hot metal and to minimize fractionation of the sample. If the capillary column is coaxially inserted into a larger tube that carries a mixture of vaporized sample in a carrier gas, the sample split is referred to as an annular split. Bauman and Gill showed that by vaporizing a sample in a carrier-gas stream and by using an annular splitter, fractionation of a sample was minimized (58). Using other splitter designs, a

smaller fraction of high-boiling components are transferred to the capillary column than of low-boiling components.

<u>Operation of the inlet system in the splitless mode</u> To inject samples by the splitless technique, valve 6 in Figure 9 was positioned to provide carrier-gas pressure on the septum side of the injection-port sleeve. Valves 4 and 5 were both in the closed position. After allowing time for the gas lines of the inlet to reach an equilibrium pressure, a microliter syringe was used to introduce liquid samples through the septum into the injection-port sleeve. At a predetermined time, usually 30 seconds for this work, valve 6 was actuated to provide carrier-gas pressure on the column side of the injection-port sleeve. After actuating valve 6, valve 4 was opened to backflush the injection-port sleeve. Needle valve 1 was adjusted to obtain the desired flow of gas through the inlet.

The time interval for splitless operation that maximizes the efficiency of a capillary-column system is a function of many variables, including: volume of the injection-port sleeve, flow rate of carrier gas through the capillary column, number of theoretical plates in the capillary column, and solvent effects (52). Because it is usually impossible to avoid broadening for all peaks, the time interval used for splitless operation is generally a compromise that gives satisfactory results. With the system used in this work,

the time interval used for splitless operation usually was 30 sec.

Optimization of exit-interface parameters To retain the separation that has been obtained on a capillary column, mixing of the column effluent before entering the detector must be avoided. Mixing of the effluent was minimized by keeping the volume between the column exit and the detector to a minimum and keeping the linear-flow rate in the transfer lines much greater than the linear-flow rate in the capillary column. A 3-in segment of 1-mm i.d. capillary glass tubing was used to connect the capillary column to the detectors. The 1-mm capillary glass tubing was used instead of standard 1/4-in glass tubing to minimize the volume of the exit interface. Make-up gas was added to the column effluent to increase the flow rate of gas through the exit interface. Capillary columns of a 0.25-mm i.d. were usually operated at flow rates of about 25 cm/sec. A linear-flow rate of 25 cm/sec through the capillary column implies a volumetric-flow rate of 0.73 ml/min. Assuming a linear-flow rate in the capillary tubing of three times the rate in the capillary column is sufficient to prevent mixing of substances eluted from the column, a linear-flow rate of 75 cm/sec or a volumetric-flow rate of 35 ml/min of make-up gas must be added to the column effluent prior to the exit interface to prevent broadening of eluted components. Typically, make-up

gas was added at a rate of 50 ml/min in this work. If volumetric-flow rate of make-up gas is too large, the sensitivities of the FID and the ECD will be reduced.

Optimization of exit-splitter parameters As with the exit interface, the two critical factors in regard to peak broadening are void volume and linear-flow rate. Since the void volume of the exit splitter was constant, the linear-flow rate of gas in the exit splitter was the factor used to limit broadening. If it is assumed that a linear-flow rate in all parts of the exit splitter must be greater than 75 cm/sec to prevent broadening, make-up gas must be added to the column effluent at a volumetric-flow rate of 140 ml/min when an exit-split ratio of 1 to 3 is used. Because the sensitivities of both the ECD and the FID are reduced by high rates of gas flow, exit-split ratios of greater than 1 to 5 were not practical with this exit splitter.

Results and Discussion

Figure 10 demonstrates the resolution that was obtained using capillary columns with the modified Tracor 550 GC. The effective number of theoretical plates for the capillary-column system, as calculated from the peak marked with an asterisk, was 44,476. The chromatogram in Figure 10 is of a mixture of polychlorinated biphenyls in Arochlor 1254. A

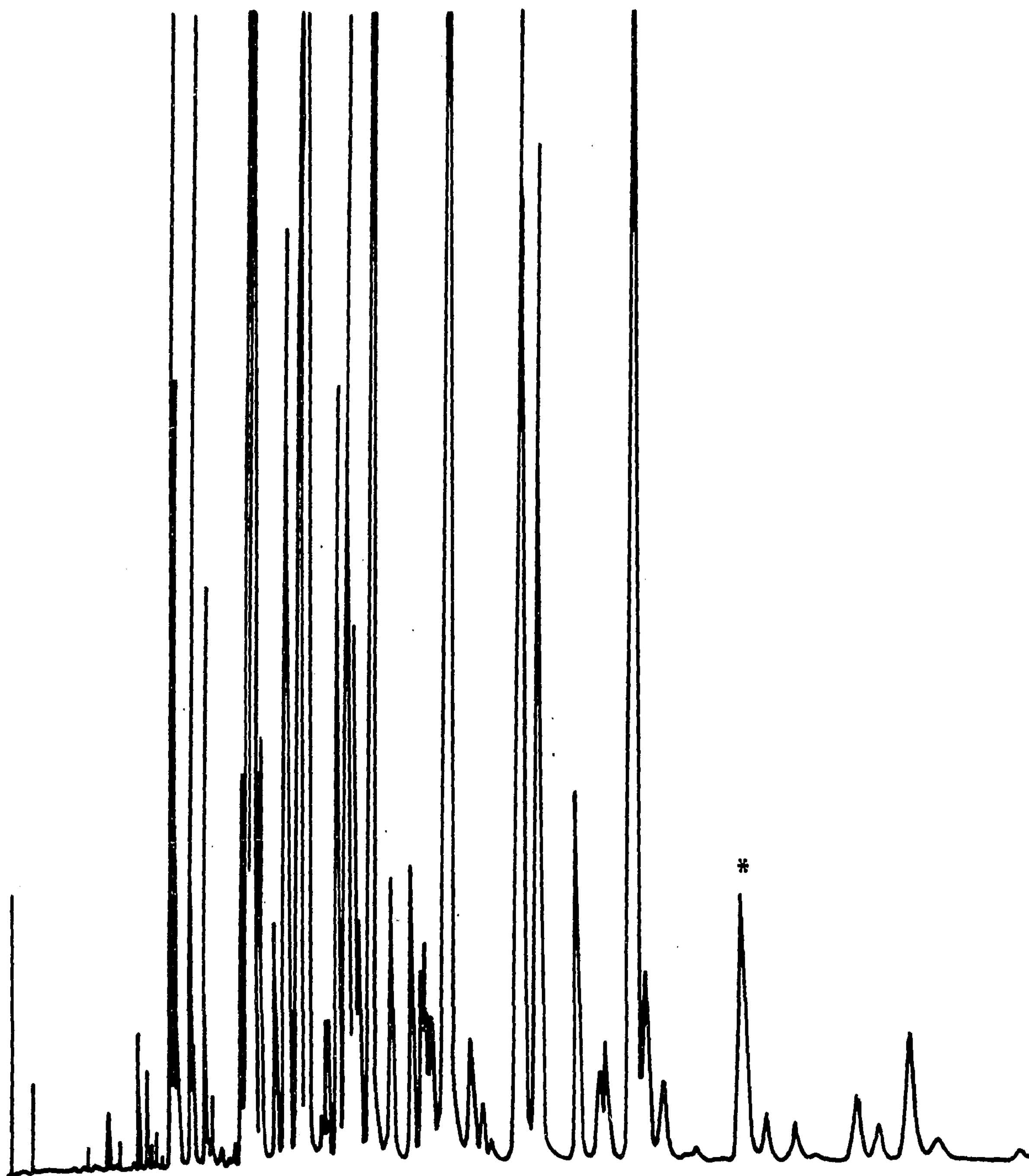


Figure 10. Separation of Arochlor 1254. Conditions of separation: Column - WCOT, SE-30 stationary phase, 30 m x 0.25 mm i.d.; Temperature - 170°C isothermal for 80 min; Carrier gas - 28 cm/sec of helium; Sample introduction - 1 µl, split ratio = 1:100; Detection - ECD, attenuation = 32; Make-up gas - 22 ml/min of nitrogen; Sample - 200 ng of Arochlor 1254 in pentane.

large split ratio was used to reduce the amount of each compound going to the ECD. By using the large split ratio, the responses for the polychlorinated biphenyls were within the linear range of the ECD.

The flexibility and sensitivity provided by temperature programming, splitless injection, and exit splitting is demonstrated in Figure 11. The chromatograms of the mixture demonstrate the ability of this system to provide good chromatograms with two detectors from a single chromatographic separation. Large peaks for 2,4-hexanedione, 2-(2-butoxyethoxy)ethyl acetate, and 1-chloro-3-nitrobenzene were observed with the ECD. All compounds in the mixture were detected by the FID. By using the splitless injection technique, one nanogram of most organic compounds can be detected by the FID with this system. The detection limit for some polychlorinated compounds is one picogram when the ECD is used with capillary columns.

Reproducibility of peak heights with the capillary-column system is a function of many variables: backflush rate, carrier-gas pressure, sample size, splitless time, etc. By introducing the sample with a microliter syringe in a reproducible manner that minimized changes of the carrier-gas pressure, peak heights were reproduced within 2 percent. If injection technique was not duplicated, large differences in peak heights were observed. If the splitless time was too

Figure 11. Separation of model compounds

Conditions of separation:

Column - WCOT, SP1000 stationary phase, 25 m x 0.25 mm i.d.

Temperature - Isothermal at 80°C for 4 min, increased temperature at 3°C/min. Maximum temperature of 220°C

Carrier Gas - 28 cm/sec of helium

Sample Introduction - 2 µl, splitless for 30 sec

Detection - Top: ECD, attenuation = 2
Bottom: FID, attenuation = 10

Make-up Gas - 105 ml/min of nitrogen

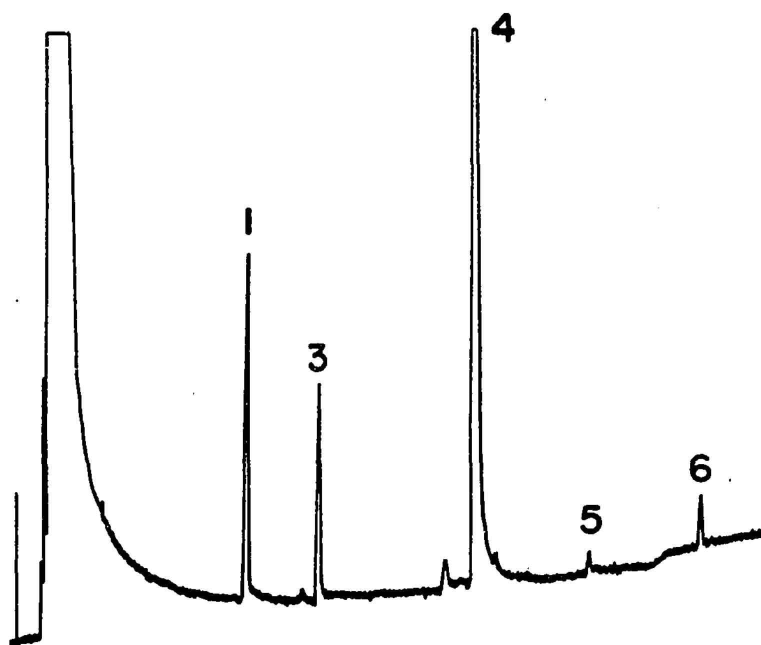
Backflush Gas - 15 ml/min of helium

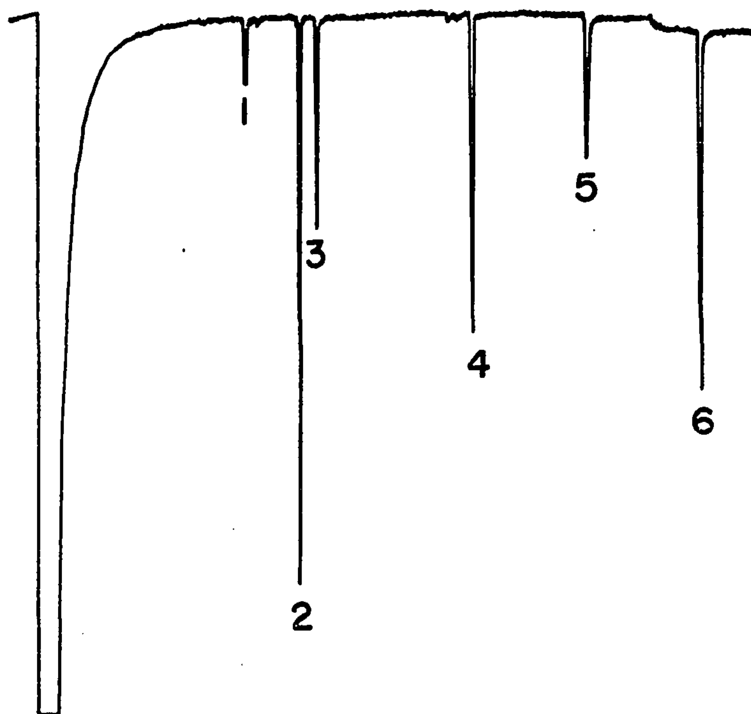
ECD Purge Gas - 70 ml/min of nitrogen

Exit-Split Ratio (ECD:FID) - 1:2

Sample Components - 8×10^{-9} g/µl each in methylene chloride

Peak Number	Compound
1	2,5-Hexanedione
2	Hexadecane
3	2-(2-Butoxyethoxy)ethyl acetate
4	1-Chloro-3-nitrobenzene
5	Butyl phosphate
6	2,6-Dimethylphenol


4
1
3
6
5


1
5
3
4
6
2

short, it was difficult to reproduce the amount of sample introduced to the column. A backflush flow great enough to quickly remove remaining sample from the inlet was necessary to obtain plug injection. A flow of 10-20 ml/min through the injection-port sleeve was sufficient to quickly purge the inlet. By trial and error, operating parameters were selected that maximized the reproducibility of the system with various capillary columns.

Retention times were reproducible with this capillary-column system. Table 7 shows the variations in retention times that were observed during three days. The average of the relative standard deviations observed for the retention times of the compounds in Table 7 was 2 percent. Large variations in retention times for early eluted compounds were the result of the inability of the Tracor 550 GC to precisely reproduce temperatures below 100°C. When an initial temperature of greater than 100°C was used, reproducibility of retention times was much better than the 2 percent variation observed for compounds in Table 7.

Conclusions

The ability of the capillary-column system with dual detection to provide retention times and relative-response factors makes the system ideal for screening samples. Specific organic compounds in samples could be determined

Table 7. Reproducibility of retention times with capillary-column gas chromatography

Compound	Average Retention Time[a] (minutes)	Number of Determinations	Standard Deviation	Percent Standard Deviation
Octane	2.8	6	0.1	4.2
Nonane	5.6	6	0.3	5.0
Decane	9.5	8	0.6	5.9
Nitrobenzene	11.4	5	0.4	3.9
Undecane	13.1	8	0.6	4.8
1,2,4-Trichlorobenzene	14.7	5	0.4	3.9
Benzethiophene	15.5	5	0.4	2.6
Benzothiozole	16.2	3	0.4	2.2
Dodecane	16.4	8	0.5	2.9
Tridecane	19.4	8	0.4	2.0
Acenaphthalene	23.8	7	0.08	0.3
Hexadecane	27.6	10	0.2	0.7
Heptadecane	29.9	5	0.07	0.2
Octadecane	32.2	5	0.05	0.2
Nonadecane	34.4	5	0.08	0.2
Eicoane	36.6	5	0.05	0.1
Fluoranthrene	37.2	7	0.07	0.2
Docosane	40.5	5	0.08	0.2
Tetracosane	44.1	5	0.00	0.0
Diethylhexyl Phthalate	46.2	8	0.2	0.4

[a]Chromatographic conditions:

Column - 30-m x 0.25-mm i.d. glass WCOT capillary column, SE-30 stationary phase

Temperature - Isothermal at 50°C for 4 min, then increased temperature at 5°C per min. Maximum temperature of 260°C was held for 10 min.

Carrier Gas: Helium flow of 90 cm/sec

and quantified using this gas-chromatographic system. Because two types of information are obtained using this system, the identities of compounds can be confirmed while obtaining information for quantitative calculations.

Reproducibility of peak heights and retention times are highly dependent on parameters of operation. Variations in sample-introduction technique and column-oven temperature cause large variations in the chromatograms obtained.

FRACTIONATION OF ORGANIC COMPOUNDS IN WATER EXTRACTS ON FLORISIL

Review of Related Work

Organic compounds in drinking waters

In recent years much interest in the organic materials present in drinking waters has developed. Although the concentrations of total-organic carbon (TOC) in drinking waters are usually less than 1 ppm (1 mg/l), the effect of chronic, low-level doses of some organic compounds is a matter of concern to many people.

