NBS TECHNICAL NOTE 889

U.S. DEPARTMENT OF COMMERCE / National Bureau of Standards

Trace Hydrocarbon Analysis:

The National Bureau of Standards Prince William Sound/Northeastern Gulf of Alaska Baseline Study

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FOREWORD

The objective of the National Bureau of Standards Analytical Chemistry Division is the development and/or improvement of analytical measurements. The Division has at its disposal the expertise of more than 80 scientists in a wide variety of disciplines. Over the years the Division has had a long-standing reputation for excellence in inorganic analysis. The increasing knowledge of, and concern over, the impact of trace organic substances upon man and his environment has necessitated an expansion of the Division's activities into trace organic analysis. The first substantial program undertaken in the area of organic environmental chemistry has been in the development of methods for the determination of petroleum hydrocarbons in sediments, water and biota. While much of this work has been and will be published in scientific journals, the NBS Technical Note series allows a more detailed description of the methodology employed and the problems that may be encountered than is possible in other modes of publication.

We believe that the work described in TN 889 will be of substantial value to those involved in determining petroleum hydrocarbons in the environment.

Philip D. LaFleur, Chief
Analytical Chemistry Division

CONTENTS

LIST OF FIGURES

LIST OF TABLES

TRACE HYDROCARBON ANALYSIS: THE NATIONAL BUREAU OF STANDARDS PRINCE WILLIAM SOUND/NORTHEASTERN GULF OF ALASKA BASELINE STUDY

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The low concentration of hydrocarbons anticipated in pollution baseline studies necessitates the development of analytical techniques sensitive at the sub-microgram per kilogram concentration level. The method of analysis developed in this laboratory involves dynamic headspace sampling for volatile hydrocarbon components of the sample followed by coupled-column liquid chromatography for the non-volatile components. These techniques require minimal sample handling, reducing the risk of sample component loss and/or sample contamination. Volatile sample components are separated from the matrix in a closed system and concentrated on a TENAX-GC packed pre-column, free from large amounts of solvent and ready for GC/GC-MS analysis. Non-volatile compounds, such as the benzpyrenes, may be extracted from large volumes of water and concentrated on a Bondapak C18 packed pre-column for coupled-column liquid chromatographic separation and analysis. Results of the application of these techniques are presented and discussed.

Key words: Baseline studies; gas chromatography; gas chromatography-mass spectrometry; hydrocarbons; liquid chromatography; petroleum analysis; trace analysis.

1. INTRODUCTION

1.1 GENERAL SUMMARY AND RATIONALE

The purpose of this investigation was to develop analytical techniques adequate to determine the low levels of hydrocarbons anticipated in pollution baseline studies in pristine environments. These techniques were to be sensitive at the sub-microgram per kilogram (ppb) level and suitable to handle the paramount problems of analytical blanks and component recoveries. Furthermore, it was desired that these analytical methods would ultimately permit the identification and quantitation of individual components among the hydrocarbons present in the samples.

To date several authors and groups have developed methods for the analysis of hydrocarbons in marine samples [1-6]. These methods are of three basic types: a screening method for total extractable hydrocarbons [1]; a headspace analysis method specific for low molecular weight hydrocarbons [3,5]; and a method involving digestion or extraction of the sample followed by clean-up and concentration procedures and gas chromatographic analysis of the hydrocarbon components from the sample [2,4,6]. Careful evaluation of each of these three basic methods revealed certain limitations when considered in terms of the goals of this study.

1) The screening technique for total extractable hydrocarbons was not sufficiently specific for this study; it would not permit single compound identification, an ultimate goal.

2) Extraction/digestion methods were generally lengthy, involved considerable handling of the sample and usually resulted in considerable losses of hydrocarbons with molecular weights of 200 and below. Hence these methods were not compatible with analyses for hydrocarbons present at the sub-microgram per kilogram levels.

3) Static headspace and extraction/digestion methods were generally utilized for molecular weight ranges other than the ones of primary interest in this study.

The scheme developed for the analysis of hydrocarbons in marine samples (see fig. 1) involves dynamic headspace sampling using a stream of purified nitrogen as a sweep gas for trapping the volatile components on a TENAX-GC packed precolumn for subsequent gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS). The non-volatile components are then analyzed by coupled-column liquid chromatography (LC). Various headspace sampling and coupled-column liquid

chromatographic techniques have been applied to other analysis systems [5,7-11] and have a number of advantages over the more classical techniques of solvent extraction and column chromatography, namely:

1) There is minimal sample handling.

2) Separation of components from the matrix occurs in closed systems which minimize both the risk of loss of trace level volatile constituents and the possibility of contamination by laboratory vapors and particulates.

3) The method affords efficient separation and concentration of the components of interest from the matrix. In particular, the headspace sampling technique is most efficient for naphthalene and substituted naphthalenes, while the coupled-column liquid chromatography is best suited for 3-6 condensed ring polynuclear aromatic hydrocarbons.

4) Water is the only solvent needed for sample preparation. The use of hydrocarbon-free water further reduces opportunities for contamination of the sample by fossil hydrocarbons usually found in organic solvents.

5) The sample components are concentrated in a form which is free of large amounts of solvents and ready for gas chromatographic or liquid chromatographic analysis.

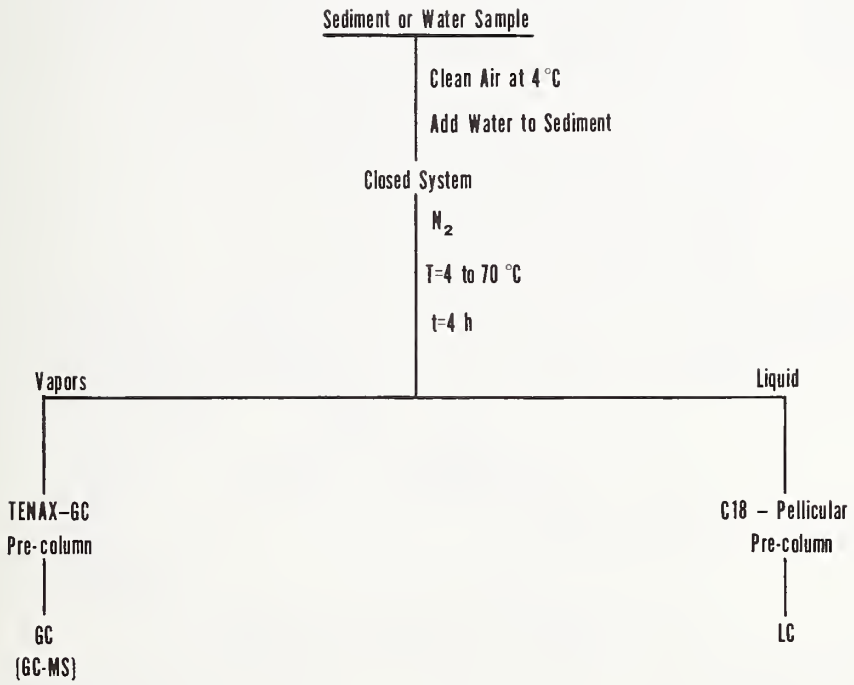

Figure 1. Scheme for analysis of hydrocarbons in marine sediments and water. Samples are headspace sampled to trap volatile components on a gas chromatographic pre-column. Non-volatile components are subsequently pumped through and collected on a liquid chromatographic pre column.

In order to analyze materials at the sub-microgram per kilogram level one must avoid significant contamination of the sample during collection, storage and analysis. Much effort must therefore be expended to insure that such contamination does not take place. An extensive procedure has been developed for cleaning all bottles and tools that come in contact with the samples. Since it has been well established that fossil hydrocarbon contaminants are present in most organic solvents [2] and in distilled water [12], these chemicals should be carefully purified (see Appendix A) and then frequently checked by sensitive analytical techniques to assure continued low contaminant levels. In addition, system blanks should be conducted with each set of samples. Continuous monitoring of these blank values permits problems in sample handling and reagent purity to be spotted quickly.

When attempting to analyze for hydrocarbons at these extremely low levels any loss from the sample must be considered as serious. Adsorption of sample components by glass and other surfaces with which the sample comes in contact can lead to significant losses. A well recognized technique for monitoring sample component losses is the use of an internal standard added to the sample, a technique that has been employed by various workers in the area of oil pollution analysis [2,4]. To be truly effective and accurately reflect the sample, the internal standard should be present in the sample from the time of collection or at least from the first step of the analysis scheme. Thus, in this procedure all water samples collected have an internal standard added at the site of collection or as soon as practical thereafter. Following addition, the samples are frozen on dry ice. It is not feasible to add the internal standard to sediment and tissue samples when collected. Such samples are immediately frozen and the internal standard is added when the samples are thawed and opened for analysis. The compound or compounds used as the internal standard must be representative of the components of interest in the sample. Only then may one assume that the internal standard will be subject to losses (through adsorption and other causes) equivalent to those of the components of interest. An internal standard consisting of a solution of aromatic hydrocarbons representative of the molecular weight range of interest was selected.

1.2 SELECTION OF SAMPLING SITES

The Prince William Sound and Northeastern Gulf region of Alaska was selected for sample collection. Prior to the development of oil transfer facilities in Port Valdez and exploratory drilling along this section of the Gulf of Alaska this area is presently relatively pristine, the principal economic activity being commercial fisheries. Except near natural oil seeps, hydrocarbons in the environment are expected to be present at the microgram per kilogram level and may well be non-petroleum in origin.

A number of sites were selected for sampling within Prince William Sound to reflect the types of environments found there (see fig. 2):

1) A site at the ocean entrance to the Sound (Hinchinbrook Island). This site is constantly being washed with water from the Gulf of Alaska.

2) Two sites in the secluded bays around the Sound where only minor traffic occurs (Siwash Bay and Wells Bay).