Because the concentrations of organic compounds in drinking waters are low, it is difficult to identify and quantify the organic compounds. Beyond quantitative analysis, it is even more difficult to determine the potential health hazards of the trace levels of organic compounds present in drinking waters. Recently, techniques have been developed for concentrating and determining some of the organic compounds present in drinking waters at the sub-part-per-billion level.

Various techniques for determining trace levels of organic pollutants in water have been developed. Although only a small portion of the TOC present in most drinking waters can be determined by gas chromatography, most analytical methods depend on gas chromatography to separate the organic compounds. Gas chromatography has been the

predominant technique for separating organic compounds found in waters because of the high resolution it offers and the sensitivity attainable when it is interfaced to a mass spectrometer. Gas chromatography/mass spectrometry (GC/MS) provides retention information and mass spectra that can be used to elucidate the structures of organic compounds. To obtain mass spectra for identifying organic compounds, approximately 10 ng of a compound must be introduced to a GC/MS. Application of GC/MS to the analysis of trace organic compounds in drinking waters requires that samples be concentrated prior to analysis. Many techniques have been implemented for concentrating the organic compounds present in drinking waters. Among the most prominent methods of concentrating organic compounds present in water are: solvent extraction (59,60,61), gas-phase extraction (33), purge and trap (17,61), carbon sorption (62,63), reverse osmosis (64), and resin sorption (5,6,7,65).

Resin sorption using Amberlite XAD-2 appears to be the most efficient analytical technique for concentrating organic compounds found in potable waters. Carbon sorption may be as effective in removing organic compounds from water; however, removal or desorption of organic compounds from activated carbon generally is incomplete (66).

Organic compounds sorbed on XAD-2 can be removed by thermal desorption or solvent elution. Chang described a method to thermally desorb organic compounds from a XAD-2

column onto a column of Tenax GC (16). Ryan and Fritz further investigated the use of thermal desorption of XAD-2 for the analysis of water for organic pollutants (67). By sorbing the organic pollutants onto a Tenax-GC column, water was removed from the sample before introducing the organic compounds to the analytical column of the gas chromatograph.

Organic compounds sorbed on columns of XAD-2 can be desorbed by solvent elution (5). By passing a small volume of diethyl ether through a resin bed, the organic compounds sorbed from a large volume of water are eluted. The concentration of the organic compounds in the diethyl-ether solution can be increased by distilling the diethyl-ether solution to a smaller volume. A small portion of the diethyl-ether solution (2 μl) is then injected into a gas chromatograph. Although greater sensitivity can be obtained for a given volume of water by thermal desorption, solvent elution yields a sample that can be treated and analyzed in many ways.

The task of identifying organic compounds extracted from waters is often extremely difficult. Infrared (IR) spectroscopy, proton magnetic resonance (PMR) spectroscopy, and mass spectrometry (MS) are useful in determining the structures of organic compounds. The spectroscopic techniques provide information about compounds' structures, but it is necessary to separate the compounds of a mixture before spectroscopic

analysis. Of these techniques, MS is the only technique that has enough sensitivity to obtain information about the trace organic pollutants present in drinking waters.

Some techniques that have been used to separate mixtures of organic compounds are: solvent extraction, liquid chromatography, and gas chromatography. Because numerous compounds are present in extracts of many waters, a high-resolution technique must be used to separate compounds in water extracts. Gas chromatography has been the most popular technique for separating organic compounds from waters because of the resolution obtained with gas chromatography. The resolution of gas chromatography can be increased further by the use of wall-coated open tubular (WCOT) capillary columns; however, the chromatogram in Figure 12 indicates the inability of capillary columns to completely resolve all the components in complex mixtures.

Gas chromatography coupled with mass spectrometry (GC/MS) provides retention information and mass spectra that can be used to determine the identities of organic compounds. The information provided by GC/MS is sufficient to determine the identities of most compounds. The identities of some compounds are difficult to determine from the mass spectra and retention time on a gas chromatographic column. If GC does not completely resolve the compounds in a sample, mass spectra obtained by GC/MS for some peaks will be of mixtures.

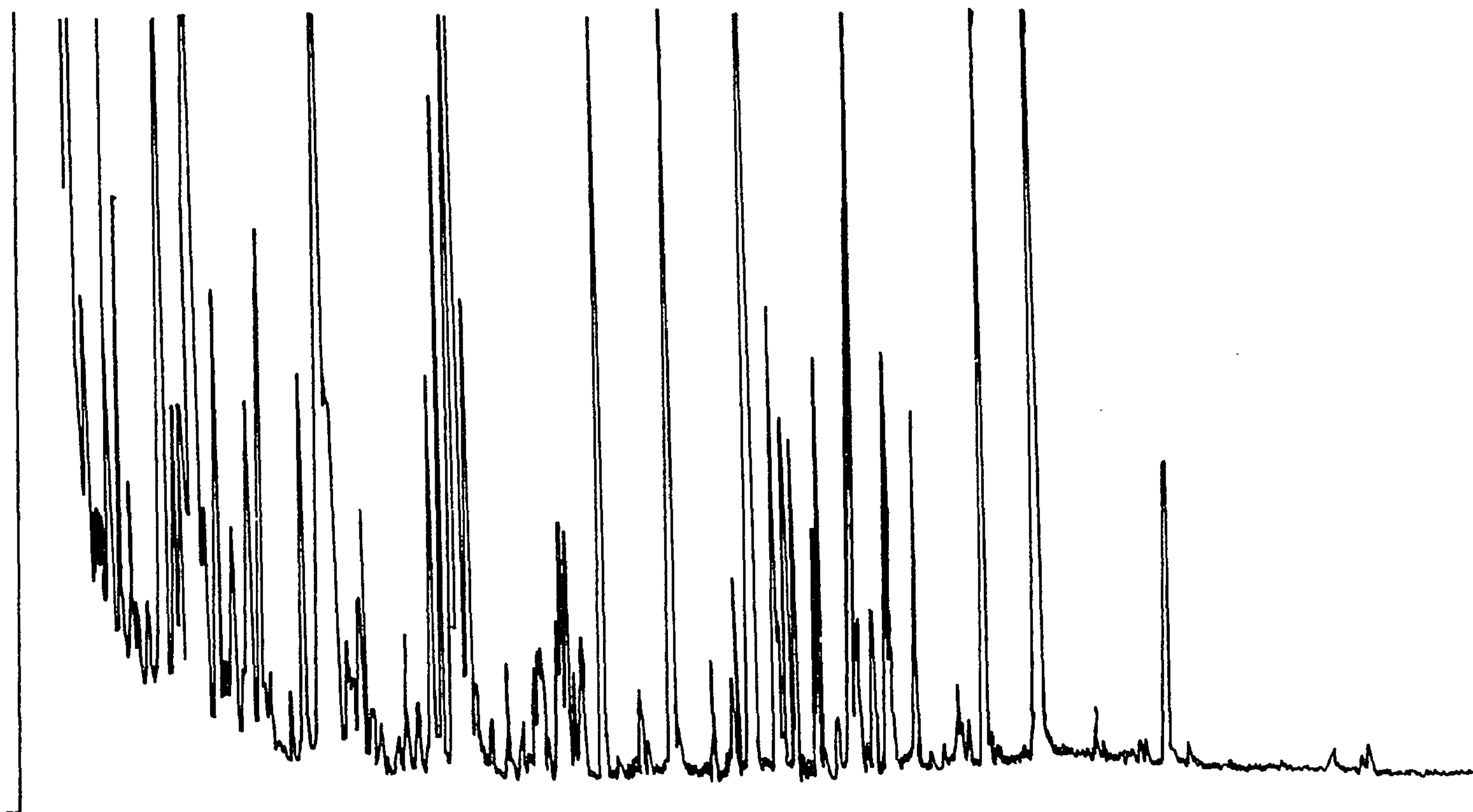


Figure 12. Separation of organic compounds extracted from water distributed in Ames, Iowa. Chromatographic conditions: Column - WCOT, Carbowax 20M stationary phase, 30 m x 0.25 mm i.d.; Temperature - Isothermal at 50°C for 4 min, then increased temperature at 3°C/min. Maximum temperature of 200°C was held for 6 min; Carrier gas - 26 cm/sec of helium; Sample introduction - 2 µl, 30 sec splitless; Make-up gas - 50 ml/min; Backflush gas - 20 ml/min; Detection - FID, attenuation = 8.

When compounds are introduced to a mass spectrometer as mixtures, it is extremely difficult and time-consuming to determine the identities of the compounds from the mass spectra.

Because analysis by GC/MS is not sufficient to determine the identities of all the organic compounds present in extracts of drinking waters, additional techniques must be used to identify some of the organic pollutants in drinking waters. A separation prior to GC/MS would help determine the identities of some compounds in samples. By dividing the compounds of a sample into fractions according to molecular properties of the compounds, information about the components is obtained. Because fewer components would be present in each fraction, fewer problems with incomplete resolution will be encountered during GC. Because fractionating a sample increases the resolution obtained for the analysis, identification of some components is easier than if the complex mixture is analyzed directly.

In addition to reducing the complexity of samples, a fractionation technique would provide additional information about the components of the sample. The information provided by the fractionation would aid in the interpretation of the data obtained by GC/MS. Information provided by a fractionation could be used to confirm the identities of compounds not well-characterized by MS.

Because WCOT capillary columns are susceptible to contamination, a fractionation could be used to remove contaminants from samples prior to introduction to the column. Only a small amount of stationary phase is present in a WCOT column; thus, the thin film of stationary phase in a capillary column can be destroyed by a small amount of a contaminant. High molecular-weight compounds that do not chromatograph build up on the column or are thermally decomposed by the heat of the gas-chromatographic oven. Water and oxygen are harmful to the thin film of stationary phase because they may cause hydrolysis of the stationary phase or the activation of the glass surface. When analyzing concentrates from water samples by capillary-column gas chromatography, a fractionation that removes nonchromatographable contaminants would increase the useful life of a WCOT capillary column.

Evaluation of fractionation techniques

Fractionating extracts of some drinking waters would help in the analysis for organic pollutants. Some properties that are desired of a fractionation scheme for water extracts are listed below.

Quantitative recovery   Complete recovery of all organic compounds in the water extracts is the ideal situation. When the analysis of the sample is to be

performed by gas chromatography, recovery of compounds that cannot be analyzed by gas chromatography is not necessary.

Informative Because it is difficult to determine the identities of some organic compounds in water extracts, information about compounds in the sample provided by the fractionation would be useful as an aid in determining their structures.

Noncontaminative The amount of organic material concentrated from a water sample is generally quite small. To maintain sample integrity, the fractionation technique must not introduce any substance that would interfere with the analysis.

Semipreparative Although most organic compounds are present in drinking waters at very dilute concentrations, the technique should have the capacity to handle any compounds that may be present in high concentrations.

Sample clean-up To aid in the analysis, the technique should provide fractions that are free of compounds that may interfere with the analysis.

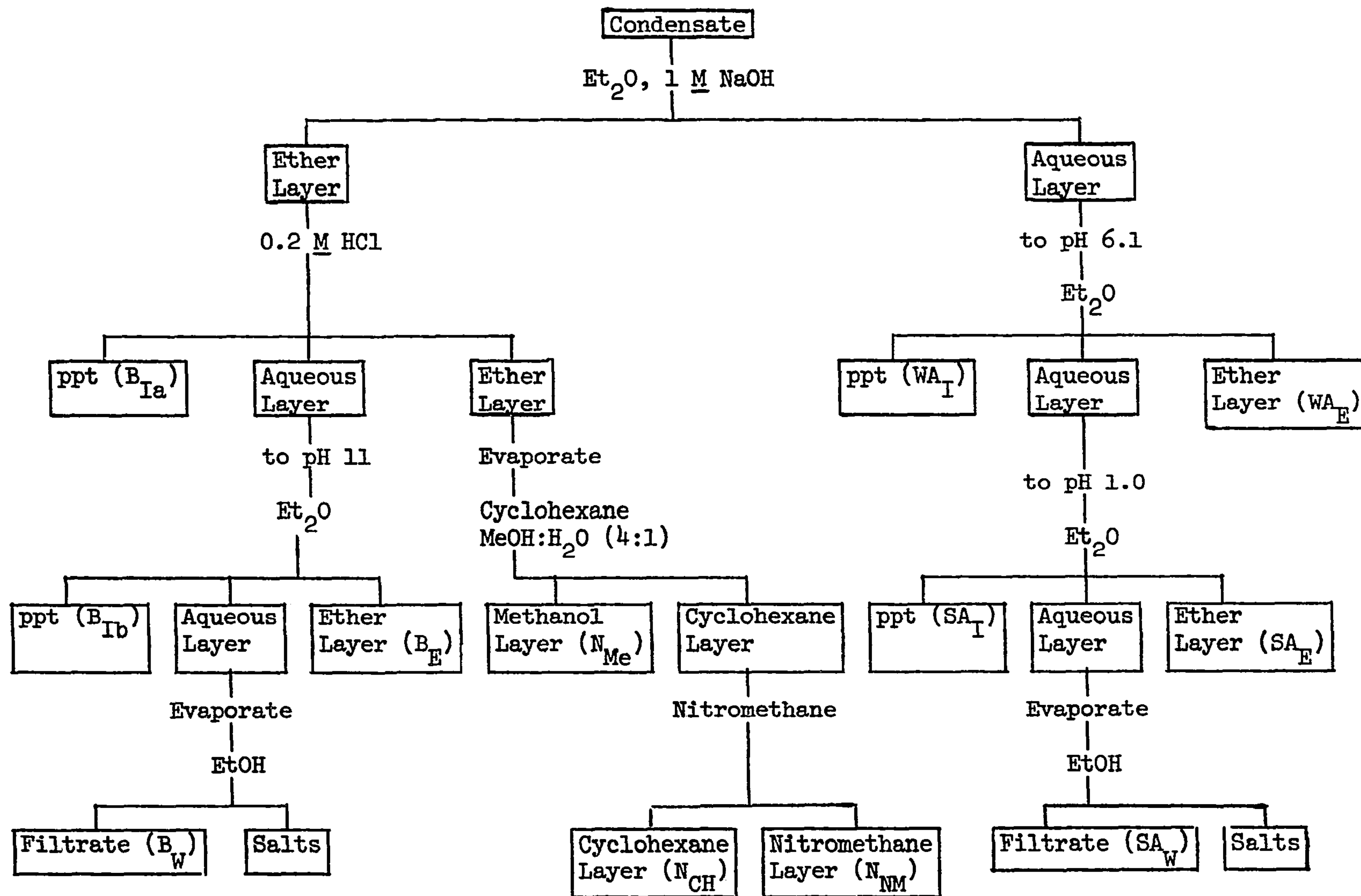
Rapid Implementation of a fractionation technique on a routine basis as an aid to an analysis would require that the technique itself not be time-consuming. If the total analysis time of the water extracts is to be minimized, the time involved in analyzing the fractions must be considered.

Inexpensive   Whether or not the cost of a fractionation technique is important may be debatable; however, a technique that is low in cost and produces the desired results would be preferred to more expensive techniques.

A number of fractionation techniques have been developed and applied to separating a variety of complex mixtures. Some fractionation techniques for complex mixtures are reviewed in the following paragraphs. Advantages and limitations in regard to fractionating water extracts are discussed.

Stedman and coworkers developed a technique for fractionating cigarette-smoke condensates based upon solvent extraction (68). Table 8 shows the extraction steps that were performed in the separation scheme. Using six-liter separatory funnels, 720-g quantities of cigarette-smoke condensates were separated into eleven fractions. The scale of this separation scheme was large to allow for the preparation of sufficient amounts of material for mutagenic testing with mice (69). The weights of the fractions indicated that 90.8 percent of the condensates were recovered. Variations in the amount recovered in each fraction were especially pronounced for the smaller fractions. The resolutions of the fractionation scheme is low; thus, small differences in the amount of a component remaining in a phase causes large differences in the amounts found in the smaller

Table 8. Fractionation scheme developed by Stedman and coworkers



fractions. For preparative fractionations, the amount of solvent used and the handling of the sample does not pose any significant problems. In addition to scaling down the scheme for trace analysis, steps to assure the purity of solvents and glassware would be necessary to avoid contamination of the sample. Studies have been performed that indicate low recoveries of trace organic compounds may occur because of sorption onto glass surfaces (6). The types of compounds in fractions B_{Ia}, B_{Ib}, WA_I, WA_E, SA_I, SA_E, SA_W, and B_E would not be sufficiently volatile nor chemically inert to analyze by gas chromatography.