3) Sites in or near traffic lanes and harbors where more extensive motor craft traffic occurs now and where extensive tanker traffic will occur in the future (Bligh Island and Orca Inlet -- across from Cordova).

4) Sites near the projected oil terminal and ballast water processing facilities in Port Valdez; the most obvious effects of chronic oil pollution would occur at these sites (Old Valdez, Dayville Mud Flats and Mineral Creek).

Similarly, a number of sites along the Northeastern Gulf of Alaska were selected to reflect the environments found there:

1) Sites on the islands that are situated around the opening of the Prince William Sound into the Gulf. These are somewhat protected from the open Gulf (Anchor Cove, MacLeod Harbor and Squirrel Bay).

2) Sites that are on the open coast or on islands out in the Gulf. These sites may be adjacent to natural oil seep areas (Cape Yakataga, Middleton Island).

3) A river site downstream from a known oil seep. This site (Katalla River) provides control samples with known hydrocarbons of petroleum origin present.

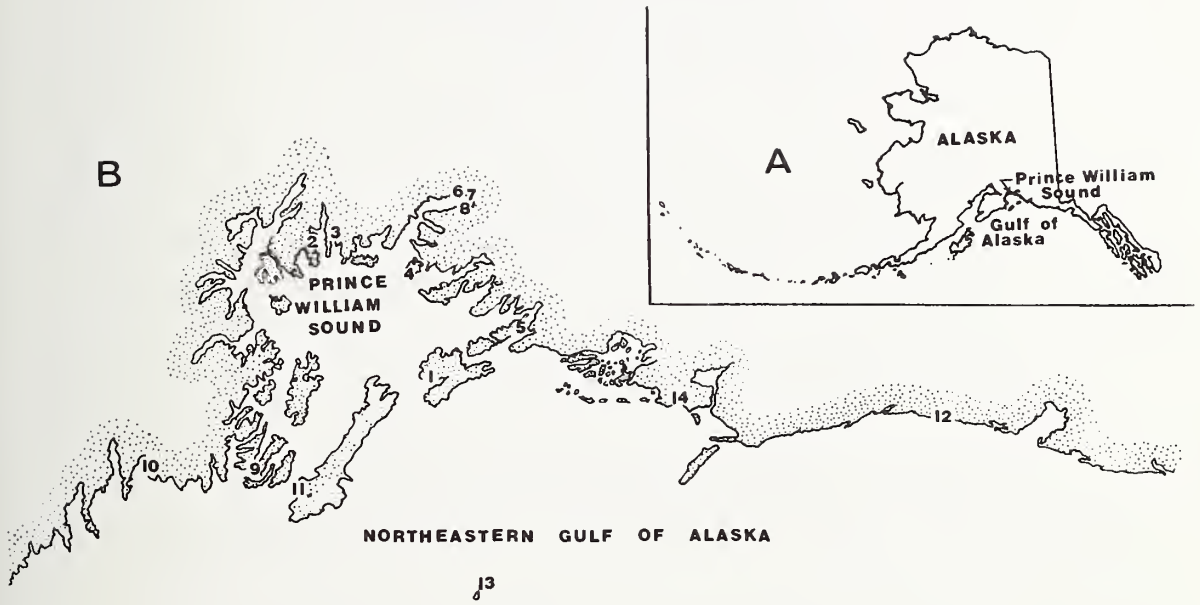

Figure 2. (A) Map of Alaska with the Gulf and Prince William Sound indicated. (B) Detailed map of the Northeastern Gulf and Prince William Sound areas showing the various sampling sites. (1) Hinchinbrook Island, (2) Siwash Bay, (3) Wells Bay, (4) Bligh Island, (5) Orca Inlet, (6) Mineral Creek, (7) Old Valdez, (8) Dayville Mud Flats, (9) Squirrel Bay, (10) Anchor Cove, (11) MacLeod Harbor, (12) Cape Yakataga, (13) Middleton Island, and (14) Katalla River.

2. PROTOCOL FOR THE SAMPLING AND ANALYSIS OF SEDIMENTS, WATER AND MARINE ORGANISMS

2.1 PREPARATION FOR SAMPLING OF SEDIMENTS, WATER, AND MARINE ORGANISMS

Disclaimer

Identification of any commercial product does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

Introduction

All samples are collected in glass bottles with plastic screw caps having aluminum foil cap liners. Since only microgram or sub-microgram quantities of hydrocarbons may be present in the samples, sample containers must be scrupulously cleaned.

Equipment

1) Sample Bottles, Screw Capped

The following bottles are used for collecting the various samples:

a) sediment -- wide-mouth bottles (16 oz);

b) Mytilus (mussel) and Macoma (small clams) -- wide-mouth bottles (32 oz);

c) Fucus (macro algae) -- wide-mouth bottles (64 oz);

d) water -- four liter (one gallon) and one liter narrow-mouth amber glass bottles.

2) Caps, Aluminum Foil Lined

Screw caps for sample bottles have slightly oversized aluminum foil liners.

3) Ultrasonic Cleaner

Cap liners are cleaned in an ultrasonic bath.

4) Tools

Various tools are used in sampling.

a) sediment -- stainless steel spatula (15 cm x 2.5 cm blade size) and Plexiglass or stainless steel right circular open-ended

cylinder (5 cm i.d. x 8.8 cm long) with sharpened edge on one end;

b) Macoma -- stainless steel spatula and tweezers;

c) Fucus -- knife with stainless steel blade.

Reagents

Details for the purification of the solvents and hydrocarbon-free water are given in the section on solvent and water purification (see Appendix A). The following reagents are used in cleaning glassware.

1) methanol -- reagent grade; singly distilled;

2) methylene chloride -- reagent grade; singly distilled;

3) n-pentane-- technical grade; triply distilled;

4) water -- distilled water and hydrocarbon-free water;

5) concentrated sulfuric acid and sodium nitrite --technical grade;

6) nitrogen gas -- purified; obtained from liquid nitrogen Dewar pressurized using nitrogen cylinder and all metal connections.

Procedure for Cleaning Sample Bottles

Two sinks are needed for cleaning glassware. One is used in the soap and water wash and the second for rinsing acid-cleaned bottles.

1) Wash each bottle with soap and water, rinse and allow to drain dry.

2) Immerse each bottle in a hot (110 °C) concentrated sulfuric acid bath for at least 30 minutes.

3) Rinse six times with distilled water.

4) Rinse with hydrocarbon-free water.

5) Rinse with methanol.

6) Rinse with n-pentane.

7) Allow n-pentane to drain.

8) Blow out remaining vapors of n-pentane with clean nitrogen gas vented from a liquid nitrogen Dewar through a flamed stainless steel line.

9) Place caps on bottles after inserting aluminum foil liner in caps.

Procedure for Preparing Aluminum Liners for Screw Caps

1) Cut liners slightly larger in diameter than the o.d. of the bottle cap. Liners are prepared from heavy-duty aluminum foil.

2) Place liners in a beaker filled with methylene chloride and agitate in ultrasonic cleaner for at least 15 minutes.

3) Decant the methylene chloride and refill the beaker with methanol. Agitate for at least 15 minutes.

4) Decant the methanol and fill the beaker with n-pentane. Agitate for at least 15 minutes.

5) Decant the n-pentane. Allow any remaining droplets of solvent to evaporate.

6) Place the liners in the screw caps using clean stainless steel tweezers.

Procedure for Cleaning Tools

1) Wash all tools with soap and water.

2) Rinse with distilled water.

3) Rinse with hydrocarbon-free water and allow to dry.

4) Wrap each tool in aluminum foil.

5) Before use in the field, rinse on-site with seawater.

2.2 SAMPLING OF SEAWATER AT SURFACE AND TEN-METER DEPTHS

Introduction

Samples of seawater are collected in four liter (one gallon) glass bottles. These samples are taken from the surface of the site and from a depth of 10 meters. Immediately following sampling, the samples are transferred to one liter glass bottles and the internal standard added. The bottles are capped, frozen and stored on dry ice.

Surface water is sampled at a site prior to any depth sampling so that the composition of the surface water is not altered. When sampling from land, carefully wade into the water to a depth suitable for filling the sample bottle. The position of the person doing the sampling must be down-wind from the sampling site. If samples are to be taken from a motorized boat, position the craft down-wind of the sampling site to prevent all exhaust hydrocarbon from floating onto the site. Sampling from a non-motorized boat is preferable. Sample at a depth of ten-meters only after completion of any surface sampling at that site. Again, position the craft down-wind of the sampling site to prevent exhaust hydrocarbon from floating onto the sampling site.

Equipment

1) Four Liter (One Gallon) and One Liter Glass Bottles

Use bottles cleaned as described in the previous section.

2) Drop-Sampling Device for Obtaining Ten-Meter Depth Water Samples

The metal frame with a Teflon bumper to hold four liter (one gallon) bottles for sampling at sub-surface depths is constructed as shown in Figure 3. Attach the drop-sampler to three 25 cm lengths of welded-link chain (3/4 in) using S-hooks. Attach upper end of chain lengths to the closed end of a 10 cm long snap shackle. Fasten the snap shackle to a suitable length of the welded link chain marked with tags at one meter intervals. Tie a length of heavy gauge fishing line to the eye hook on top of the spring-loaded bottle closure unit. Detailed drawings of the drop sampling device are given in Appendix B.

3) Disposable Micropipettes

Use disposable 5.0 µl pipettes for adding the internal standard to collected water samples. (Five microliter Microcaps are supplied by Drummond Scientific Co., U.S.A.).

4) pH Meter

A Corning Model 3 Portable pH Meter or equivalent is used for measuring the pH of the water samples.

5) Salinometer

A combination temperature,conductivity and salinity meter and probe are used to obtain these readings (Electrodeless Induction Salinometer, Beckman Instrument, Inc. or equivalent).

Reagents

1) Internal standard -- prepared to contain approximately 2.0 mg/ml each of

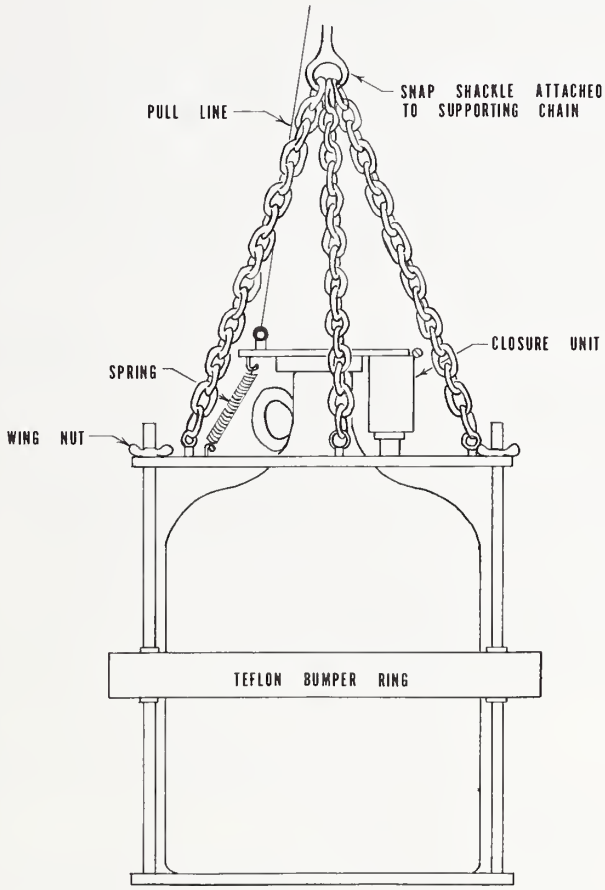

Figure 3. Drop Sampling Device for Obtaining Sub-Surface Water Samples.

various aromatic hydrocarbons in triply distilled n-pentane. Store internal standard in a sulfuric acid washed vial. The internal standard solution is added to a sample by the following procedure.

a) Fit a 5 µl micropipette into its rubber bulb holder.

b) Add 5 ml of hydrocarbon-free water to a clean beaker.

c) Transfer 5.0 µl of internal standard from the pipette to the beaker. Rinse the pipette three times with water from the beaker by filling and expelling.

d) Pour the contents of the beaker into the sample bottle. Rinse beaker three times with 5 ml portions of hydrocarbon-free water, adding the rinses to the sample bottle.

Procedure for Obtaining Surface Samples

1) Remove the foil-lined cap from a clean, unused sampling bottle.

2) Collect approximately three liters of water and transfer 750-800 ml to each of three, clean one liter bottles.

3) Cap the bottles being careful not to crumple the foil cap liners. Record bottle numbers and pertinent information about the samples and the site in a notebook.

4) Measure and record the pH, salinity (conductivity) and temperature of the water at the sampling site.

5) As soon as feasible add 5 µl of the internal standard solution to each of the one liter sample bottles. Shake vigorously for one minute to mix the sample and internal standard.

6) Freeze the water samples by laying each bottle on its side on a bed of dry ice. Once frozen, pack the bottles standing upright using sufficient packing material around each bottle to prevent breakage and sufficient dry ice to prevent thawing.

Procedure for Obtaining Sub-Surface Samples

1) Rinse drop sampler frame with hydrocarbon-free water to remove possible surface contamination. Place a clean bottle (one gallon) into the drop-sampling device.

2) Fit top plate of sampler on the threaded rods and just tighten the plate against the bottle top with the wingnuts.

3) Adjust the height of the bottle closure unit until closure plate fits flat across the top of the bottle neck. Tighten nut at bottom of closure unit to prevent movement during sampling. Attach closure spring.

4) Fasten snap-shackle to the length of 1.9 cm welded link chain marked with tags at one meter intervals.

5) Lower drop-sampler by chain into the water. Simultaneously and carefully (so that the sampling bottle is not prematurely opened) play out the heavy gauge line attached to the eye bolt on top of the bottle closure unit.

6) At the depth of 10 meters pull on line to open the closure unit and allow the sample bottle to fill. Release tension on line to effect closure.

7) Retrieve drop-sampler and transfer 750-800 ml to each of three, clean one liter bottles.

8) Cap the bottles, being careful not to crumple the foil cap liners. Record the bottle numbers and pertinent information about the samples and site in a notebook.

9) Measure and record the pH, salinity (conductivity) and temperature of the water at 10 meters depth.

10) As soon as feasible, add 5 µl of the internal standard solution to each of the samples. Mix, freeze the samples and pack the bottles for storage as previously described.

11) Use a clean sampling bottle at each site sampled.

2.3 SAMPLING OF SEDIMENT AND INTERTIDAL ORGANISMS

Introduction

Sediment and intertidal organisms are collected to reflect seasonal, species and geographic variations. All samples are collected during low tide and, therefore, only hand collection methods are used.

The main objectives to bear in mind when sampling marine materials and organisms for hydrocarbon analysis are to obtain the sample with a minimum degree of contamination and to preserve the sample so that artifacts are not introduced.

Equipment

1) Wide-Mouth Bottles

Use bottles (16, 32, 64 oz) with aluminum foil lined caps. These bottles are cleaned as previously described.

2) Tools

Various tools are used in sampling.

a) Sediment -- Plexiglass or stainless steel cylinder and stainless steel spatula for transferring sediment to sample bottles;

b) Macoma -- shovel and 0.6 cm (1/4 in) wire mesh shaker screen for obtaining sediment-free Macoma; stainless steel spatula and tweezers for transferring to sample bottles;

c) Fucus -- knife with stainless steel blade.

3) Freezer Chest

This chest, filled with dry ice, is carried to each site for immediate freezing of the samples.

Procedure for Collecting Sediment Samples

1) Select a site in the intertidal zone and scrape off any vegetation present. Since rubberized rain gear is worn, all samples should be collected with personnel and equipment located so that the tidal wash is away from the sample.

2) Push the sharpened end of the sediment corer into the sediment.

3) Use a clean spatula to dig around the circumference of the cylinder until it is possible to insert the spatula beneath the core sampler.

4) With the spatula, thus positioned, remove the core sampler.

5) Transfer the sediment sample to a sample bottle (16 oz). No attempt should be made to drain water or remove debris, rocks or biota from the sediment sample.

6) Cap the sample bottle being careful not to crumple the foil cap liner. Freeze the sample on dry ice and store.

Procedure for Collecting *Macoma* Samples

1) Select a site on the beach that appears to be slightly mounded and has several small holes visible. Inspect the sand for the presence of Macoma.

2) Shovel surface sediment (top 1-2 cm) into the shaker screen.

3) When the screen is filled, recover the Macoma by sifting out sediment, silt and other small debris in tidal water.

4) Transfer Macoma with clean spatulas to the sample bottles (32 oz).

5) Cap the sample bottles, freeze and store.

Procedure for Collecting *Mytilus* Samples

1) Pick adult Mytilus by hand, avoiding contamination by carefully washing hands prior to collection.

2) Remove any clinging rocks and other debris from each sample.

3) Place samples into the sample bottles (32 oz), cap and freeze.

Procedure for Collecting *Fucus* Samples

1) Only Fucus attached to a rock or sediment base is used to insure that the sample is taken from a stable colony.

2) Detach samples from rocks and sediment by cutting the specimen at the base of the stem.

3) Place the Fucus in bottles (64 oz), cap and freeze.

2.4 PREPARATION OF SEDIMENTS, WATER AND MYTILUS FOR GAS CHROMATOGRAPHIC ANALYSIS

Introduction

The work-up of frozen samples in the laboratory is such that the final method of analysis is identical for sediment, water, and tissue samples. With the exception of the minor variations described below the method of sample preparation is also identical for these various samples. In all cases, the compounds of interest are adsorbed onto a 6.5 x 0.6 cm stainless steel column packed with TENAX-GC*. This column is used -- as subsequently described -- as a gas chromatographic pre-column in the analysis for trace level hydrocarbons. An estimate of the variability in sampling and analysis is obtained by running all samples at least in triplicate and running system blanks with all work-ups and analyses. In order to avoid the introduction of contamination by laboratory personnel, no glassware surface which will come into contact with the sample is handled once the glassware has been acid cleaned.

Equipment

1) Cold room

All sample handling takes place in laminar flow hoods located in a cold room maintained at 4 °C. For efficient operation the room is large enough for two people to work simultaneously.

2) Class-100 Laminar Flow Hoods

Two model 100-Plus laminar flow hoods (Environmental Air Control, Hagerstown, Maryland) are strapped together and used in the cold room. Each hood has a particulate and a charcoal filter for scrubbing the air circulated around the samples. In these hoods the samples are allowed to thaw and are transferred

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* TENAX-GC is a registered trademark of Enka N.V., the Netherlands. It is a porous polymer of 2,6-diphenyl-p-phenylene-oxide.

from the sample bottles to the flasks used in the sample work-up.

3) Sample Preparation Laboratory

One laboratory is used solely for the purpose of sample work-up. Air flow in this laboratory is designed to assure a constant flow of air out of the lab, thus minimizing air entering from the hallways. The tops of all laboratory benches are covered with Teflon sheet.

4) Sulfuric Acid Baths

All glassware is cleaned in one of two 110 °C concentrated sulfuric acid baths located in a hood in the sample preparation laboratory. Each bath is contained in a large Pyrex battery jar (9&1/2 gallon) and heated by a hot plate. An eye shield is provided for each bath and the complete set-up sits in a tray large enough to contain all the acid in case a battery jar should break.

Extreme caution is required in working with these baths. Glassware is handled only with metal tongs and safety glasses are an absolute necessity.