The separation scheme by Stedman and coworkers has been expanded by other workers. Novotny et al. scaled the scheme down and used it as part of a scheme to prepare samples of cigarette-smoke condensates for analysis of polycyclic aromatic hydrocarbons (70,71). The polycyclic aromatic hydrocarbons were contained in the N_{NM} fraction of Table 8. The N_{NM} fraction was evaporated to dryness, dissolved in isopropanol, and 1.5 g of the extract introduced to a 115-cm x 1.5-cm column packed with Sephadex LH-20. Organic compounds were eluted from the Sephadex column by pumping isopropanol at a rate of 6 ml/hr. The size of each fraction collected and a representative compound for each fraction are listed in Table 9. These fractions were analyzed by gas chromatography with capillary columns. If the

Table 9. Fractions collected from Sephadex LH-20 column by Novotny and coworkers

Fraction Number	Representative Compound	Collection Volume (ml)
I	---	0 - 240
II	Naphthalene	241 - 345
III	Anthracene	346 - 415
IV	Fluoranthrene	416 - 470
V	Chrysene	471 - 585
VI	Benzo[a]pyrene	586 - 740
VII	Dibenz[a,h]anthracene	741 - 860
VIII to XIV	---	861 - 4360

capillary-column chromatogram was too complex, the fraction was further separated by liquid chromatography on a 50-cm x 2.6-mm reverse-phase column. The reverse-phase column was packed with OPN/Porisil C and hexane was used as the eluent. The procedure was shown to be an effective, but time-consuming, way of obtaining samples containing polycyclic aromatic hydrocarbons with specific numbers of rings. Many more steps would be required to analyze for compounds other than aromatic hydrocarbons.

Rubin *et al*. also investigated the further fractionation of the N_{NM} fraction from Stedman's scheme (72). Organic

compounds were eluted from Florisil columns, 30 cm x 2.6 cm, with ten bed volumes of the following solvents: hexane, hexane:benzene (4:1), benzene:ether (4:1), and methanol. Polycyclic aromatic hydrocarbons were observed to elute in the hexane and the hexane:benzene fractions. Samples of cigarette-smoke condensates and synthetic oils were fractionated then examined for mutagenic activity by a microbacterial technique developed by Ames (73). This is a rapid technique that provided sufficient material for mutagenic testing. Compounds from cigarette-smoke condensates were separated more rapidly on the Florisil columns than by Novotny using Sephadex LH-20. Results of the fractionation indicate that not all aliphatic hydrocarbons are resolved from the aromatic hydrocarbons. Elution behavior of compounds other than hydrocarbons was not investigated.

Many methods of fractionation by adsorption chromatography have been developed for complex mixtures of organic compounds. A commonly used fractionation scheme was developed by Mills for samples to be analyzed for pesticides (74). Samples were separated on 22-mm i.d. columns containing 20 g of 60/100-mesh Florisil that had been activated at 130°C. Organic compounds were eluted from the Florisil columns by the following solvents: 6% diethyl ether in petroleum ether, 15% diethyl ether in petroleum ether, and 50% diethyl ether in petroleum ether. Two-hundred milliliters of each solvent

were passed through the columns by gravity flow and collected as independent fractions. The pesticides eluted in each fraction are listed in Table 10. Nonpolar compounds were

Table 10. Pesticides grouped according to the fraction the compounds eluted from Florisil

Fraction I[a]	Fraction II[b]	Fraction III[c]
Aldrin	Dieldrin	Malathion
o,o'-DDT	Endrin	
o,p'-DDT	Methyl Parathion	
p,p'-DDT	Ethyl Parathion	
Heptachlor Epoxide		
α-BHC		
Lindane		

[a]6% diethyl ether in petroleum ether.

[b]15% diethyl ether in petroleum ether.

[c]50% diethyl ether in petroleum ether.

eluted by the first solvent and the polar compounds were divided between the second and third fractions. Mills developed the technique to confirm the identities of pesticides present in the samples and to remove substances, such as lipids, that may interfere with the gas-chromatographic

determination of the pesticides. Reproducibility of elution was obtained by adjusting the amount of Florisil used in the columns according to the activity of the Florisil (75). With the solvent system used by Mills, not all compounds that could be analyzed by gas chromatography were eluted from the Florisil. Because the fractionation is rapid, inexpensive, and effective, the technique is a part of most standard methods for the analysis of samples for pesticides. With slight modifications, the Florisil fractionation technique has been applied to the analysis of grapefruit for aromatic compounds (76), and the analysis of animal and vegetable oils for hydrocarbons (77), steroids (78), and alcohols (79).

Another adsorption-chromatography scheme was developed by Jones et al. for fractionating organic compounds present in process streams (80). Organic compounds were eluted from a 25-cm x 1.0-cm column packed with 200-mesh silica gel that had been activated at 200°C. Fractions were collected from the column by eluting with 25 ml of eight solvents. The solvents used and the types of compounds eluted by each solvent are listed in Table 11. The volumes of the fractions were reduced by distillation. The two techniques employed to analyze the fractions were weighing the residue and infrared spectroscopy. The scale of the scheme was large enough that a sensitive analytical balance could be used to determine the weights of the fractions. Although the complexity of

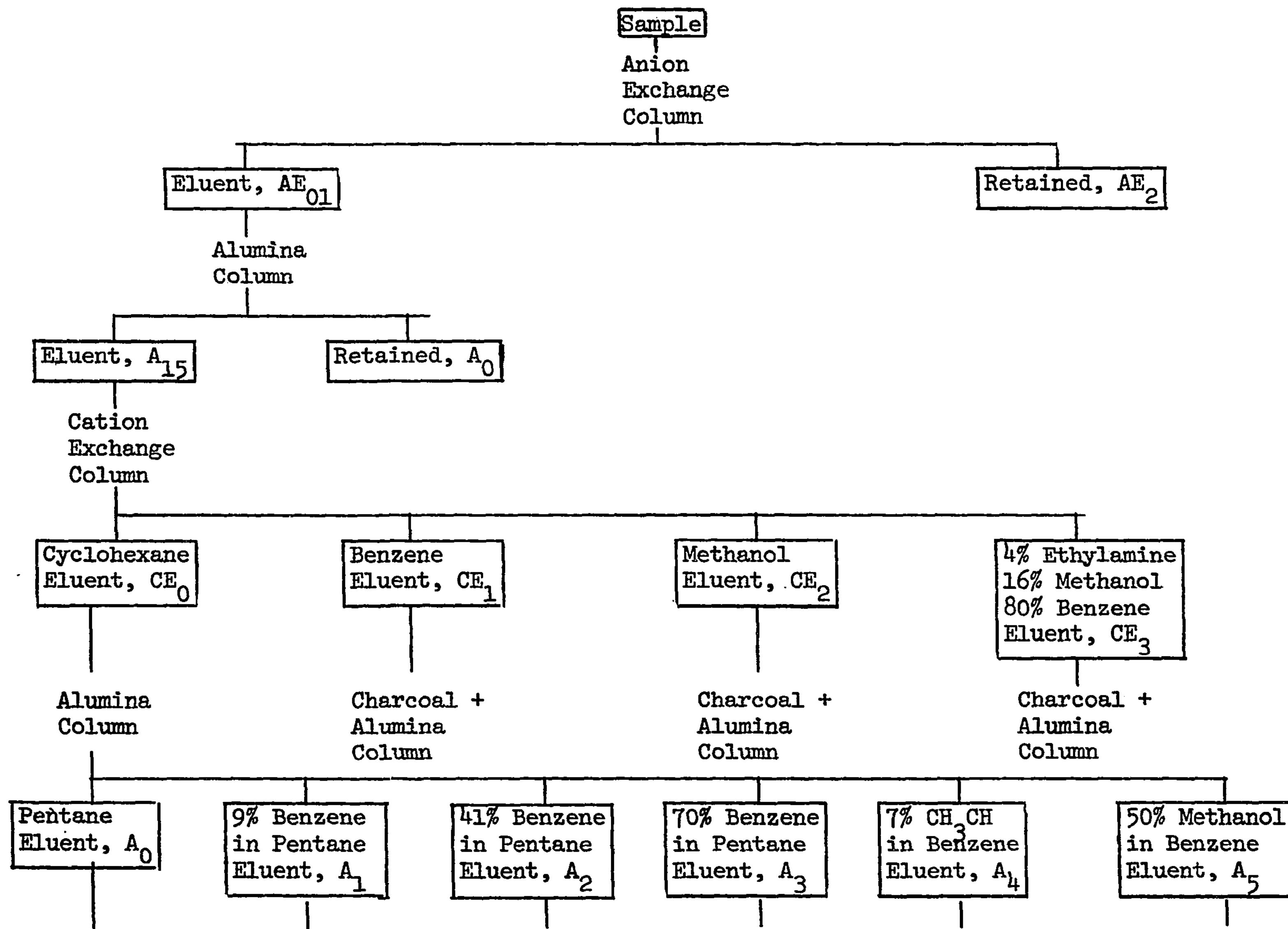
Table 11. Fractions collected from the Jones scheme

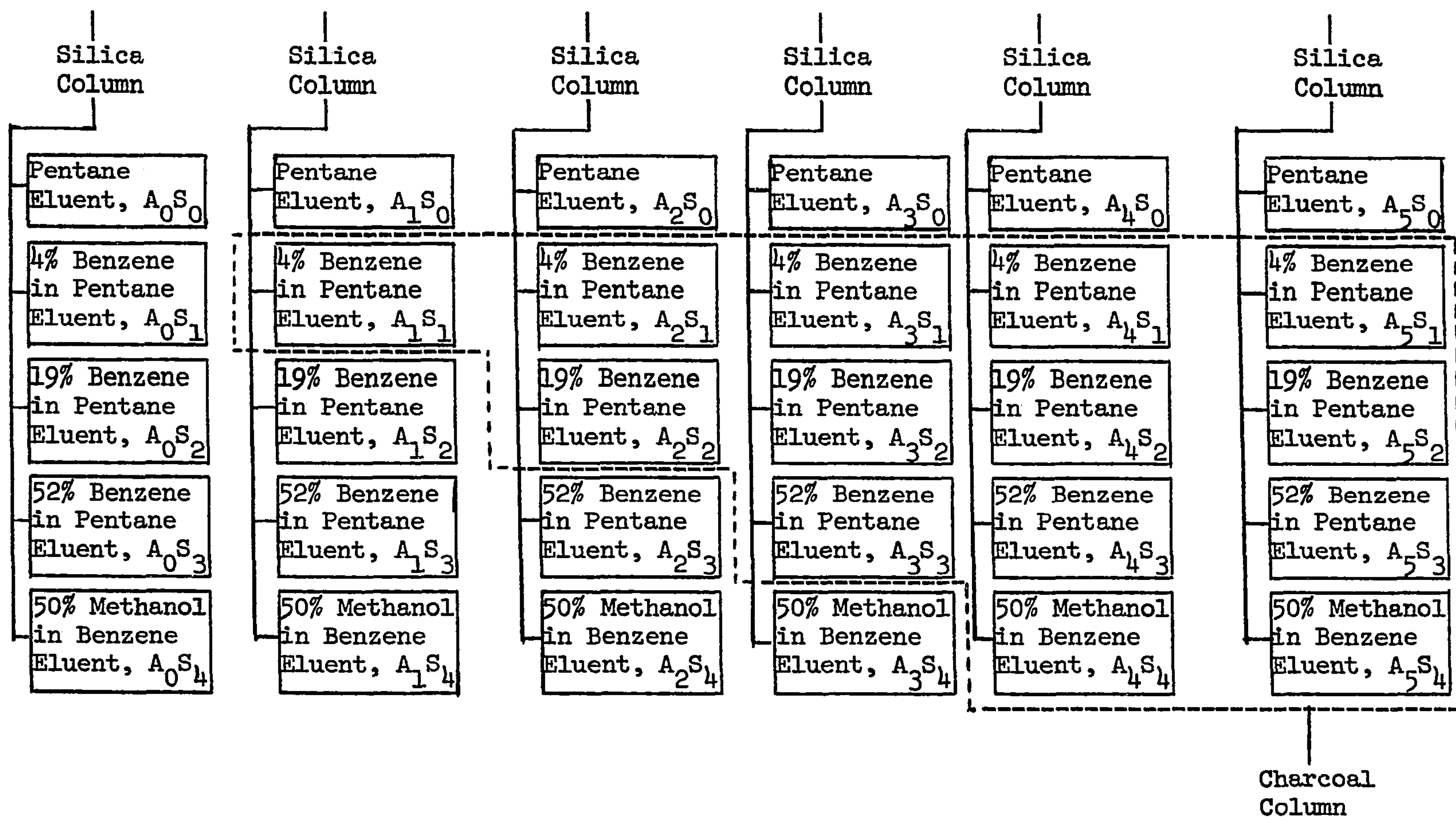
Fraction Number	Solvent	Compounds Eluted
I	Petroleum Ether	Aliphatic Hydrocarbons
II	20% Methylene Chloride in Petroleum Ether	Aromatic Hydrocarbons, Halides
III	50% Methylene Chloride in Petroleum Ether	Esters, Ethers, Epoxides, Nitro Compounds
IV	Methylene Chloride	Esters, Ketones, Aldehydes, Phenols
V	5% Methanol in Methylene Chloride	Esters, Phenols, Alcohols, Amines
VI	20% Methanol in Methylene Chloride	Amides, Sulfonates, Carboxylic Acids, Acid Salts
VII	50% Methanol in Methylene Chloride	Sulfonates, Sulfoxides, Sulfonic Acids
VIII	Methanol	Sulfonic Acids

samples was reduced, analysis of fractions by infrared spectroscopy was not suitable for extracts of most process streams. The information gained by the fractionation was empirical; thus, the elution behavior of all compounds of interest must be determined. Reproducibility of the technique was not explored, but other studies indicate that retention behaviors of compounds on activated silica gel are difficult to reproduce (81).

Snyder and Buell combined the separating abilities of alumina, silica gel, carbon, and ion-exchange resins to develop a technique to fractionate petroleum (82,83). The scheme is outlined in Table 12. The technique had the flexibility of deciding sample size, column size, and eluent volumes that best suited the needs of the analysis. The sorbents required for the fractionation scheme were: Amberlyst A-29 anion-exchange resin, Alcoa F-20 alumina, Amberlyst XN-1005 cation-exchange resin, Darco G-60 charcoal mixed with Celite 545, and Davison Code 62 silica gel. Each adsorbent was prepared in a manner that tailored its adsorptive properties. Although many fractions were obtained by this fractionation scheme, complete resolution was not obtained with the three to five milliliters of solvent per gram of adsorbent used to collect the fractions. By employing the additional steps of chromatography on charcoal and alumina, many components of these were determined by high-resolution mass spectrometry. The scheme may be unsatisfactory for fractionating trace levels of organic compounds, because the sample may be contaminated by impurities present in most commerical ion-exchange resins (84). The difficulty in assuring the purity of all of these sorbents and solvents makes this technique undesirable for trace analysis.