5) TENAX Trapping Columns

Organic constituents extracted from the sample are adsorbed on a TENAX-GC packed gas chromatographic pre-column. Details for fabricating these columns are given in the section: "Construction of The Gas Chromatographic Analysis System."

6) Headspace-Sampling Apparatus and Flow System

Headspace sampling is accomplished by blowing purified nitrogen through the water or sediment sample, or by blowing purified nitrogen over the surface of a tissue sample dispersed in hydrocarbon-free water. Figure 4 contains a flow diagram for the headspace-sampling apparatus.

In the flow system dry nitrogen is passed through a trap containing XAD-2 co-polymer, molecular sieves and activated charcoal to remove any trace organics in the nitrogen. Flow from the control panel can then be directed to any number of sampling flasks. Nitrogen lines are made of copper tubing, which is flamed to remove any trace organics that may be originally present. All metal to glass junctions are made with Swagelok unions. Seals on glass are made with stainless steel back ferrules and Teflon front ferrules. Thick-walled glass tubing is used to reduce breakage when seals are made. The nitrogen flowing out of the TENAX column goes through a rotometer in the control panel and then is vented.

Figure 5 shows in greater detail the configuration of the round-bottom flask used as the headspace sampling vessel. Figure 6 is a photograph of one of the sampling vessels and the control panel for the six set-ups.

Equipment and Parts for Headspace Sampling Apparatus

a) copper tubing -- 0.3 cm (1/8 in) and 0.6 cm (1/4 in) for connecting lines and cooling coils.

b) glass tubing -- thick-walled 0.6 cm (1/4 in) o.d.

c) flexible Teflon tubing -- 0.3 cm (1/8 in) for connections to rotometers.

d) toggle valves -- for use in the control panel, should not contain any organic lubricant.

e) needle valves -- for controlling flow in each line out of the control panel.

f) rotometers -- capable of measuring flow rates to 250 ml/min.

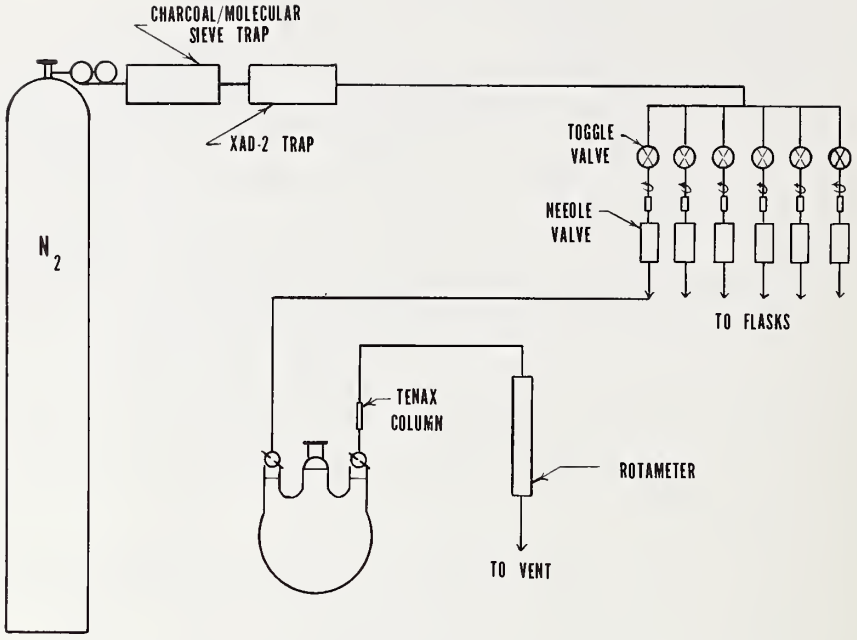


Figure 4. Flow Diagram of Headspace Sampling Apparatus.

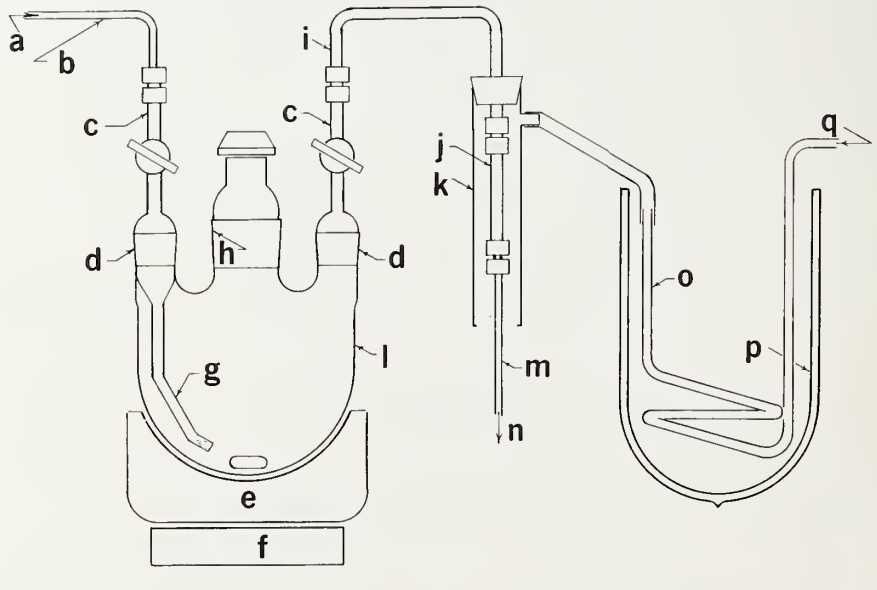


Figure 5. Headspace sampling vessel. (a) N_2 gas inlet, (b) 0.3 cm copper tubing (1/8 in), (c) 0.6 cm glass tubing (1/4 in), (d) ℔ 24/40 joint, (e) heating mantle, (f) magnetic stirrer, (g) dispersion tube with coarse frit, (h) ℔ 45/50 joint, (i) 0.6 cm glass transfer line, (j) TENAX trap, (k) glass cooling jacket maintained at 15 °C with air, (l) 2-liter round-bottom flask, (m) 0.3 cm Teflon tubing, (n) to rotometer, (o) copper coil, (p) ice bath, and (q) compressed air inlet.

g) magnetic stirrers and stirring bars -- powerful stirring motors are required in order to stir sediment; egg-shaped stirring bars are used to facilitate stirring.

h) heating mantles -- two liter hemispherical mantles.

i) Variable voltage regulators -- Variacs are used for controlling temperature of heating mantles.

j) glassware shown in Figure 5 -- all stopcocks are Teflon; joints are not greased.

k) ice bath with cooling coils -- to provide cold air for TENAX column cooling towers.

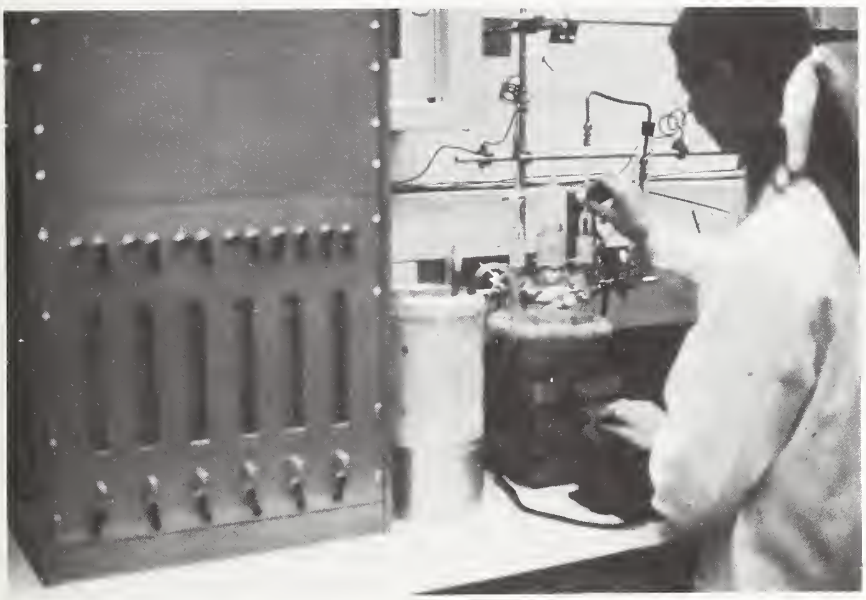


Figure 6. Headspace sampling vessel and control panel.

7) Miscellaneous Additional Equipment

In addition to the above items, the following items are either necessary or convenient for handling various samples:

a) ultrasonic probe -- for disruption of tissue structure (Brinkman Polytron PT 10 20 350D with a special ₹ 45/50 stainless steel male adapter for insertion into the center neck of the two liter round-bottom flask).

b) crystallizing dish and stainless steel spatula -- for mixing sediment samples and transferring them to the headspace sampling flasks.

c) tweezers -- for handling and shucking mussels.

d) micropipettes and 10 ml beaker -- for addition of an internal standard.

e) heavy-duty aluminum foil -- for covering cleaned glassware.

f) ovens -- for thermally cleaning stainless steel and Teflon fittings and stirbars.

Reagents

1) hydrocarbon-free water.

2) technical grade concentrated sulfuric acid and sodium nitrite -- for use in the sulfuric acid baths. One dozen bottles (9-pound) of concentrated sulfuric acid and 5-10 g of sodium nitrite are used for each bath.

3) compressed air -- for use in the cooling coils.

4) prepurified dry nitrogen -- for use in headspace sampling.

5) activated charcoal, molecular sieve 4A, and XAD-2 copolymer -- for use in scrubbing the nitrogen to remove any trace impurities.

6) TENAX-GC -- used as the adsorbent for headspace analysis.

7) triply-distilled n-pentane and approximately 2.0 mg/ml each of several hydrocarbons -- used as internal standards.

Procedure for Cleaning Glassware

1) Wash glassware with soap and water, rinse and allow to drain dry.

2) Immerse glassware in the hot concentrated sulfuric acid bath for at least 30 minutes.

3) Rinse six times with distilled water.

4) Rinse with hydrocarbon-free water.

5) Allow glassware to drain dry.

6) Wrap all openings and all additional glass which will come into contact with the sample in aluminum foil.

Procedure for Preparing Sediment for Headspace Analysis

1) Remove sample from freezer and allow to thaw overnight in the cold room in the laminar air flow hood.

2) Place side-arms in the two liter flasks and close the stopcocks. Using long forceps, insert an acid-cleaned and thermally cleaned (220 °C oven for 45 minutes) Teflon stirring bar through the center neck and stopper each flask.

3) Bring the flasks into the cold room, and obtain the tare weight of each assembled flask.

4) Sample handling is carried out in the laminar flow hood.

5) Transfer the sediment to a crystallizing dish and mix with a cleaned spatula.

6) Using the spatula, put approximately 100 g of sediment into each of three flasks, reseal the flasks and obtain the sediment weight for each flask. Return the flasks to the hood.

7) Remove the center stopper from each flask and add 600 ml of hydrocarbon-free water.

8) Using an acid-washed Teflon squeeze bottle filled with hydrocarbon-free water, wash any small particles from inside the center joint into the flask. (This is to insure a subsequent leak-tight seal of the flask.)

9) Using the procedure detailed in the Section, "Sampling of Seawater at Surface and Ten-meter Depths," add 5 µl of the internal standard to each flask.

10) Stopper the center neck of each flask and check that the stopcocks in the side-arms are shut.

Procedure for Preparing *Mytilus* (Mussel) for Headspace Analysis

1) Remove sample from freezer and allow to thaw for two hours in the cold room in the laminar air flow hoods.

2) Place side-arms in the two liter flasks and close the stopcocks.

3) After the sample has thawed, obtain a tare weight on each assembled flask.

4) Sample handling is carried out in the laminar flow hood.

5) Using two sets of cleaned tweezers, shuck the mussels, and transfer all tissue material, by tweezer, to a round-bottom flask. Fill each of three flasks with approximately 25 g of tissue and reseal the flasks. Obtain a sample weight for each flask. Return the flasks to the hood.

6) Remove the center stopper from each flask and add 400 ml of the hydrocarbon-free water.

7) Using an acid- and base-washed Teflon squeeze bottle filled with hydrocarbon-free water, wash any small particles from inside the center joint into the flask. This is to insure a subsequent leak-tight seal of the flask.

8) Insert the ultrasonic probe into the center neck of the flask and agitate the mixture for 1.5 minutes. Remove probe and rinse off with hydrocarbon-free water from Teflon squeeze bottle. Using long forceps, insert an acid cleaned and thermally cleaned (220 °C oven for 45 minutes) Teflon stirring bar through the center neck and stopper each flask.

9) Using the procedure detailed in the water sampling section, add the internal standard to each flask.

10) Place a stopper in the center neck of each flask and check that the stopcocks in the side-arms are shut.

Procedure for Preparing Water for Headspace Analysis

1) Remove sample from freezer and briefly place sealed bottle under stream of warm running water. (This insures that the bottle will not crack from ice expansion during thawing.) Allow sample to thaw overnight in the cold room in the laminar flow hood.

2) Place side-arms in the two liter flasks and close the stopcocks. Using long forceps, insert an acid-cleaned and thermally cleaned (220 °C oven for 45 minutes) Teflon stirring bar through the center neck and stopper each flask.

3) After the sample has thawed, bring the flasks into the cold room. Obtain a tare weight on each assembled flask.

4) All sample handling is carried out in the laminar flow hood.

5) Remove the center stopper from each flask and add the water sample (~750 ml).

6) Replace the stopper in the center neck of each flask, check that the stopcocks in the side-arms are shut and obtain a sample weight.

Running A System Blank

The procedure for a system blank is analogous to that for a water sample:

hydrocarbon-free water (which is not frozen) is used and the internal standard is added prior to running the blank (the standard is added to the water samples in the field.)

Procedure for Headspace Analysis

1) For each sample to be run a TENAX column needs to be pre-flashed at 350 °C for one hour. (This insures a clean TENAX column for headspace analysis.)

2) Place all stainless steel Swagelok caps, unions, nuts and ferrules in a clean 500-ml beaker and put beaker in 350 °C oven for 45 min. Place all Teflon ferrules and Teflon coated stirbars in a 220 °C oven for 45 min.

3) Replace Teflon ferrules after 8 to 10 analyses. Wash new ferrules with reagent grade methylene chloride prior to thermal clean-up.

4) Fill large ice bath with ice and water.

5) Attach each TENAX column to a 0.6 cm o.d. glass transfer line (see Figure 5) and to a 0.3 o.d. cm Teflon line.

6) Insert each TENAX column into a glass cooling jacket.

7) Bring the round-bottom flasks into the sample preparation laboratory. Attach the nitrogen inlet lines to the respective glass dispersion tubes using Swagelok fittings. Attach the outlet of each flask to its respective glass connecting tube. The flask containing the system blank is placed in a different position from day to day to insure that all transfer lines are clean.

8) Start the compressed air running through the cooling coils and glass cooling jackets.

9) Start the magnetic stirrers.

10) Connect the Teflon lines to the rotometers.

11) Open the stopcocks on each flask and then open the toggle valves on the control panel to initiate flow through the flasks, TENAX columns and rotometers.

12) Check that the rotometers show a flow of 150 ml/min through each set-up. (If not, locate the leak and correct). The flow through each nitrogen line should be pre-set at 150 ml/min through use of the needle valves on the control panel.

13) Continue headspace sampling for two hours at ambient temperature. Periodically confirm that flow in each system remains constant.

14) After two hours, disconnect the 0.3 cm Teflon lines from the rotometers (to prevent their filling with moisture) and heat the flasks to 70 °C while continuing headspace sampling for two additional hours.

15) Disconnect connecting lines from the flasks and take the flasks apart for cleaning. Transfer water to beakers for cooling prior to liquid chromatographic analysis.

16) Attach the nitrogen inlet lines directly to the TENAX columns to blow off any water trapped on the columns. Briefly attach the Teflon lines to the rotometers to confirm that flow is still 150 ml/min. Disconnect rotometers after checking flows. Continue drying the columns for two hours.

17) Stop nitrogen and cold air flow. Remove and cap all TENAX columns using thermally cleaned stainless steel Swagelok column caps. Place columns in the cold room until they are ready to be analyzed.

2.5 CONSTRUCTION OF THE GAS CHROMATOGRAPHIC ANALYSIS SYSTEM

Introduction

The organic constituents that condense on a TENAX column are subjected to gas chromatographic analysis. This is done by using the TENAX trap as a pre-column in a glass, support-coated open tubular (capillary) column chromatographic system. This system is capable of resolving trace organic constituents present on the TENAX polymer and enhances quantitative measurement of the individual chromatographic peaks.

Equipment

1) Stainless Steel Seamless Tubing

Use 0.6 cm o.d. (1/4 in) seamless stainless steel tubing for fabricating the TENAX columns.

2) Swagelok Fittings

Swagelok 316 stainless steel ferrules, nuts and fittings are used as needed.

3) Ultrasonic Cleaner

Use for cleaning the stainless steel tubing.

4) Borosilicate Glass Tubing

A 20 mm o.d. borosilicate glass tube is used for cleaning glass wool for the TENAX traps. Lengths of 8 mm o.d. x 240 cm borosilicate glass tubing are used for pulling the capillary columns.

5) Capillary Drawing Machine

Capillary columns are drawn and coiled in a Hewlett-Packard (or equivalent) drawing machine.

6) Capillary Coating Apparatus

a) A capillary coating reservoir is assembled from sections of borosilicate glass tubing by joining short lengths of 0.6 cm o.d. glass tubing to each end of a 10 cm length of 2.5 cm o.d. tubing. This reservoir holds approximately 20 ml of coating solution.

b) The coating reservoir is connected to a nitrogen cylinder using a Swagelok tee (1/4 in) and copper tubing (1/4 in). The copper tubing is attached to the side arm of the tee. A cap nut (removed while filling the reservoir) is placed on the top arm of the tee.

c) A Swagelok reducing union (1/4 in to 1/16 in) connects the capillary column to the bottom of the reservoir. The small end of this union is filled with silver solder and center drilled to 0.1 cm to make a snug fit for the capillary which is then attached with a Swagelok nut (1/16 in) and a piece of silicone rubber septum stock (cut with a #1 cork borer) used as a compression seal. A Swagelok nut (1/4 in) with Teflon ferrules attaches the union to the reservoir.

7) Gas Chromatograph

A gas chromatograph with an oven large enough to accommodate the TENAX pre-column and a glass capillary analytical column is used.

Reagents

The following reagents are used for cleaning or packing the TENAX columns and for preparing and coating the glass capillary columns;

1) methylene chloride -- technical grade used for cleaning TENAX columns; reagent grade for rinsing borosilicate glass tubing.

2) glass wool -- used as plugs to hold the TENAX resin in the packed columns.

3) stainless steel screen (100 mesh) -- used as a particle filter to prevent TENAX from leaking out of the column.

4) TENAX-GC -- a porous polymer of 2,6-diphenyl-p-phenyleneoxide acts as a trapping pre-column for the gas chromatographic system.

5) acetone -- reagent grade for rinsing borosilicate glass tubing.

6) potassium hydroxide -- a 1% aqueous solution (wt/wt) for etching the inside wall of borosilicate glass tubing.

7) methanol -- reagent grade for rinsing borosilicate glass tubing and the glass capillary.

8) graphite -- used for lubricating the coiling oven on the glass capillary drawing machine.

9) toluene -- reagent grade for preparing the capillary silanizing solution and for rinsing the capillary column.

10) carbon tetrachloride -- reagent grade for preparing a capillary coating solution and for rinsing the capillary column.