Table 12. Fractionation scheme developed by Snyder and Buell



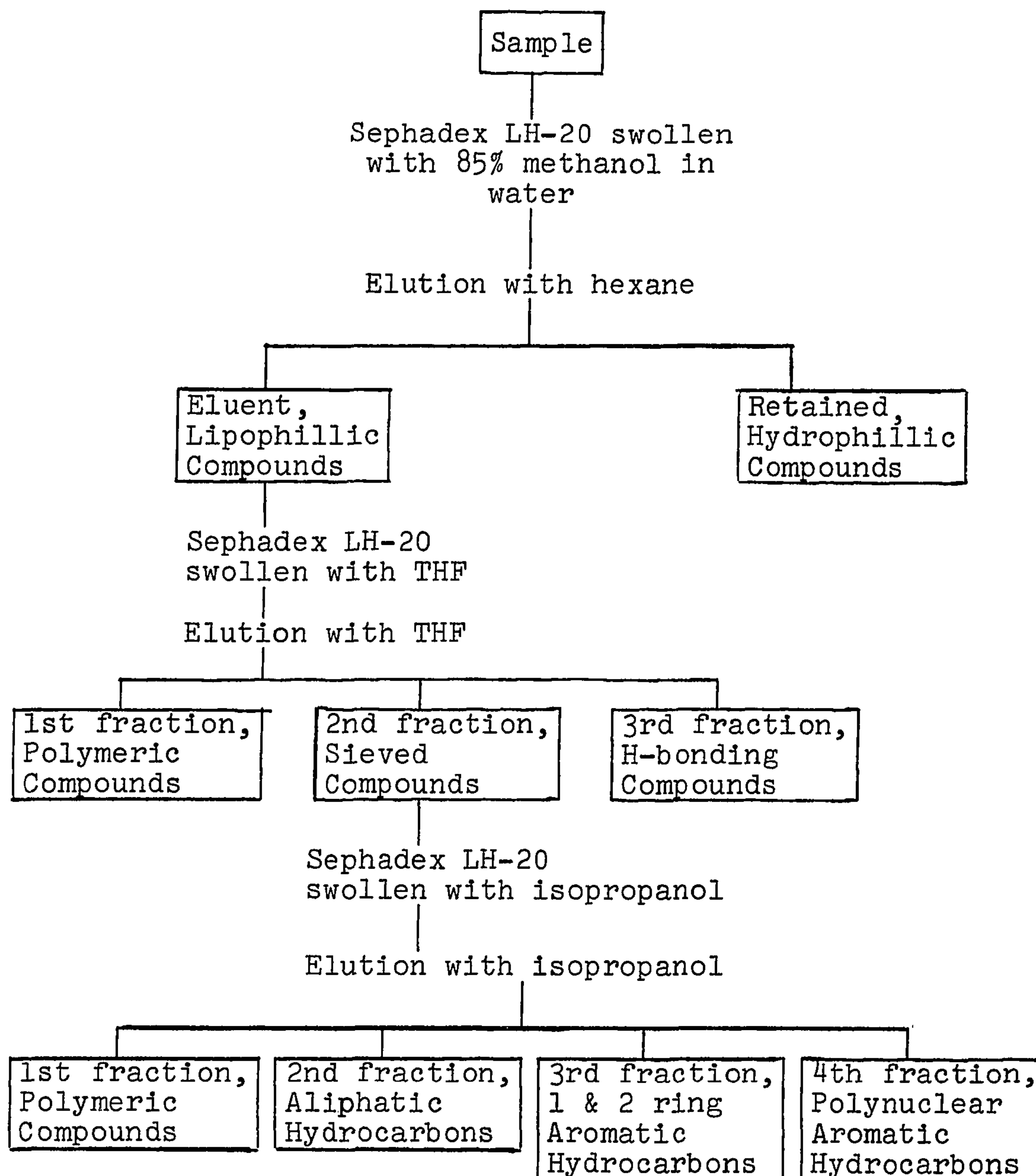


Silica Column
Pentane Eluent, A_0S_0
4% Benzene in Pentane Eluent, A_0S_1
19% Benzene in Pentane Eluent, A_0S_2
52% Benzene in Pentane Eluent, A_0S_3
50% Methanol in Benzene Eluent, A_0S_4
Silica Column
Pentane Eluent, A_1S_0
4% Benzene in Pentane Eluent, A_1S_1
19% Benzene in Pentane Eluent, A_1S_2
52% Benzene in Pentane Eluent, A_1S_3
50% Methanol in Benzene Eluent, A_1S_4
Silica Column
Pentane Eluent, A_2S_0
4% Benzene in Pentane Eluent, A_2S_1
19% Benzene in Pentane Eluent, A_2S_2
52% Benzene in Pentane Eluent, A_2S_3
50% Methanol in Benzene Eluent, A_2S_4
Silica Column
Pentane Eluent, A_3S_0
4% Benzene in Pentane Eluent, A_3S_1
19% Benzene in Pentane Eluent, A_3S_2
52% Benzene in Pentane Eluent, A_3S_3
50% Methanol in Benzene Eluent, A_3S_4
Silica Column
Pentane Eluent, A_4S_0
4% Benzene in Pentane Eluent, A_4S_1
19% Benzene in Pentane Eluent, A_4S_2
52% Benzene in Pentane Eluent, A_4S_3
50% Methanol in Benzene Eluent, A_4S_4
Silica Column
Pentane Eluent, A_5S_0
4% Benzene in Pentane Eluent, A_5S_1
19% Benzene in Pentane Eluent, A_5S_2
52% Benzene in Pentane Eluent, A_5S_3
50% Methanol in Benzene Eluent, A_5S_4
Charcoal Column

Gel-permeation chromatography is another technique that has been used to fractionate mixtures. Gjessing and Lee eluted humic material from Sephadex G columns with water (85). The fractionation provided information about the molecular-weight distribution of macro-molecules present in natural waters. Low molecular-weight compounds were not separated by this technique.

A. R. Jones _et al_. used three solvents to elute organic compounds in crude oils from three 1-m x 5-cm columns packed with Sephadex LH-20 (86). The samples of crude oils were separated according to the scheme in Table 13. Because of the effects different solvents have on the elution behavior of compounds from Sephadex LH-20, three types of separations were performed on the Sephadex LH-20 columns. The first separation was the result of the gel being a support for a methanol/water mixture that retains hydrophilic compounds (87). To elute hydrophillic compounds from the first Sephadex LH-20 column, it was necessary to use methanol or acetone and swell the gel again in 85% methanol/water before reusing the column. Elution of Sephadex LH-20 with tetrahydrofuran (THF) produced separations as a result of molecular exclusion and hydrogen bonding (88). Nonpolar compounds were separated according to the principles of gel-permeation chromatography, while compounds that hydrogen bonded with the gel were retained longer than the nonpolar

Table 13. Fractionation scheme for crude oils by A. R. Jones *et al.*



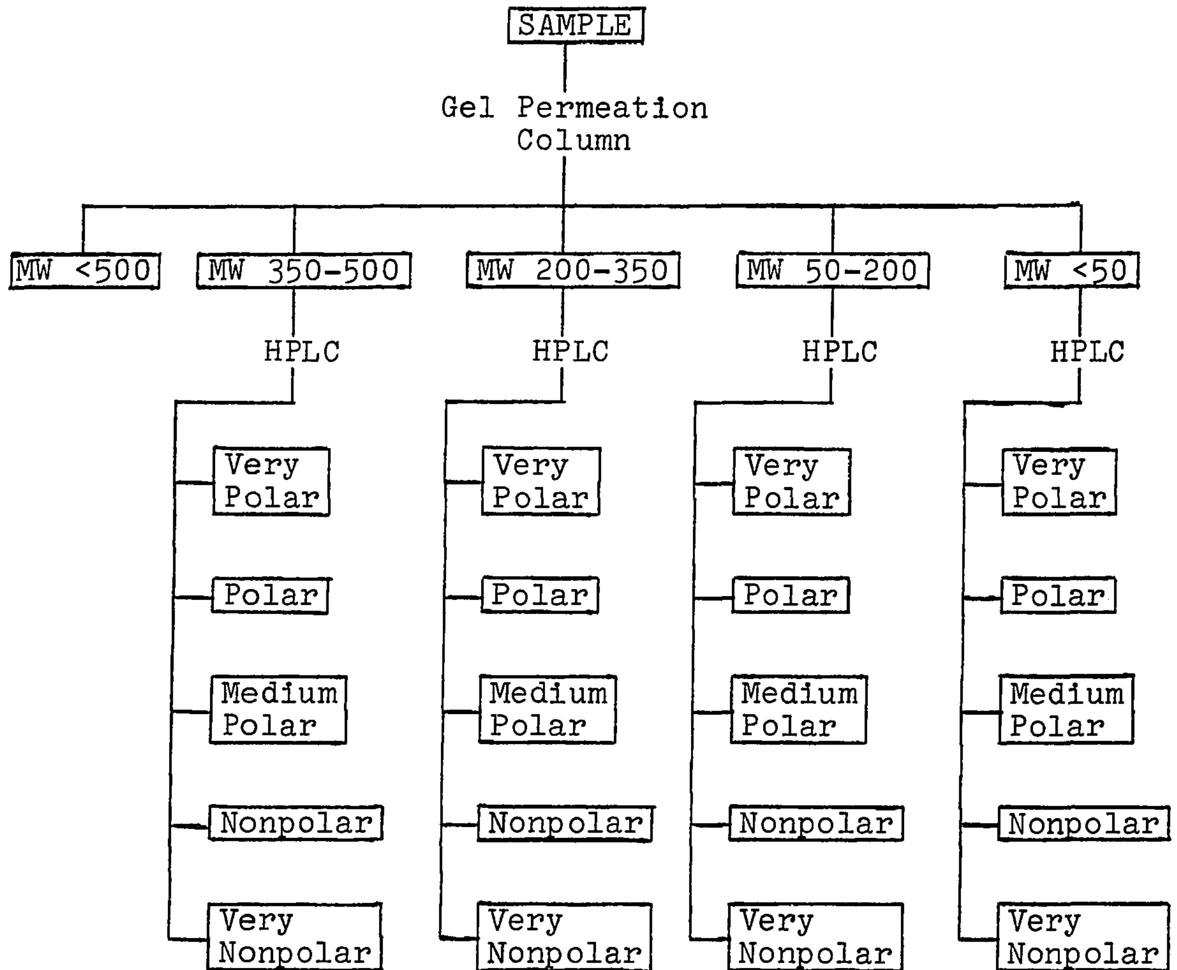
compounds. The third separation occurred by the mechanisms of size exclusion and π-bonding to the gel (89). Aliphatic hydrocarbons eluted from the column according to molecular sizes. Aromatic hydrocarbons were retained on the third column by π-bonding and eluted in order to increasing aromaticity. Sample sizes from 10-300 g were separated on the columns. The scale of the scheme was preparative to allow biological testing of the fractions. Elution of the columns was performed by pumping solvents at a flow rate of approximately 500 ml/hr. The volumes collected for each fraction ranged from 250 ml to 6 l. The resolution between fractions was not great; hence, some compounds were present in more than one fraction. Because the compounds of interest were hydrocarbons, the elution behavior of other compounds was not studied. Studies have shown that little losses of polar and reactive compounds occurs when samples are chromatographed on Sephadex LH-20 (70).

Giger and Schaffner used columns packed with Sephadex LH-20 and silica gel to fractionate environmental samples prior to analysis by capillary-column gas chromatography (90). Extracts were separated on a 50-cm x 1.6-cm column packed with Sephadex LH-20. Organic compounds were eluted from the column with 50% benzene in methanol. The first 50-ml fraction contained compounds that could not π-bond to the Sephadex LH-20. Compounds that π-bonded to the Sephadex

LH-20 were collected in the second 50-ml fraction. After reducing the volumes of the two fractions that were obtained by GPC, the fractions were added to 13-cm x 1-cm columns packed with Kieselgel 40 silica gel. A fraction eluted with 25 ml of pentane and a fraction eluted with 25 ml of methylene chloride were collected from the silica gel columns. The volumes of the fractions were reduced, then the fractions were analyzed by gas chromatography. Although only four fractions were obtained from this scheme, good information was provided about the components of each fraction. Chromatography on the Sephadex LH-20 separated the samples into nonaromatic and aromatic fractions, while chromatography on silica gel separated the samples into polar and nonpolar fractions. Using these solvents, incomplete elution of sample components and cross contamination of samples on Sephadex LH-20 column occurred.

P. W. Jones et al. developed a scheme for fractionating extracts of process streams using gel-permeation chromatography (GPC) and high-performance liquid chromatography (HPLC) (80). The combination of these chromatographic techniques allows for the sample to be separated into fractions determined by both molecular weight and molecular polarity. Table 14 shows the scheme suggested by Jones et al. In the description of the technique, details were not given in regard to column packings and fraction sizes. Important

Table 14. Fractionation scheme for process stream developed by P. W. Jones *et al.*



factors such as recovery, reproducibility, and sample contamination would have to be examined to determine the applicability of the scheme to the analysis of samples on a routine basis. The combination of GPC and HPLC seems to be an effective way of obtaining much information about the components of samples. By separating samples by GPC before introducing the samples to a HPLC column, contamination of the reverse-phase packing should be minimized and the useful life of the expensive column extended. The instrumental costs and the preparation time required to perform both GPC and HPLC separations on samples are apparent disadvantages of the scheme.

A major disadvantage in using most of the described fractionation schemes for analysis of water extracts is that much time would be required to analyze all of the fractions. Chromatograms on high-performance capillary columns for gas chromatography may take as long as two hours to obtain. Although more information can be derived about sample components by fractionation schemes yielding many fractions, the time required to analyze the fractions must be considered.

Selection of fractionation technique for water extracts

After evaluating some fractionation schemes, it appears that no fractionation scheme can completely satisfy the properties listed for water extracts. To obtain a balance between analysis time and the information obtained, a

gravity-flow chromatography technique was selected. Three fractions collected from small columns packed with an absorbent will provide information about the sample components without adding unnecessarily to the analysis time of water extracts.

Silica gel, alumina, and Florisil are the three most common adsorbents for adsorption chromatography. Various grades and activities of silica gel and alumina have been prepared. Snyder has shown the adsorption characteristics of these adsorbents can be tailored to fit various needs by deactivation with water (81,91,92,93). Although the properties of silica gel, alumina, and Florisil are very similar, a number of advantages exist for the use of Florisil to fractionate water extracts.

Florisil is commonly used to fractionate and clean-up complex mixtures (74,94). The success of the fractionation scheme for pesticide analysis indicates the usefulness of Florisil fractionations for organic compounds. Florisil fractionation of pesticides has been useful for providing information about the pesticides present in samples and for the removal of components that interfere with the gas-chromatographic determination of the pesticides. The simple technique developed by Mills for measuring the activity of Florisil (75) makes elution behaviors of compounds reproducible for columns packed with Florisil. For these

reasons, Florisil was the adsorbent selected for fractionating water extracts.

Mutagenic testing

Mutagens have been determined in extracts of drinking waters (95). Mutagens can be detected in samples by a test developed by B. N. Ames *et al*. (73,96). Mutations of various strains of *Salmonella typhimurium* indicate the presence of mutagens in a water sample. The mutagenic activities of compounds, as determined by the Ames Test, have been correlated to the carcinogenic potential of the compounds (97,98). The strains of *Salmonella* used by Ames were histidine auxotrophs, which means they were unable to produce histidine. The histidine auxotrophs could not reproduce without having histidine present in their environment. Some substances caused mutations in these strains of *Salmonella* which made the bacteria able to produce histidine. The histidine producing bacteria, histidine prototrophs, could grow and reproduce. By culturing these strains of bacteria with some nutrients and a finite amount of histidine, the population of the culture was limited by the amount of histidine present. If mutagens were introduced to the culture, the population was proportional to the number of mutations from histidine auxotrophs to histidine prototrophs caused by the mutagens. The mutagenic

activities of numerous compounds (97,98), cigarette-smoke condensates (73,99), and shale oil (100) have been evaluated using the Ames Test.

Experimental

Apparatus and reagents

Gas chromatography A Tracor model 550 gas chromatograph equipped with an automatic linear temperature programmer, a flame ionization detector (FID), and a linearized electron capture detector (ECD) was used for this work. Modifications as previously described were implemented to operate the chromatograph with WCOT capillary columns and simultaneous detection with both detectors. Chromatograms were recorded with a Fisher Recordall series 5000 strip chart recorder.

Gravity-flow columns Glass columns of 8-mm o.d., 6-mm i.d. were fabricated. At one end of a 14-cm segment of 8-mm glass tubing, a 1.5-cm segment of 4-mm glass tubing was attached. An 8-cm segment of 22-mm glass tubing was attached to the remaining end of the 8-mm glass tubing. The 22-mm glass tubing functioned as a reservoir for solvents.

Solution concentrators A solution concentrator fitted with a Snyder column was used to reduce the volumes of solutions by evaporative distillation. A diagram of a solution concentrator and Snyder column is shown in Figure 13.

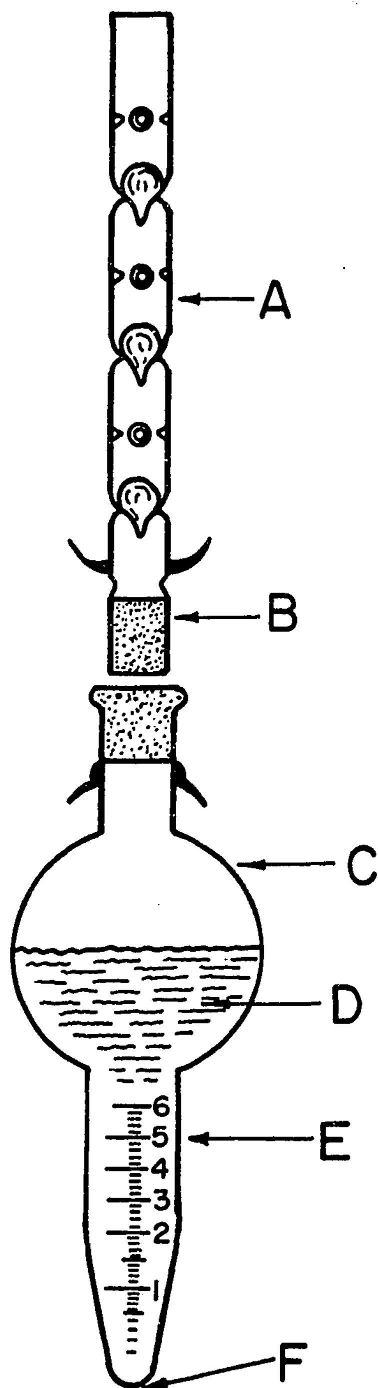


Figure 13. Solution concentrator: (A) Snyder distillation column; (B) 14/20 standard taper; (C) 50 ml flask; (D) solution; (E) graduated and calibrated taper; (F) boiling chip

The concentrators were fabricated after the design described by Junk _et al_. to minimize the loss of compounds during concentration (6). Longer sections from calibrated centrifuge tubes were used than previously described to allow measurement of sample volumes in the range of 0.5-5.0 ml.