11) iso-octane -- reagent grade for preparing the capillary coating solution.

12) dimethyldichlorosilane -- for preparing the capillary silanizing solution.

13) silicone gum rubber SE-30 -- for preparing the capillary coating solutions.

14) silicone rubber septum stock -- used to prepare compression seals for attaching the glass capillary to the coating solution reservoir.

15) microsilica 6-10 μm -- for coating the wall of the capillary (Silanox, Cabot Corp.).

Procedure for Fabricating the Headspace TENAX Traps

1) Cut lengths of seamless stainless steel tubing into 6.5 cm sections.

2) Remove rough edges at tube ends with a suitable deburring tool. Attach ferrules and nuts (1/4 in) to both ends of columns.

3) Place 20 g of glass wool in a 20 mm o.d. borosilicate tube, and heat the tube with a burner to 500 °C while passing a stream of oxygen through the tube. Continue heating for about 15 minutes or until all visible traces of organic char are removed.

4) Place newly fabricated columns in a glass beaker and cover the columns completely with technical grade methylene chloride.

5) Place beaker and contents in ultrasonic cleaner for 10 min, then decant solvent, and place beaker containing columns in a 125 °C drying oven for one hour.

6) Form a glass wool plug approximately 3 mm thick and push it into one end of a metal column just slightly past the position of the Swagelok ferrules. Handle glass wool as little as possible.

7) Fill the column volume between the ferrules with TENAX polymer. Tap the column gently to settle the polymer.

8) Form an additional glass wool plug and insert it on top of the firmly tamped TENAX polymer bed.

9) Cut 1 cm square pieces of 100 mesh stainless steel screen. Using a 2 mm o.d. steel rod as a ramrod, force screen into both ends of column forming a barrier which holds the packing in place. Be sure not to compress the packing during this

operation.

10) Place the TENAX trapping columns on a manifold (constructed of Swagelok fittings) and connect the manifold to an injection port inside a gas chromatographic oven.

11) Adjust carrier gas flow rate such that the flow through each TENAX column is approximately 20 ml/min.

12) Heat the chromatographic oven to 350 °C and maintain this temperature for one hour.

13) Let the oven cool, remove the TENAX traps and immediately seal column ends with clean stainless steel Swagelok caps. Conditioned columns should be used immediately.

Procedure for Drawing and Coating Glass Capillary Gas Chromatographic Columns [13]

1) Attach one end of an 8 mm o.d. x 240 cm length of borosilicate glass tubing to a house vacuum line.

2) Rinse the inside surface of the tubing with 50 ml of reagent grade acetone and let dry for one minute.

3) Repeat with 50 ml of reagent grade methylene chloride and let dry for one minute.

4) Remove the tubing from the vacuum line and stopper one end with a cork.

5) Fill the tubing with a 1% aqueous solution (wt/wt) of potassium hydroxide and let the solution stand in the tubing for 20 min.

6) Drain solution from the tubing; attach the tubing to the vacuum line.

7) Rinse tubing with 50 ml of reagent grade methanol and dry one minute.

8) Place tubing in capillary drawing machine and turn on both drawing and coiling ovens.

9) Heat the drawing oven to an "orange" heat and the coiling oven to a "dull red" heat. (Beware of burn hazards).

10) Position the tubing in the drawing oven so that approximately 10 cm of the tube extends from the exit end of the oven.

11) When the heated tubing is fairly flexible begin pulling the capillary by grasping the tubing at both ends of the drawing oven keeping the feed side stationary and pulling at the exit side with a steady pressure.

12) When the emerging capillary is approximately 0.75 mm i.d., set the controls to provide a capillary of 0.65 mm i.d. and turn on the feed motor. Break off and discard the first section of tubing, and insert the capillary end into the feed rollers.

13) After a few minutes the emerging capillary will reach a constant diameter of approximately 0.65 mm i.d. Break the capillary at this point and guide the new end into the coiling oven.

14) Set the length counter to zero when the first coil emerges from the coiling oven. Lubricate the coiling oven with powdered graphite every 15 minutes.

15) Turn off the capillary drawing machine when a 100-110 m length of capillary column has been pulled.

16) Add 25 ml of dimethyldichlorosilane to 225 ml of reagent grade toluene. Mix thoroughly and store in a labeled glass-stoppered bottle.

17) Prepare the coating solutions by adding 5 g of silicone gum rubber, SE-30, to one liter of reagent grade carbon tetrachloride and by adding 20 g of SE-30

to one liter of reagent grade iso-octane. Place the two coating solutions into glass bottles and agitate for 12 hr to dissolve the silicone gum rubber.

18) Add 20 ml of the silanizing solution to the capillary coating reservoir. Pressurize the reservoir to approximately 210 kPa (30 psig) and completely fill the capillary with solution.

19) Remove the cap nut and turn off nitrogen pressure to the reservoir. Let the solution stand in the capillary for 45 minutes.

20) Replace the cap nut, pressurize to approximately 345 kPa (50 psig) and blow out all the silanizing solution from reservoir and capillary. Turn off the nitrogen pressure and remove the cap nut.

21) Add 20 ml of reagent grade toluene to the reservoir. Replace the cap nut and pressurize to approximately 345 kPa forcing the toluene rinse solution through the capillary.

22) Turn off nitrogen pressure and remove the cap nut.

23) Add 20 ml of reagent grade methanol to the reservoir. Replace the cap nut and repressurize to approximately 345 kPa forcing the methanol rinse solution through the capillary.

24) Keep reservoir pressurized for one hour to dry the capillary.

25) Remove the cap nut and fill the reservoir with 5 ml of reagent grade carbon tetrachloride. Pressurize the reservoir to approximately 70 kPa (10 psig) and slowly fill the first 25% of the column with carbon tetrachloride.

26) When approximately 1 ml of carbon tetrachloride remains in the reservoir, remove the cap nut and turn off nitrogen pressure.

27) Add 0.5 g of microsilica to 20 ml of the SE-30-carbon tetrachloride solution previously prepared and mix thoroughly.

28) Add this solution to the reservoir and pressurize so that the solution flows through the capillary at a linear rate of between 5 and 10 cm/sec. The pressure will have to be increased and then decreased to maintain this flow rate.

29) When all the solution has been forced through the capillary, adjust the pressure to 344 kPa and maintain for two hours to dry the capillary. A layer of silica will be visible on the inside surface when the capillary is dry.

30) Turn off the nitrogen pressure and remove the cap nut.

31) Add 20 ml of the SE-30 iso-octane solution to the reservoir. Replace the cap nut and pressurize the reservoir so that the coating solution flows slowly through the capillary.

32) Dry the capillary as previously described.

2.6 GAS CHROMATOGRAPHIC ANALYSIS OF HYDROCARBONS

Introduction

The gas chromatographic system used for analysis of the constituents condensed onto a TENAX trapping pre-column is schematically shown in Figure 7. Helium carrier gas from a cylinder and two stage regulator flows through a molecular sieve and particle filter. The carrier gas then flows through an unheated injection port and the removable TENAX pre-column. Surrounding the TENAX column is a removable aluminum heating block which is powered by a high intensity cartridge heater and Variac. From the TENAX column flow is directed through the capillary column,

which is capable of being cryogenically cooled, and into the flame ionization detector. Additional make-up carrier gas must be introduced into the detector to reduce the effective detector dead volume. This system is capable of resolving various organic constituents on the TENAX column. When attached to a suitable recorder or data acquisition system, it permits quantitative measurements of the separated constituents.

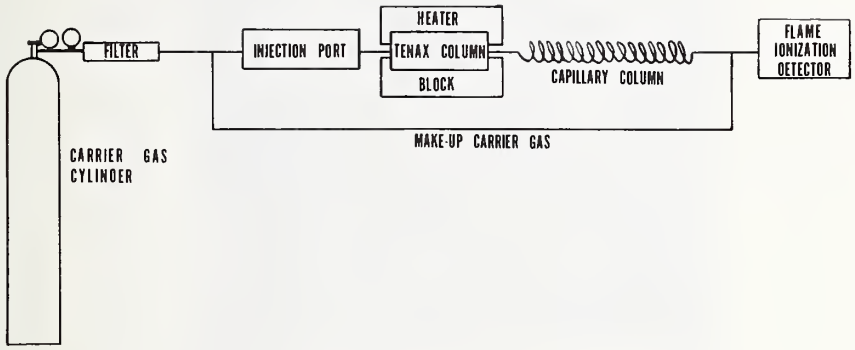


Figure 7. Gas chromatographic flow system.

Equipment

1) Gas Chromatograph

A gas chromatograph having an oven large enough to accommodate the TENAX pre-column and a glass capillary analytical column is used (see Figure 8).

a) The gas chromatograph is suitably modified to supply the flow system described in the introduction to this section.

b) A molecular sieve trap capable of containing 750 ml of molecular sieves 5A is attached to the carrier gas cylinder. These sieves must be reconditioned every six months in an oven.

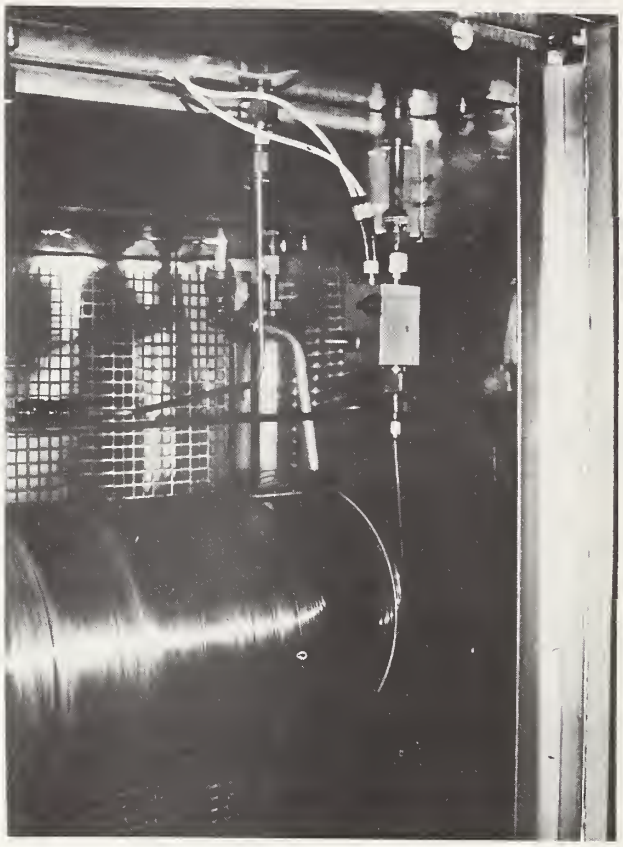


Figure 8. Gas chromatographic oven with flash heater, cryogenic cooler and capillary column.

2) Cryogenic Cooling System

A cryogenic cooling system is installed within the gas chromatographic oven for cooling the first coils of the capillary column during the time the TENAX pre-column is being flash heated.

a) The first eight coils of the capillary column are isolated from the remaining coils on the support frame.

b) An aluminum transfer line is installed between a 50 liter non-self-pressurizing liquid nitrogen Dewar and a point 1 cm above the isolated capillary coils in the chromatographic oven [see fig. 8].

3) Cartridge Heater

A fast heating, cartridge heater is used to heat the aluminum block surrounding the TENAX pre-column (200 watts, Hotwatt Corp. Cat. #HS 3715). The cartridge heater is controlled with a laboratory Variac.

4) Swagelok Fittings

Swagelok nuts (1/16 in) and back ferrules are used to attach the capillary column into the chromatographic oven. The fittings to which the capillary ends are connected are Swagelok reducing unions (1/4 in to 1/16 in) which have the small

end filled with silver solder and center drilled to 0.10 cm.

Reagents

1) Silicon Rubber Septum Stock -- used to prepare compression seals for attaching the glass capillary in the chromatographic oven.

Procedure for Installation and Conditioning of Capillary Columns

1) Straighten the ends of the glass capillary column by passing them through a 0.3 cm o.d. (1/8 in) x 6 cm stainless steel tube which is maintained at "red" heat by a small gas burner.

2) Cut out two plugs of septa material with a #1 cork borer and punch a small hole in the center of each plug with a piece of glass capillary tubing.

3) On the inlet end of the capillary fit a Swagelok nut (1/16 in) with reversed rear ferrule and a septum plug.

4) Support the capillary in the gas chromatographic oven with a suitable frame and insert the end of the column into the injector fitting (which couples the TENAX pre-column and the capillary column together) an appropriate distance. Do not attach the detector end of the column until the capillary column has been conditioned.

5) Tighten the nut firmly to assure a satisfactory compression seal.

6) Adjust the carrier gas flow rate through the capillary column to six ml/min.

7) Program the column oven at a rate of 1 °C/min from room temperature to 200 °C and maintain at 200 °C for six hours.

8) Program the column oven at 1 °C/min to 300 °C and then cool. The column is now fully conditioned.

9) Install the detector end of the column following steps 3) to 5). To reduce dead volume position end of capillary close to flame tip.

10) Heat the chromatographic oven to 275 °C and maintain for 12 hours to cure the compression seal. Cool the oven.

11) Isolate the first eight coils of the capillary column on the support frame for subsequent cryogenic cooling.

Procedure for Gas Chromatographic Analysis

1) Zero the recorder and annotate both the chart and log book.

2) Obtain a sample from cold storage and enter sample information in the log book. Be sure sample records are complete and accurate.

3) Open the oven door, turn off the oven heater and let the fan cool oven to room temperature.

4) Remove block heater from around the TENAX trap in the oven.

5) Loosen and remove the old TENAX trap being careful not to break the capillary column.

6) Remove Swagelok plugs from new TENAX column and carefully install in the gas chromatograph.

7) Replace the block heater around the TENAX trap being sure that the cartridge heater in the block is fully seated.

8) Turn on the flow of liquid nitrogen by pressurizing the Dewar to

approximately 30 kPa (4 psig) using a high pressure nitrogen cylinder and regulator.

9) When the isolated front coils of the capillary column are completely bathed in liquid nitrogen, turn on Variac control to heat block heater at maximum rate until it reaches 350 °C. This should take no longer than three minutes.

10) Turn the Variac control down so that a temperature of 375 °C is maintained in the heater block and increase the carrier gas flow rate through the TENAX trap from the analytical flow rate of six ml/min to 20 ml/min. (This can be accomplished by connecting an auxillary carrier gas line to the flow system at the injection port connector).

11) Maintain these temperature and flow conditions for six minutes.

12) Turn off the block heater, reduce the carrier gas flow rate back to six ml/min, stop the liquid nitrogen flow, close the oven door and start the oven preset for a temperature of 50 °C. Turn on the recorder and/or start the data acquisition system.

13) Maintain the oven temperature at 50 °C for eight minutes, then heat at 6 °C/min to 275 °C and hold until the analysis is complete. (The isothermal time delay and heating rate should be selected to provide the desired peak resolution. The selections presented were appropriate for the specific capillary column specified.)

2.7 DATA ACQUISITION AND REDUCTION

Introduction

Analog data is reduced using an internal standard peak height calibration technique. The compounds chosen as internal standards span the retention range normally exhibited during the gas chromatographic analysis. The computer algorithm used for quantitating the compounds analyzed is shown in Figure 9. Each compound eluted is placed into one of the retention-time regions surrounding each of the internal standard compounds used. Compounds eluting prior to the first internal standard compound or after the last internal standard compound are quantitated by relating them only to the first and last internal standard compound, respectively, as shown in equation 1.

$$C_c = C_s \, (H_c/H_s) \qquad (1)$$

where C_c = the concentration of the compound measured

C_s = the concentration of the internal standard compound

H_c = peak height of the measured compound

H_s = peak height of the internal standard compound

Since the efficiency of the headspace analysis varies with the specific compounds analyzed (and therefore with chromatographic retention time), it is necessary to utilize more than one internal standard when quantitating all intermediate compounds. The concentration of these intermediate eluting compounds is calculated using equation 2.

$$C_c = (1-k)C_1(H_c/H_1) + kC_2(H_c/H_2) \qquad (2)$$

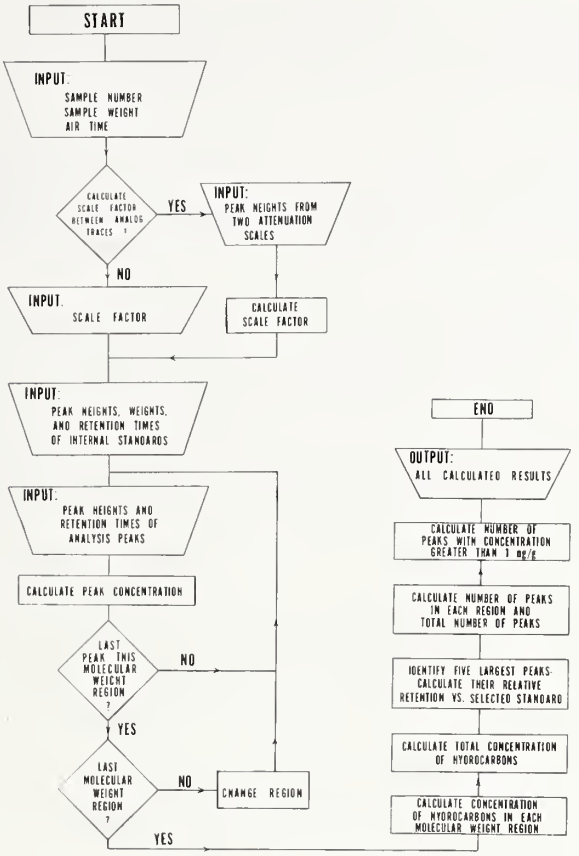


Figure 9. Computer algorithm used for quantitating GC data.

where C_1 and C_2 = the concentrations of the internal standard compounds eluting prior to and after, respectively, the compound whose concentration is being calculated.

H_1 and H_2 = the corresponding peak heights of the two internal standard compounds.

k = the retention time ratio as defined by equation (3);

$$k = \frac{t_{Rc} - t_{R_1}}{t_{R_2} - t_{R_1}} \qquad (3)$$

where t_{Rc} = the retention time of the compound of interest

tR_1 and t_{R_2} = the retention times of the two internal standard compounds

Procedure for Preparing the Chromatograms for Data Reduction

1) Identify and label the internal standard peaks.

2) Tabulate the peak heights and retention times for the internal standard peaks.

3) From the laboratory notebook, note the amount (in micrograms) of the internal standard compounds added to the sample prior to analysis.

4) From the laboratory notebook, note the weight of sample analyzed.

5) Identify the first compound peak to be eluted and determine its retention time.

6) Measure the time between the first and the second internal standard peaks. Mark the interval from one-half of this time before the first internal standard peak to one-half of this time after this first peak. This region is designated as "region I."

7) Measure the time between the second and third internal standard peaks and mark the midpoint time. The section between this mark and the end of "region I" is designated as "region II."

8) In the same fashion delineate other internal standard regions on the chromatograms.

9) Beyond the last internal standard peak, mark a point equal to the time difference between it and the previous internal standard peak and number appropriately.

10) Mark the best tangential baseline on the chromatogram and measure all peak heights from this baseline.

11) Determine the peak heights and retention times of all of the peaks in each region. Enter the data into the computer as required for the quantitative calculations.