Florisil Two batches of pesticide grade Florisil (a product of Floridin, Pittsburgh, Pennsylvania) were obtained from Fisher Scientific Company, Fair Lawn, New Jersey. Florisil was stored in a drying oven at 130°C for at least 24 hrs prior to use in the gravity-flow column or measuring its activity.

Amberlite XAD-2 resin Amberlite XAD-2 resin, a macroporous styrene-divynylbenzene copolymer, was obtained from Rohm and Haas, Philadelphia, Pennsylvania. Soxhlet extraction was used to clean the resin before sampling waters (6).

Solvents Diethyl ether for water extracts was obtained from Fisher Scientific Company, Fair Lawn, New Jersey. The diethyl ether was redistilled to remove the sodium diethyldithiocarbamate present as a free-radical inhibitor.

Petroleum ether (30-60°C bp), methylene chloride, and acetonitrile used in solutions to elute organic compounds from Florisil columns were distilled-in-glass grade obtained from Burdick and Jackson Laboratories, Inc., Muskegon, Michigan.

Hydrofluoric acid, 48% in water, was obtained from Fisher Scientific Company, Fair Lawn, New Jersey.

Techniques and procedures

Determination of Florisil activity   The activity of Florisil used in this work was measured by the procedure developed by Mills (75). Two grams of Florisil and 20 ml of hexane containing 400 mg of lauric acid were stirred for 15 min. After allowing the Florisil to settle from the hexane solution, 10 ml of the hexane solution above the Florisil were pipetted into a beaker containing 50 ml of ethanol that had been neutralized to a phenolphthalein end-point. The lauric acid remaining in the 10 ml of hexane was titrated with 0.05 N aqueous sodium hydroxide solution. By dividing the amount of lauric acid sorbed on the Florisil by the weight of Florisil used, a value for the activity of the Florisil was obtained.

Fractionation procedure

Preparation of Florisil columns   A quantity of Florisil was weighed out to give a total activity of 125 mg lauric acid as measured by the procedure developed by Mills. If a batch of Florisil had an activity of 100 mg lauric acid per gram, 1.25 g of Florisil was weighed out to prepare a column.

A plug of Pyrex glass wool, approximately 1 cm thick, was placed at the bottom of a gravity-flow column. The

correct amount of Florisil was added to the column and was made to settle by gently rapping the column. Another 1-cm plug of Pyrex glass wool was placed on the top of the Florisil bed.

Prior to addition of a sample to the column, 15 ml of petroleum ether was added to the column and the effluent from the column was discarded.

Elution of compounds from Florisil columns

Compounds were eluted from the Florisil columns in three fractions according to the following steps:

1. When the level of petroleum ether used to prepare the column reached the top of the glass-wool plug, the sample was pipetted onto the column.

2. A concentrator was placed under the column and collection of the first fraction started.

3. When the level of liquid in the column again reached the glass-wool plug, 25 ml of a 2% (volume/volume) solution of methylene chloride in petroleum ether was added to the column.

4. When the level of liquid approached the glass-wool plug, 25 ml of a 50% (volume/volume) solution of methylene chloride in petroleum ether was added to the column.

5. Immediately after addition of the 60% solution, an empty solvent concentrator was placed under the column to collect the second fraction.

6. As the level of the solution in the column again reached the glass-wool plug, 25 ml of a petroleum ether solution containing 60% (volume/volume) methylene chloride plus 2% (volume/volume) acetonitrile was added to the column.

7. After addition of the 60% plus 2% solution, an empty solution concentrator was placed under the column to collect the third fraction.

Fraction concentration After collecting the fractions from the Florisil columns, the volume of each fraction was reduced to the volume of the initial sample. After adding a couple of boiling chips to each solution concentrator, the solutions were heated to a boil. When the desired volumes were achieved, the solution concentrators were removed from the heat and allowed to cool. Fractions were removed from the solution concentrators and stored in glass vials with Teflon-faced septa under refrigeration.

Dissolution and extraction of Florisil with hydrofluoric acid To test for compounds that were not eluted, Florisil was dissolved and extracted by a method similar to that used by Kukreja and Bove (101). The steps of the dissolution and extraction procedure are as follows:

1. The glass-wool plug was removed from the top of the Florisil bed.

2. By rapping the side of the column, the top inch of Florisil was emptied from the column into a 50-ml glass beaker.

3. Slowly 25 ml of hydrofluoric acid was added to the beaker, then stirred periodically during the next five minutes.

4. The contents of the beaker and 25 ml of petroleum ether were added to a separatory funnel. The contents of the separatory funnel were shaken, being careful to vent off gas pressure that builds up.

5. After two layers formed, the lower layer was removed into the 50-ml beaker.

6. The upper layer was removed into a solution concentrator.

7. Steps 4, 5, and 6 were repeated and the upper layer was added to the same solution concentrator.

8. The volume of the petroleum ether solution was reduced as described above.

Model compound recoveries   Solutions containing between 1×10^{-8} and 1×10^{-6} g/µl of model compounds were made up in petroleum ether. The petroleum-ether solutions were introduced to Florisil columns and fractionated according to the described procedure. After decreasing the volume of each fraction, gas chromatograms of each fraction and the initial sample were obtained. To evaluate the total recovery efficiency of the fractionation scheme, the fractions from the Florisil column were combined before concentration.

Preparation of water extracts Extracts of water in diethyl ether were obtained by passing water through beds of XAD-2, eluting compounds from the XAD-2 with diethyl ether, and concentrating the volume of the effluent to a small volume (6). Prior to introduction to Florisil columns, the diethyl-ether solutions were placed in solution concentrators with approximately twenty times the volume of petroleum ether. A couple of boiling chips were added to each solution concentrator, a Snyder column placed on the concentrators, and the volumes of the solutions reduced to that of the initial solutions by boiling. The petroleum-ether solutions were then ready to be fractionated on columns packed with Florisil. If a precipitate formed, the precipitate was allowed to settle before removing the solution to be fractionated.

Mutagenicity testing The potential health hazards of waters were estimated by testing the extracts for mutagens by the procedure described by Ames, McCann, and Yamasaki (102). Work performed at Iowa State University indicates that organic compounds concentrated from some drinking waters were mutagenic (95,103). Samples in volatile solvents were added to 500 µl of dimethyl sulfoxide (DMSO). The volatile solvents were allowed to evaporate; concentrating the organic compounds into the DMSO. Mutagenic activities of the DMSO solutions were determined by adding 10 µl of the DMSO solutions to petri

dishes of agar seeded with Salmonella according to the procedure described by Ames, McCann, and Yamasaki (102). *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were used for testing the samples. Positive or mutagenic activities were recorded if the number of colonies on the test plates were at least twice the number of colonies on solvent control plates. Marginal positives were recorded if only small increases in the numbers of colonies were observed.

Results and Discussion

Development of analytical technique

The usefulness of fractionating water extracts on Florisil columns was evaluated. Various parameters that affect the separation on Florisil were examined.

Selection of solvent system The widely used technique for fractionating pesticide samples on Florisil by eluting with solutions of petroleum ether and diethyl ether (74) was applied to fractionation of mixtures of model compounds. The data in Table 15 indicate the poor resolution obtained by eluting small columns that were packed with Florisil with mixtures of diethyl ether and petroleum ether. Because compounds eluted faster from the smaller columns, the fraction of each compound in the mobile phase was significant. The reproducibility of the fractionation with these solvents would

Table 15. Percentage of model compounds in fractions eluted from Florisil with diethyl ether in petroleum ether

Compound Column	Tridecane		1-Octanol		2-Undecanone		1-Chlorododecane		Naphthalene		Benzyl Alcohol	
	A[a]	B[b]	A	B	A	B	A	B	A	B	A	B
Fraction[c]												
0%	83	100	0	0	0	0	22	100	0	9	0	0
10%	17	0	0	0	0	57	78	0	97	91	0	0
25%	0	0	0	100	0	43	0	0	3	0	0	63
50%	0	0	0	0	83	0	0	0	0	0	0	37
100%	0	0	100	0	17	0	0	0	0	0	100	0

[a]Column bed 2.2 x 10 cm.

[b]Column bed 1.5 x 10 cm.

[c]100 ml of eluent per fraction, percentage indicates the amount of diethyl ether in the eluent used to collect the fraction.

be dependent on the reproducibility of fraction volumes. To ensure reproducibility of elution behavior, the number of bed volumes of each eluent would have to be reproduced. Rather than accurately reproduce the amount of eluent used, it would be preferable to use eluents that greatly changed the distribution coefficients of compounds. If a compound is to be present in a fraction, the solvent used to collect the fraction should cause the compound to have a very small distribution coefficient. With solvents not used to elute the compound from the column, the distribution coefficient should be very large.

Distribution coefficients (D) for some model compounds between Florisil and various solvents are shown in Table 16. Approximate values for distribution coefficients were obtained by collecting fractions from Florisil columns. Small fractions from gravity-flow columns were collected, then the fractions were analyzed by gas chromatography. By measuring the peak heights for compounds in each fraction, the retention volume (V_R) for each compound was approximated. After measuring the void volume of the column (V_V), the distribution coefficient was calculated from the equation below. The great differences in distribution coefficients

$$D = \frac{V_R - V_V}{V_V}$$

Table 16. Distribution coefficients (D) for some model compounds between Florisil and various solvents

Compound	Petroleum Ether	10T Diethyl Ether	10% CH_2Cl_2	40% CH_2Cl_2 2% CH_3CN
d-Linonene	↑	↑	↑	↑
Tridecane				
Butylbenzene	<4	<4	<4	
Bromobenzene				
3,4-Dimethyl-chlorobenzene				
1,2-Dichlorobenzene				
1-Chlorododecane	↓			
Naphthalene	5			
Biphenyl	16	↓		<6
Isopentyl Benzoate	↑	6		
Butrophenone		9		
Benzaldehyde		23	↓	
2-Undecanone	>50	24	↑ >50	
4-Methylcyclo-hexanone		↑		
Cyclohexanol		not run		
1-Octanol				
Methyl Undecylenate				
4-Methylacetophenone				
Benzyl Cyanide				
4-Methylphenol	↓	↓	↓	↓

obtained with 10% methylene chloride in petroleum ether vs 40% methylene chloride plus 2% acetonitrile in petroleum ether indicate that elution with these solvents would minimize the variation of elution behaviors of compounds. The effects of sample volume, Florisil activity, fraction size, eluent composition, etc. on the elution behaviors of compounds would be minimized if the distribution coefficients of compounds are greatly different with the solvents used for elution of the column.

Snyder has measured the eluent strengths (ε°) of many solvents for different sorbents (91,93,104). The eluent strength of a solvent is a measure of the ability of a solvent to elute strongly sorbed compounds. A list of the eluent strengths of some solvents as determined by Snyder for Florisil is in Table 17. The eluent strength of a solvent of

Table 17. Elution strengths (ε°) of some solvents for Florisil

Solvent	ε°
Pentane	0.00
Carbon Tetrachloride	0.07
Benzene	0.28
Chloroform	0.31
Methylene Chloride	0.37
Diethyl Ether	0.49

mixed composition can be calculated by summing the fraction of eluent strength provided by each component of the mixture.

Additional solvents containing petroleum ether, diethyl ether, methylene chloride, acetonitrile, methanol, benzene, and acetone were evaluated for eluting organic compounds from Florisil. Results showed that even small amounts of acetone or acetonitrile in a solvent greatly increased the elution strength of a solvent. From these experiments, the three solvents that caused the distribution coefficients for compounds to change greatly were selected for elution of the Florisil columns. In order of their addition to the Florisil, the solvents that were selected for elution of the Florisil columns were: 2% methylene chloride in petroleum ether, 60% methylene chloride in petroleum ether, and 60% methylene chloride plus 2% acetonitrile in petroleum ether. The Florisil columns were eluted with 25 ml of each of the solvents.

Effects of water in samples The distribution coefficients of compounds onto Florisil from organic solutions vary with the water content of the Florisil (93). Water is strongly adsorbed onto the surface of Florisil, altering the activity of the Florisil. Approximate distribution coefficients obtained by collecting fractions from 6-mm x 10-cm gravity-flow columns are listed in Table 18. The data in Table 18 indicate that water present in a sample has only small affects on the elution behaviors of model

Table 18. Variation in distribution coefficients (D) on Florisil with water present[a]

Compound	D			
	0 µl H_2O	50 µl H_2O	200 µl H_2O	500 µl H_2O
Bromobenzene	0.85	1.1	2.0	1.1
Butylbenzene	4.6	3.4	3.9	2.5
Naphthalene	4.4	3.3	2.9	2.5
Dodecane	2.3	2.2	2.0	1.1
1-Tetradecane	0.7	0.8	0.8	2.3
1-Chlorodecane	4.4	3.5	3.0	1.7
Hexadecane	6.4	4.5	6.4	6.8

[a]Column - 6 mm packed with 1.2 g Florisil (activity = 109 $\frac{\text{mg lauric acid}}{\text{g}}$).

compounds. Because the solubility of water in hexane is approximately 1.38×10^{-4} g/ml (105), the amount of water introduced to a column of Florisil by adding samples in petroleum ether should have negligible effects on the retention behavior of compounds in the sample.

Effects of Florisil activity The effectiveness of adjusting the amount of Florisil used in a column, based upon the activity of the Florisil, was evaluated. A technique developed by Mills was used to measure the activity of

Florisil (75). Distribution coefficients ($D = \frac{t_R - t_V}{t_V}$) calculated from retention times (t_R) on Florisil columns of 6-mm i.d. are listed in Table 19. A Chromatronix Cheminert metering pump and a Chromatronix Model 220 UV detector were used to determine the retention times of model compounds. The time for a nonretained substance to reach the detector (t_V) was determined by introducing hexane to the column. By dividing the activity of the Florisil bed by a compound's distribution coefficient, the activity of Florisil per distribution coefficient unit (activity/D) was obtained. The ratios of activity per distribution coefficient unit for the low-activity Florisil *versus* the high-activity Florisil are shown in the last column of Table 19. Because these ratios are near unity, the retention behavior of compounds can be reproduced by adjusting the amount of Florisil packed into the column. If the total activity of Florisil in a column is equal to the total activity of Florisil in another column, the elution behaviors of compounds on the two columns will be the same.

Elution behaviors of model compounds Elution behaviors of organic compounds were determined by fractionating samples of model compounds on Florisil columns. The compounds in each fraction were determined by gas chromatography. Chromatograms for a sample of model compounds and fractions from the sample are in Figure 14. Except for

Table 19. Retention behavior of compounds on different activities of Florisil

Compound	Solvent	Low-Activity Florisil[a] D[c]	Low-Activity Florisil[a] Activity/D[d]	High-Activity Florisil[b] D	High-Activity Florisil[b] Activity/D	Ratio D/Activity[e]
Benzene	pet. ether	0.90	210	2.0	188	1.12
Bromobenzene	pet. ether	0.60	315	1.0	375	0.84
Naphthalene	pet. ether	2.9	65	8.2	46	1.41
	2% CH_2Cl_2	1.4	135	2.8	134	1.01
Nitrobenzene	60% CH_2Cl_2	1.6	118	3.2	117	1.01
3-*tert*-Butylphenol	60% CH_2Cl_2	1.8	105	4.2	89	1.18
Benzyl Alcohol	60% CH_2Cl_2	2.4	78	7.6	49	1.60

[a]Florisil activity equal to 77 mg of lauric acid sorbed per gram. Column of 6 mm i.d. was packed with 2.45 g of Florisil to make total activity of the column equal to 189 mg of lauric acid.

[b]Florisil activity equal to 109 mg of lauric acid sorbed per gram. Column of 6 mm i.d. was packed with 3.44 g of Florisil to make total activity of the column equal to 375 mg of lauric acid.

[c]Distribution coefficient.

[d]Activity of Florisil bed divided by the distribution coefficient.

[e]Activity per D unit for the low-activity Florisil divided by the activity per D unit for the high-activity Florisil.

Figure 14. Chromatograms from a mixture and fractions from the mixture: (a) separation of model compounds in a mixture; (b) separation of compounds in first fraction; (c) separation of compounds in second fraction; (d) separation of compounds in third fraction. Chromatographic conditions:

Column - WCOT, Carbowax 20M stationary phase, 28 m x 0.25 mm i.d.

Temperature - Isothermal at 80°C for 4 min, then increased temperature at 4°C/min. Maximum temperature of 200°C held for 4 min

Carrier gas - 30 cm/sec of helium

Sample introduction - 2 µl, 30 sec spitless

Make-up gas - 100 ml/min of nitrogen

Backflush gas - 20 ml/min of helium

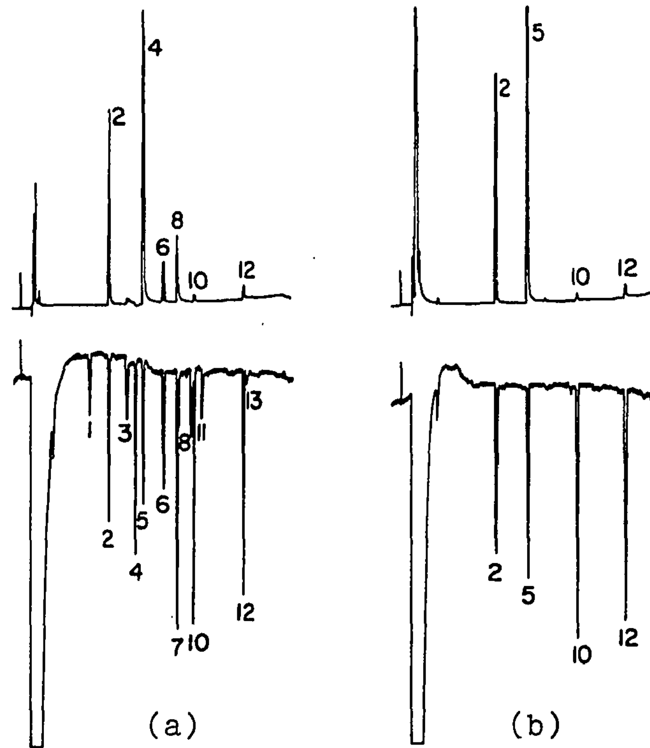
ECD purge gas - 65 ml/min of nitrogen

Detection - Top: ECD, attenuation = 8
Bottom: FID, attenuation = 8

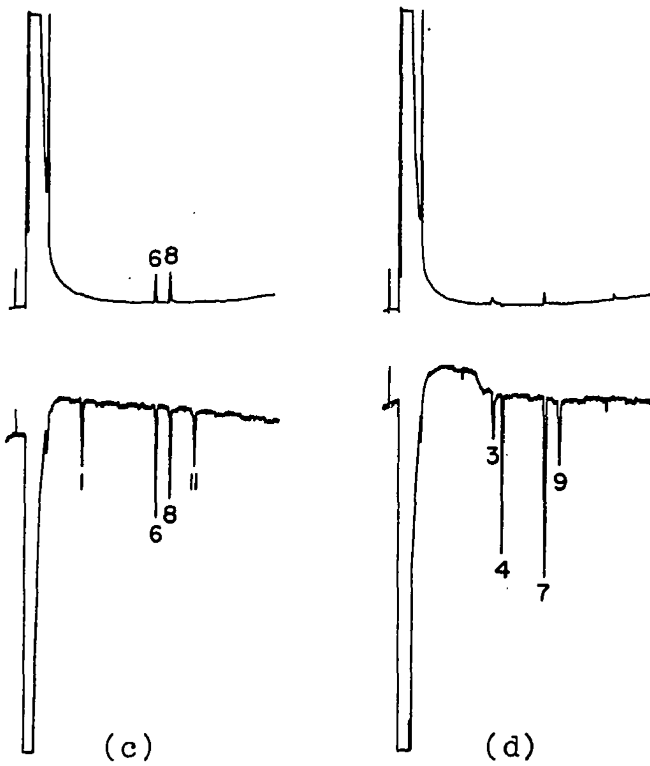
Exit-split ratio (ECD:FID) - 2:5

Sample components - 6×10^{-9} g/µl each

Peak Number	Compound
1	2-Ethylhexyl acetate
2	1,2-Dichlorobenzene
3	1-Octanol
4	Benzyl nitrile
5	1,2,4-Trichlorobenzene
6	Nitrobenzene
7	Benzothiophene
8	*n*-Butyrophenone
9	Benzyl alcohol
10	1-Methylnaphthalene
11	Isopentyl benzoate
12	Acenaphthalene
13	Tributyl phosphate



4
2
5
8
6
10
12
1
3
13
8
11
6
5
2
4
12
7
10
(a)
2
5
10
12
(b)



6
8
1
11
3
9
4
7
(c)
(d)

tributyl phosphate, all of the organic compounds in the mixture were present in one of the fractions. Table 20 lists compounds according to the fraction they were eluted from Florisil. Of all the organic compounds introduced to the Florisil columns, tributyl phosphate was the only compound not eluted by the solvents. Of the compounds listed in Table 20, only a few compounds were present in more than one fraction. Interpretation of chromatograms obtained from samples fractionated by this technique was simplified by the reproducibility and the resolution obtained.

For clarity, the fractions collected from the Florisil columns will be referred to in an abbreviated form. The fraction eluted from Florisil with 2% methylene chloride in petroleum ether will be referred to as the first fraction. The fractions eluted with 60% methylene chloride in petroleum ether and 60% methylene chloride plus 2% acetonitrile in petroleum ether will be referred to as the second fraction and third fraction, respectively.

The first fraction contained hydrocarbons and halogenated hydrocarbons. The eluent strength of the first solvent was low; thus, only nonpolar compounds that were weakly retained by Florisil were eluted from the column with the first solvent.

The boundary between the second and third fractions was not as well defined as the boundary between the first and

second fractions. Compounds having the following functional groups were eluted in the second fraction: nitro, carbonyl, or ester. Compounds found in the third fraction were alcohols, nitriles, and esters of dibasic acids. Elution behaviors of some classes of compounds were not well established by the data in Table 20. For example, some phenols, amines, and polyfunctional compounds were eluted in the second fraction, while other phenols, amines, and polyfunctional compounds were eluted in the third fraction.

Recovery Recoveries of compounds from samples were determined by measuring peak heights from chromatograms for samples before and after passing the samples through Florisil columns. Recoveries of model compounds and gas-chromatographable components from water samples were determined. Although large deviations were caused by volume measurements and peak height measurements, recoveries of most compounds from the Florisil columns were 80-120%. The complexity of chromatograms obtained from water extracts made accurate measurements of recoveries difficult.

It was discovered that small amounts of some polar compounds were not eluted from the Florisil columns. Approximately 10 μg of tributyl phosphate was present in a water extract. The tributyl phosphate in the water extract was not present in any of the fractions collected from the Florisil column, but the tributyl phosphate was recovered by

Table 20. Model compounds grouped according to the fraction the compounds eluted from Florisil

First Fraction	Second Fraction	Third Fraction
Hexadecane	Nitrobenzene	2,5-Hexanedione
Octadecane	1-Chloro-3-nitrobenzene	4-Methylcyclohexanone[a]
1-Chlorodecane	1-Nitronaphthalene	Methyl undecylenate
1-Bromododecane		2-(2-Butoxyethoxy)-ethyl acetate
1-Iodooctane	Phenylacetonitrile	Diethyl phthalate
Bromoform	2-Undecanone	Dibutyl phthalate
4-Methyldecane	Cyclohexanone[b]	Diethyl malonate
1-Tetradecene	*n*-Butyrophenone	1-Octanol
	4-Methyl acetophenone[b]	
d-Limonene	Benzaldehyde	Benzyl alcohol
Pinene	Dibutoxybenzene	2,4-Dichlorophenol
Biphenyl	2-Ethylhexyl acetate	N,N-Dimethylaniline
	Isopentyl benzoate	
Naphthalene	Phenol[b]	2,4-Dimethylaniline
Acenaphthalene		
Butylbenzene	3-*tert*-Butylphenol	N-Ethylaniline
1-Methylnaphthalene		
Bromobenzene	2,6-Dimethylphenol	Benzyl nitrile
α-Chloronaphthalene	1-Naphthylamine	Benzothiophene
1,2-Dichlorobenzene	Dibenzylamine	
1,2,4-Trichlorobenzene		
3,4-Dimethylchlorobenzene		
Polychlorinated biphenyls		
4-Iodotoluene		

[a]Some of this compound was found in the previous fraction.

[b]Some of this compound was found in the following fraction.

dissolving the Florisil in hydrofluoric acid. When 1 mg of tributyl phosphate was introduced to a Florisil column, greater than 90% of the tributyl phosphate was eluted in the third fraction. The greater retention of tributyl phosphate at low concentrations indicates that some heterogeneity of Florisil exists. Recoveries obtained for tributyl phosphate indicate that recoveries of other phosphorus and polar compounds may be incomplete.

Analytical determinations

Gas-chromatographic analysis Extracts of drinking waters from fifteen utilities were fractionated on Florisil columns. Water samples were obtained from fourteen cities that participated in an American Water Works Association (AWWA) sponsored water quality survey (106) and Ames, Iowa. Sampling was performed in the fall of 1976. The amount of water sampled from each city was approximately 400 liters. The organic compounds from 100-l. water samples were sorbed on 6-in x 5/8-in brass columns packed with Amberlite XAD-2. The organic compounds were eluted from the XAD-2 with 100 ml of diethyl ether. The diethyl-ether solutions from each city were combined and reduced to a volume of 5 ml by evaporative distillation. Half of the volume from the diethyl-ether solutions were taken for fractionation on Florisil columns.

The identities of the fourteen cities participating in the AWWA sponsored survey are to remain confidential. For this survey, it was agreed that results of this survey would remain confidential, unless the utilities themselves wished to release information obtained from this survey. In the following discussion, the water sources will be referred to as Cities #1-14.

Chromatograms obtained from two water extracts and fractions from those extracts are in Figures 15 - 24. The identities and concentrations of most of the components of these samples have not been determined, but the chromatograms demonstrate the separations that are possible by fractionating water extracts on small columns packed with Florisil.

Chromatograms of the organic compounds concentrated from City #1's water are in Figures 15 - 20. Chromatographic conditions for the separations in Figures 15 - 20 are given in Table 21. By comparing the chromatograms in Figure 17 to those in Figure 15, it can be concluded that the majority of compounds that give a large response on the electron capture detector (ECD) are nonpolar. Large peaks are observed for bromodichloromethane and dibromochloromethane using the ECD. The haloforms present in the water are probably formed during the treatment procedure by the action of chlorine on humic matter and bromide present in the water (27,28). Because halogenated hydrocarbons are the only nonpolar compounds

that give a large relative response on the ECD *versus* the FID, the chromatograms in Figure 17 indicate that only a small portion of the gas-chromatographable material in this water was haloforms.

Figure 16 demonstrates that only a small portion of the gas-chromatographable material was precipitated when changing the solvent from diethyl ether to petroleum ether. The total amount of precipitate formed for the extract from City #1 was 7 mg. The precipitate was dissolved in a volume of methanol that equaled the volume of the extract. A number of compounds were present in the second and third fractions from City #1.

The chromatograms in Figure 18 and Figure 19 contain peaks for a number of polar compounds that were extracted from the water of City #1. It is apparent by inspecting the chromatograms that it would be easier to determine a polar compound in this sample after fractionating than before fractionating the sample.

Tributyl phosphate present in the water extract from City #1 was not eluted from the Florisil column by any of the solvents. Figure 20 demonstrates that tributyl phosphate was recovered from the Florisil column by dissolving the Florisil in hydrofluoric acid, then extracting the hydrofluoric-acid solution with petroleum ether. As previously discussed, small amounts of tributyl phosphate were not eluted from the Florisil with the solvents used.

Table 21. Chromatographic conditions for Figures 15 - 20

Column - WCOT, SP1000 stationary phase,
25 m x 0.25 mm i.d.

Temperature - Isothermal at 60°C for 4 min, then increased temperature at 3°C/min. Maximum temperature of 220°C was held for 10 min.

Carrier gas - 27 cm/sec of helium

Sample introduction - 2 µl, 30 sec splitless

Make-up gas - 110 ml/min of nitrogen

Backflush gas - 20 ml/min of helium

ECD purge gas - 75 ml/min of nitrogen

Detection - Top: ECD, attenuation = 2
Bottom: FID, attenuation = 8

Exit-split ratio (ECD:FID) - 2:5

Known sample components -

Peak Number	Compound
1	Bromodichloromethane
2	Dibromochloromethane
3	Tributyl phosphate

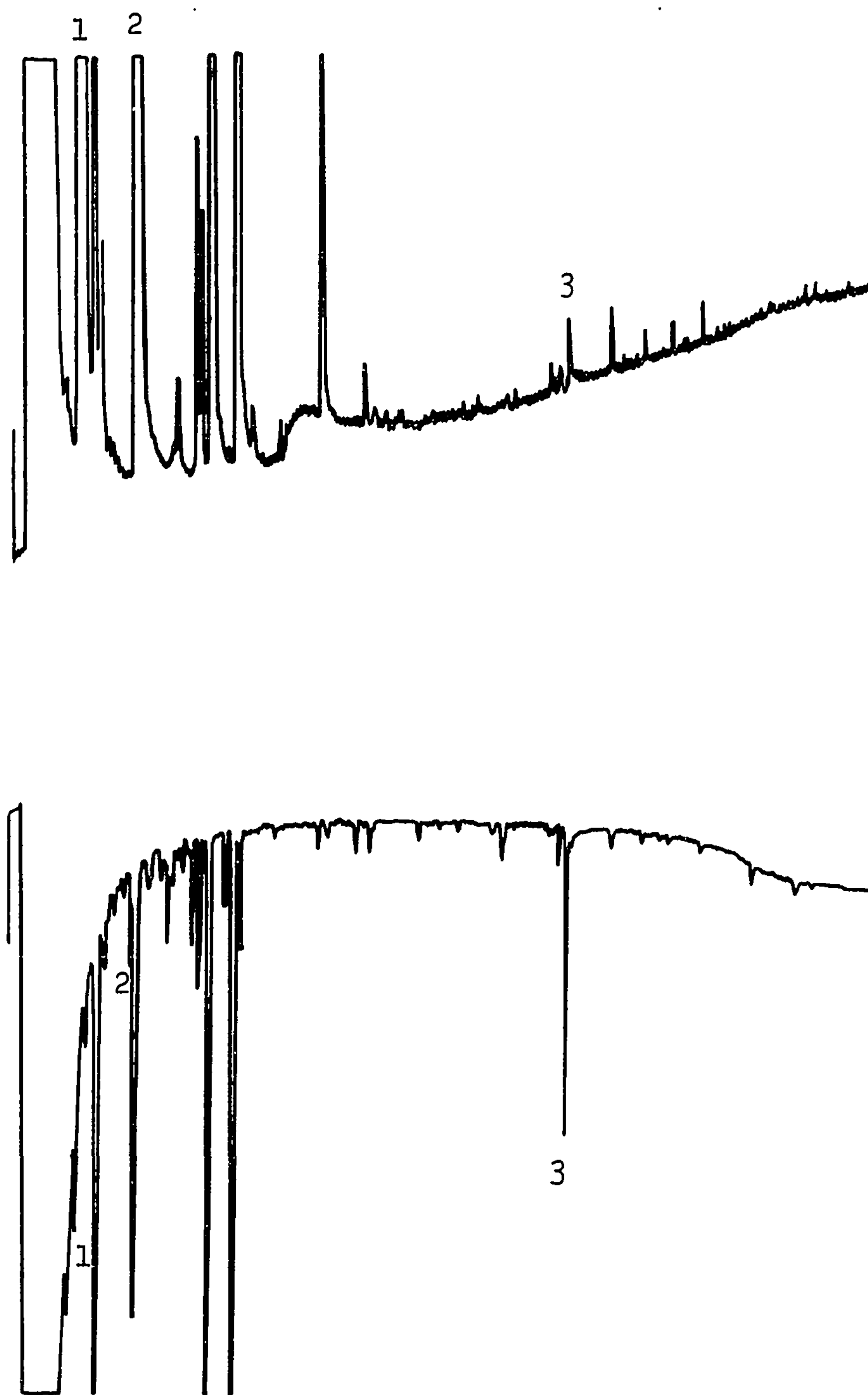


Figure 15. Separation of compounds extracted from City #1's water supply

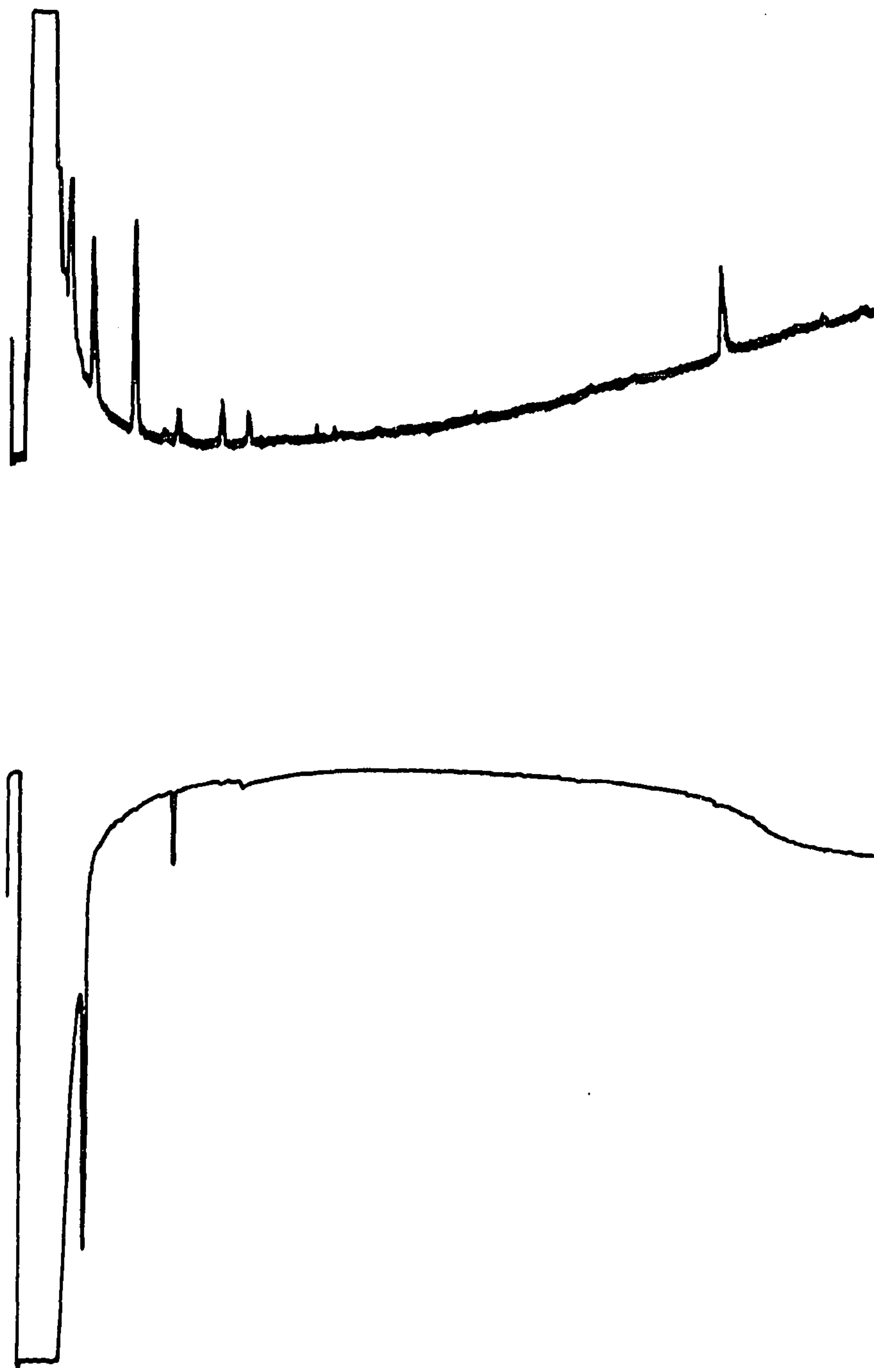


Figure 16. Separation of compounds from City #1's water supply that were not soluble in petroleum ether

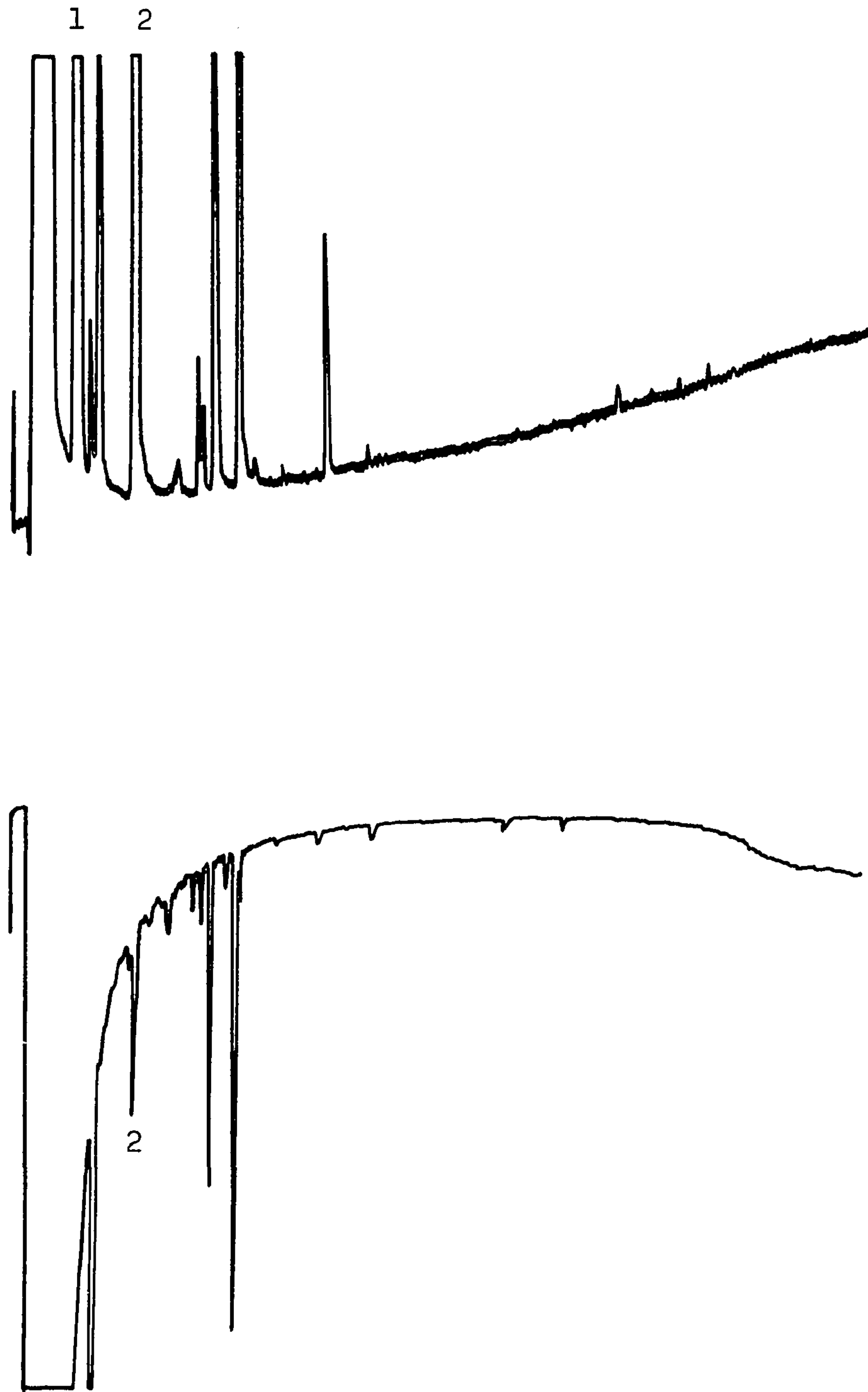


Figure 17. Separation of compounds from City #1's water supply that were eluted in the first fraction from the Florisil column

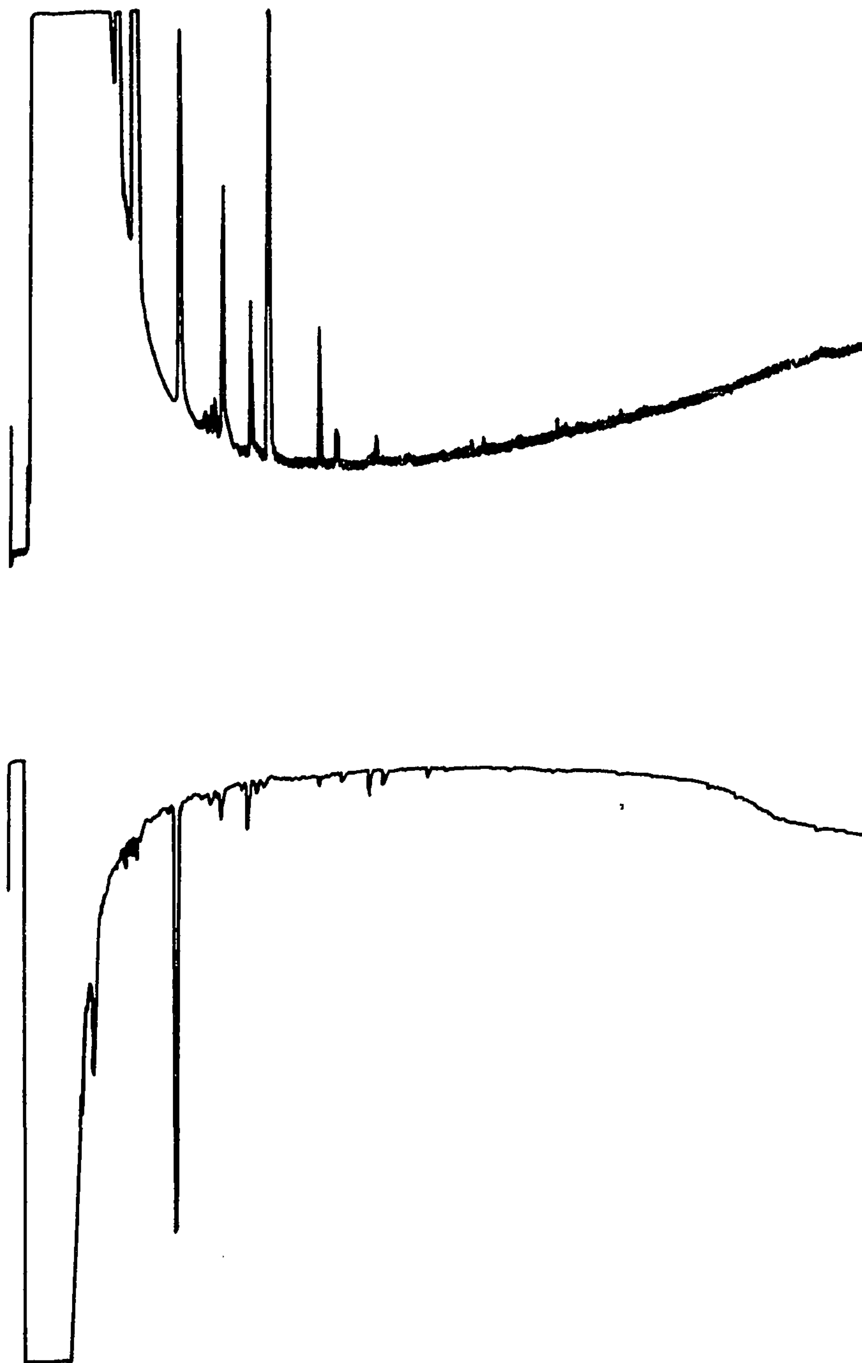


Figure 18. Separation of compounds from City #1's water supply that were eluted in the second fraction from the Florisil column

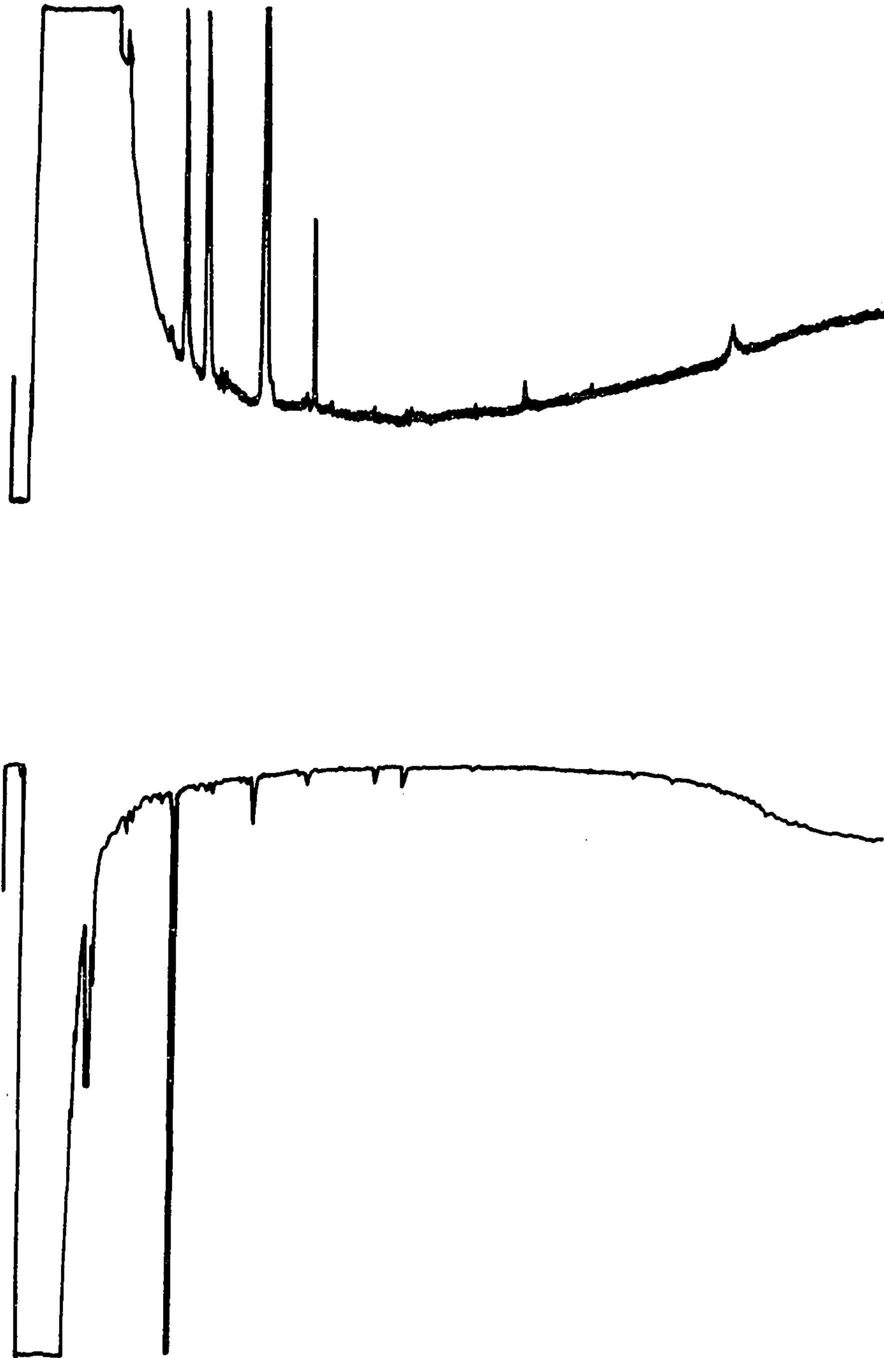


Figure 19. Separation of compounds from City #1's water supply that were eluted in the third fraction from the Florisil column

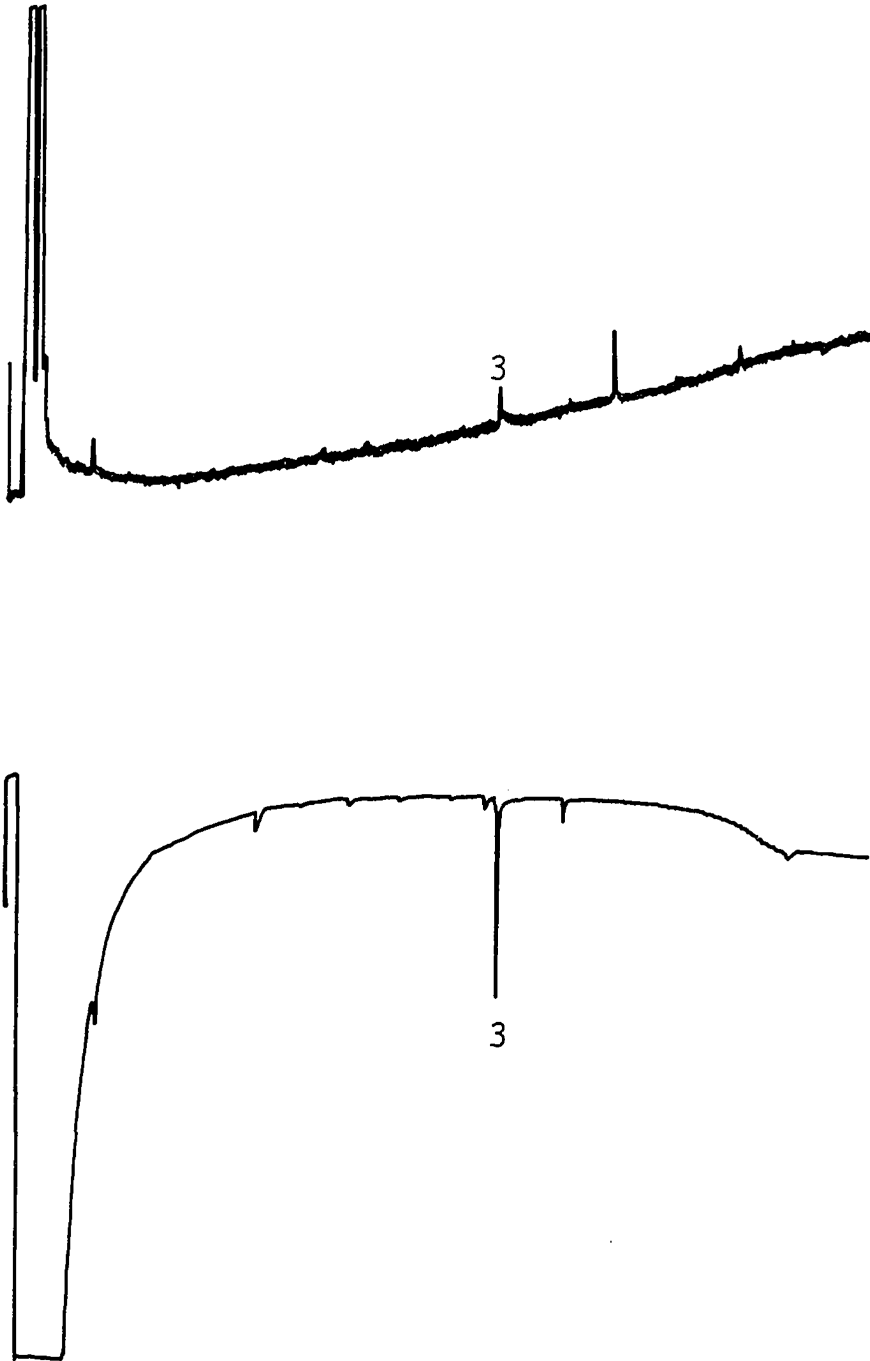


Figure 20. Separation of compounds from City #1's water supply that were recovered from Florisil by dissolution in hydrofluoric acid

Figures 21 - 24 are chromatograms of organic compounds concentrated from the water distributed in City #13. Table 22 lists the chromatographic conditions used to obtain the chromatograms in Figures 21 - 24. Presence of halogenated hydrocarbons in this water sample is indicated by the large responses obtained with the ECD in Figure 22. The presence of halogenated hydrocarbons in the first fraction is indicated by larger peaks using the ECD than FID in Figure 22. Assuming the sensitivity of the FID is about the same for all organic compounds, the bottom chromatogram in Figure 23 indicates that the majority of gas-chromatographable compounds from this extract were eluted in the second fraction. Because the majority of gas-chromatographable compounds were present in the second fraction, the majority of the gas-chromatographable compounds in the extract from City #13 were polar. The lack of response with the FID for the first fraction indicates that little nonpolar material was present in the extract of water from City #13. Chromatograms in Figure 24 show that only a small amount of the gas-chromatographable material from this water extract was eluted in the third fraction. Figures 23 and 24 indicate that a number of the polar compounds in the extract have a large cross-section for low-energy electrons; thus, peaks were observed using the ECD.

Table 22. Chromatographic conditions for Figures 21 - 24

Column - WCOT, Carbowax 20M stationary phase, 30 m x 0.25 mm i.d.

Temperature - Isothermal at 50°C for 4 min, then increased temperature at 3°C/min. Maximum temperature of 200°C was held for 6 min.

Carrier gas - 27 cm/sec of helium

Sample introduction - 2 μl, 30 sec splitless

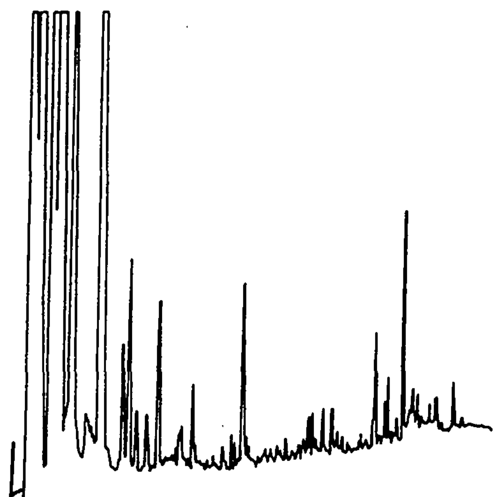
Make-up gas - 110 ml/min of nitrogen

Backflush gas - 20 ml/min of helium

ECD purge gas - 60 ml/min of nitrogen

Detection - Top: ECD, attenuation = 4
Bottom: FID, attenuation = 10

Exit-split ratio (ECD:FID) - 1:2



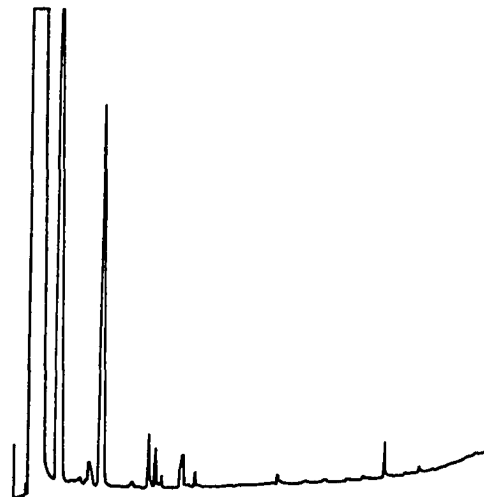
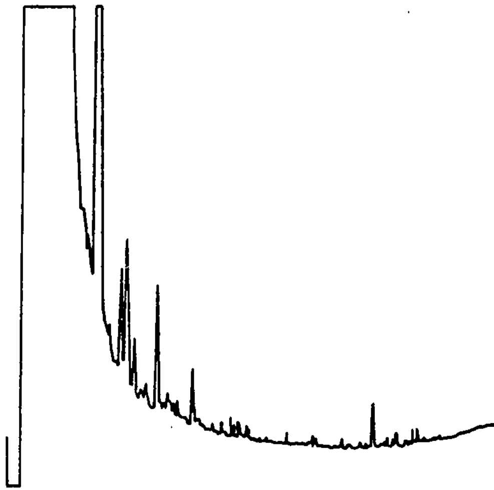


Figure 21.

Figure 22.

Figure 21. Separation of compounds extracted from City #13's water supply

Figure 22. Separation of compounds from City #13's water supply that were eluted from Florisil in the first fraction



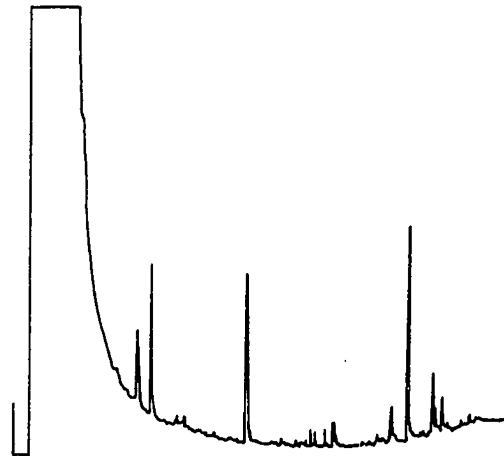


Figure 23.

Figure 24.

Figure 23. Separation of compounds from City #13's water supply that were eluted from Florisil in the second fraction

Figure 24. Separation of compounds from City #13's water supply that were eluted from Florisil in the third fraction

The results of fractionating extracts from the various cities were significantly different. For some of the water extracts, compounds were present in each of the three fractions from the Florisil columns. The gas-chromatographable compounds from some of the water extracts were completely eluted from Florisil in one of the fractions. Although the fractionation on Florisil was ineffective for separating the organic compounds from some of the water extracts, the fractionation provided information about the compounds in the samples. In some cases, the organic compounds from water extracts gave little or no response on the FID or ECD. Very little material was detected by the ECD or FID for water extracts from Cities #2 and 4. Because the amounts of gas-chromatographable matter in these extracts were low, the fractionation on Florisil was of little utility.

For most of the cities, the majority of organic compounds detected by gas chromatography in the water extracts were nonpolar. The vast majority of the peaks in the water extracts from City #10 and Ames, Iowa, were for nonpolar compounds. The chromatograms of the first fractions from these cities looked the same as the chromatograms of the water extracts. Only a few, small peaks were observed in chromatograms of the second and third fractions from these cities. Previous research has shown that most of the gas-chromatographable compounds in the drinking water of Ames,

Iowa, were aromatic hydrocarbons (5). Because aromatic hydrocarbons were nonpolar, most of the gas-chromatographable compounds in a water extract from Ames, Iowa, were eluted from the Florisil column in the first fraction.

Most of the compounds that gave responses on the FID from Cities # 5, 6, 7, 8, 11, 12, and 14 were present in the first fraction. Unlike City #10 and Ames, Iowa, significant amounts of the gas-chromatographable compounds were eluted in the second and third fractions. The compounds that gave a response on the ECD from Cities #8 and 12 were eluted in the first fraction, while the compounds that gave large FID responses were eluted in the second fractions. This information indicates that halogenated hydrocarbons and some polar compounds were present in the water from Cities #8 and 12. In the case of City #14, most of the compounds that eluted quickly from the gas-chromatographic column were present in the first fraction. Peaks for the compounds that eluted later from the gas-chromatographic column were present in the second fraction. Because a nonpolar column was used for the gas chromatography of samples from City #14, the information from the fractionation indicates that most of the volatile compounds in the water from City #14 were nonpolar, while most of the polar compounds in the water were less volatile.

The organic compounds that could be determined by gas chromatography from City #3 were polar. Only a few, small

peaks were observed in the first and second fractions from City #3. The vast majority of gas-chromatographable matter in the sample from City #3 was present in the third fraction. Because only small responses were observed with the ECD for compounds extracted from the water of City #3, apparently few halogenated compounds were present in this water.

The gas-chromatographable compounds in the water extract from City #9 were divided into three approximately equal portions by fractionating on Florisil. Numerous peaks were present in each of the fractions from City #9. Because of the complexity of the chromatograms for the water extract from City #9, the fractionation was useful for simplifying the chromatograms of the organic compounds. The fractionation of this water extract indicates that many types of compounds were present in the water distributed in City #9.

Some of the organic compounds present in water extracts were not present in any of the fractions because some of the compounds were not soluble in petroleum ether. When the solvents for the water extracts were changed from diethyl ether to petroleum ether, precipitates formed in many of the water extracts. Elemental analysis of the precipitate from City #1 showed the composition of the precipitate to be as follows: 65% carbon, 7.7% hydrogen, and 1.5% nitrogen. Although the composition of the precipitate was organic, the chromatograms in Figure 16 indicate that very little

gas-chromatographable matter was present in the precipitate. Attempts to gain information about the petroleum-ether insoluble matter by proton magnetic resonance (PMR) and infrared (IR) spectroscopy were unsuccessful. Using one-percent solutions of the precipitate, only short and broad peaks were obtained by PMR and IR spectroscopy. This information indicates that the precipitate was composed of high molecular-weight, polar compounds that are commonly referred to as humic matter.

Many water utilities have a problem with a musty or earthy odor in the water (107). Most of the water extracts from the cities participating in the AWWA survey had an earthy odor. After fractionating the water extracts, the earthy odor was only present or was the strongest in the second fraction. Geosmin and 2-methylisoborneol are two cyclic alcohols that impart an earthy odor to drinking waters (108,109). Chromatograms of some fractions that had an earthy odor had no peaks or only a few small peaks. Because the sensitivities for geosmin and 2-methylisoborneol with the FID are approximately 1×10^{-8} g, the amounts of geosmin or 2-methylisoborneol in the extract were very small or some other compounds were responsible for the odor.

<u>Mutagenic testing</u> Table 23 presents the results of mutagenic testing on raw and finished water extracts from various sources. Results in Table 23 indicate that the

mutagenic activity of a mixture of compounds is not the sum of the mutagenic activities of the compounds present in the mixture. Pelroy and Peterson reported the mutagenic activity of a mixture may be inhibited or enhanced by some compounds (100).

Results of mutagenic testing indicate that mutagenic activities of the organic compounds in drinking water may be increased by the treatment processes. In seven of the fourteen cities participating in the AWWA survey, the mutagenic activity of an extract from the finished water was greater than an extract from the raw water.

Data in Table 23 indicate the mutagenic activities of water extracts were not related to the mutagenic activities of any one fraction from the Florisil columns. Because chloroform is a mutagen (26), concern has developed over the fact that most drinking waters are treated with chlorine. Because the mutagenic activities of the water extracts were not correlated with mutagenic activities of the first fractions, the mutagenic activities of the water extracts was not the result of halogenated hydrocarbons present in the waters.

Although little gas-chromatographable material was present in the water extract from City #2, mutagenic activity was detected in the water extract from City #2. The compounds that caused the mutagenic activity from

Table 23. Mutagenic activities of selected water extracts

Source		Total	First Fraction	Second Fraction	Third Fraction	Precipitate
City #1,	Finished	+	-	-	+	-
	Raw	-	-	-	-	?
City #2,	Finished	+	?	-	?	-
	Raw	+	-	?	-	-
City #3,	Finished	+	?	-	?	-
	Raw	-	-	?	-	-
City #4,	Finished	+	-	-	-	-
	Raw	-	-	-	-	-
City #5,	Finished	?	-	?	-	-
	Raw	-	-	-	-	-
City #6,	Finished	?	-	-	-	-
	Raw	?	-	-	-	-
City #7,	Finished	+	-	-	-	-
	Raw	?	?	-	?	-
City #8,	Finished	-	-	?	-	-
	Raw	-	-	-	-	-
City #9,	Finished	-	-	-	-	-
	Raw	-	-	-	-	-
City #10,	Finished	+	-	-	-	-
	Raw	-	-	-	-	-
City #11,	Finished	+	?	?	-	?
	Raw	?	-	-	-	-
City #12,	Finished	+	-	-	+	-
	Raw	+	-	-	-	-
City #13,	Finished	+	+	+	-	-
	Raw	+	+	+	-	?
City #14,	Finished	-	-	-	-	-
	Raw	-	-	-	?	-
Ames, IA,	Finished	-	-	-	-	-

+ = Positive mutagenic activity by at least one strain of *Salmonella*.

? = Marginal mutagenic activity by at least one strain of *Salmonella*.

- = No observed mutagenic activity with any of the strains of *Salmonella*.

this water extract were probably not eluted from the gas-chromatographic column. Because only a small portion of the organic matter from water can be detected by gas chromatography, techniques such as liquid chromatography are needed to determine the mutagens present in the water extract.

Conclusions

The usefulness of Florisil fractionation as an aid to the analysis of water for trace organic compounds has been demonstrated by this work. Although the information obtained by the fractionation is not enough to determine the identities of compounds, the information provided by the fractionation and clean-up is useful when combined with other analytical techniques.

Application of the technique of fractionation is limited to cases where recovery of polar, high molecular-weight compounds, such as humic matter, is not important. Low solubility of humic matter in petroleum either and strong adsorption of humic matter on Florisil result in little humic matter being recovered in fractions from Florisil.

The combination of mutagenic testing and fractionation has demonstrated that compound identification and quantification is not necessarily the best approach to evaluating

the toxilogical effects of drinking waters. Even if the identities, concentrations, and potential health hazards of all components present in a mixture are known, the potential health hazard of the mixture is not known directly from this information. Because the mutagenic activities of compounds in a mixture are not accumulative, methods other than identification of compounds present in waters must be applied to determine the potential health effects of the organic compounds injested with drinking water.

SUGGESTIONS FOR FUTURE WORK

The potential of capillary-column gas chromatography with two different types of detectors for determination and verification of compound identities is great. If the outputs from a flame ionization detector and an electron capture detector or a photo ionization detector were connected to a microprocessor, retention times on capillary columns and relative response factors of sample components on two detectors could be obtained from a single chromatographic separation. By fractionating samples on Florisil columns prior to analysis on capillary columns with dual detection, enough information could be obtained to verify the presence of preselected compounds and determine their concentrations.

Further characterization of the organic compounds present in drinking waters could be accomplished by identifying compounds present in fractions by GC/MS. After determining the compounds that are present in fractions from water extracts, specific compounds could be determined in samples by fractionating the samples and analyzing the fractions by capillary-column gas chromatography with dual detection. This technique of analysis would be more rapid and sensitive, provide about the same quality of information for compound verification, and be much less expensive than capillary-column GC/MS.

Although the fractionation was developed to aid in the analysis of organic compounds present in drinking waters, it is applicable to other types of analysis. Air pollutants and waste-water effluents are areas which organic compounds in complex mixtures are analyzed by gas chromatography and in which fractionation and capillary-column chromatography with dual detection could be useful.

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