2.8 GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS OF HYDROCARBONS

Introduction

The GC-MS flow system used in analysis of the constituents condensed onto a TENAX pre-column is similar to that described for the GC analysis of hydrocarbons. Some changes are necessary at the exit of the capillary column. The column effluent enters the ion source of the mass spectrometer through a separator which vents the carrier gas to the laboratory atmosphere. Spectra are scanned from m/e 40 to m/e 500 every 4.3 seconds during the course of the entire gas chromatogram. All total ionization and mass spectrometric data are transferred by the data system to magnetic disc storage in real-time. After completion of data accumulation, the stored information may be recalled from disc for spectrum interpretation.

Equipment

1) Gas Chromatograph-Mass Spectrometer-Computer System

The gas chromatograph must be capable of handling capillary columns and the data system must have a storage device capable of recording the mass spectrometric data in real-time. Stainless steel capillary columns (61 m x 0.61 mm) may be used in the GC-MS analyses, if the GC oven used is not large enough to easily accommodate glass capillary columns.

2) Cryogenic Cooling System

For stainless steel columns, the first three meters of the capillary column are formed into 5 cm diameter coils and immersed in a small Dewar of liquid nitrogen during the time the TENAX pre-column is being flash heated.

Procedure for GC-MS analysis

1) Remove a TENAX pre-column from cold storage and enter sample information in the log book. Prepare the data system for spectrum acquisition and key in the sample information.

2) Remove Swagelok plugs from the TENAX column and install the column in the GC-MS flow system.

3) Place the block heater around the TENAX pre-column.

4) Place the cryogenic trapping coils of the analytical column in a Dewar filled with liquid nitrogen.

5) Start helium flow through the TENAX column (20 ml/min); turn on the Variac control to heat the block heater to 350 °C at the maximum rate.

6) Turn down the Variac control so that a temperature of 375 °C is maintained in the heater block.

7) Maintain this temperature and carrier gas flow rate for six minutes.

8) Turn off the block heater, discontinue carrier gas flow through the TENAX column and remove the Dewar of liquid nitrogen.

9) Briefly place the cryogenic trapping coils of the analytical column in a beaker of tepid water to warm the coils to room temperature.

10) Close the oven door and set the temperature program for 4 min at 50 °C followed by a 4 °C/min temperature rise to 270 °C. (The isothermal time delay and heating rate should be selected to provide the desired peak resolution. The selections presented were appropriate for the specific capillary column used.)

11) Open the isolation valve between the gas chromatograph and mass spectrometer.

12) After the 4 min isothermal period turn on the filament and begin the computer-controlled data acquisition.

13) At completion of the chromatogram, discontinue data acquisition and save the data on a bulk storage device for subsequent spectrum interpretation.

2.9 ANALYSIS OF POLYNUCLEAR AROMATIC HYDROCARBONS IN WATER

Introduction

Dynamic headspace analysis has little sensitivity for polynuclear aromatic hydrocarbons (PAH) due to their low volatility. Recoveries of phenanthrene and pyrene are 19% and 2%, respectively. Therefore, these compounds are analyzed by a complimentary coupled-column liquid chromatographic technique which has maximum sensitivity for the 3-6 condensed ring PAH's. Recoveries of both phenanthrene and pyrene are greater than 90%.

The compounds of interest are concentrated by passing the desired volume of sample through a Bondapak C18 pre-column. The analysis is continued by eluting adsorbed components onto a μBondapak C18 column for analytical separation.

The eluate from the analytical column may be collected and the compounds identified by spectrometric techniques. Once identified, response factors for individual components are readily calculated and accurate quantitative analysis may be performed.

When collection and identification of individual compounds in a complex mixture of polynuclear aromatic hydrocarbons is not feasible, absolute quantitation is not possible. Peak heights (or areas) are not directly convertible into concentration units for two reasons: (1) Recovery of these compounds from water increases as an inverse function of their solubility in water. (2) Since the UV detector (254 nm) monitors a single wavelength (generally not that of maximum absorbance for the polynuclear aromatic hydrocarbons), the sensitivity of detection varies randomly with elution time. Approximate quantitation is obtained in this case by expressing quantitative results in terms of equivalents of one particular compound whose recovery from water has been determined and whose UV response at 254 nm has been measured. For the purposes of this baseline study, quantitative results are expressed in terms of phenanthrene equivalents.

Equipment

1) Stainless Steel Tubing

Use 0.6 cm o.d. (1/4 in) stainless steel tubing for fabricating the Bondapak C18 pre-columns.

2) Swagelok Fittings

Swagelok 316 stainless steel ferrules, nuts and fittings are used as needed. All pre-columns are fitted on both ends with a reducing union (1/4" to 1/16") with 2 μm snubbers.

3) Ultrasonic Cleaner

Use for cleaning the stainless steel tubing.

4) μBondapak C18 Columns (analytical column)

Purchase commercially from Waters Associates, Milford, Massachusetts.

5) Milton-Roy Minipump

Use to meter flow in the concentration of sample on the pre-column.

6) Liquid Chromatograph

A research grade liquid chromatograph is needed. It should have gradient elution capability and at least a 2.1 x 10^4 kPa (3000 psi) pressure rating.

7) Bondapak-C18

Use this superficially porous liquid chromatographic column material in the pre-column.

8) Recorder

A recorder with a 10 mv output is necessary.

Reagents

1) methanol -- singly distilled for running elution gradients.
2) hydrocarbon-free water -- for running elution gradients.

Procedure for Fabricating Bondapak C18 Pre-Columns

Use the procedure previously described in the section, "Construction of the Gas Chromatographic Analysis System," for fabricating headspace TENAX traps with two exceptions:

1) The columns are packed with a pellicular liquid chromatographic packing (Bondapak C18).

2) The columns are fitted on both ends with 2 µm reducing union snubbers.

Procedure for Coupled-Column Liquid Chromatography

The procedure is outlined in Figure 10.

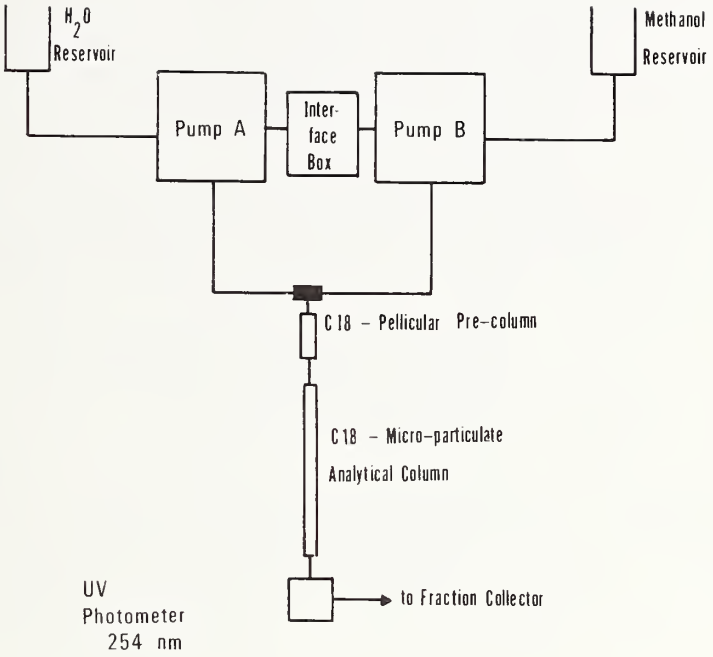


Figure 10. Analysis of polynuclear aromatic hydrocarbons by coupled-column LC. Non-volatile components trapped on a C18-pellicular pre-column are stripped onto the C18-micro-particulate analytical column for analysis.

1) (a) Obtain a sample from storage and allow it to thaw; or

(b) Allow the headspace sampled water to cool to room temperature.

2) Immediately pump the desired volume of sample through the Bondapak C18 (6 x 0.6 cm) pre-column at a flow rate of 10 ml/min.

3) Connect the pre-column to the output side of the analytical pumping system, past the solvent mixing tee.

4) Flush the pre-column with 10 ml of hydrocarbon-free water.

5) Connect the outlet end of the pre-column to the inlet side of the µBondapak C18 analytical column.

6) Zero the recorder, set the initial conditions as follows and run the analysis:

Flow rate: 3 ml/min

Linear Gradient: 30 to 100% methanol in water in 40 minutes. Hold at 100% MeOH for 10 minutes.

Procedure for Quantitating Mixtures of Polynuclear Aromatic Hydrocarbons in Terms of Phenanthrene Units

1) Identify and label the internal standard peak (phenanthrene).

2) Tabulate the peak height (or area) of the phenanthrene peak .

3) From the laboratory notebook, note the amount (in micrograms) of phenanthrene added to the sample prior to analysis.

4) From the laboratory notebook, note the weight of sample analyzed.

5) Mark the best tangential baseline on the chromatogram and measure all peak heights (areas) from this baseline.

6) Calculate the total PAH concentration in terms of phenanthrene equivalents, as shown in equation 1.

$$\frac{\sum_{p=1}^{p=n} h_p \cdot W_{phen}\ (\mu g)}{h_{phen} \cdot W_{sample}\ (kg)} = \text{Concentration } (\mu g/kg) \text{ in phenanthrene equivalents} \qquad (1)$$

where

h_p = peak height (or area) of peak p in the chromatogram

W_{phen} = weight (µg) of the phenanthrene internal standard added to the sample

h_{phen} = height (or area) of the phenanthrene peak

W_{sample} = weight of the sample analyzed (kg)

7) Convert phenanthrene equivalents into concentration units for any single identified component in the chromatogram using equation 2.

$$\frac{R_{phen}}{R_x} \cdot \text{phenanthrene equivalents of compound x} = \text{Concentration of x } (\mu g/kg) \qquad (2)$$

where

R_{phen} = the response factor of phenanthrene (units/mg)

R_x = the response factor for the compound of interest (units/mg)

8) Calculate response factors by doing the following:

a) Add a known amount of the compounds of interest to approximately 500 ml of water in a headspace-sampling flask.

b) Headspace sample for four hours, as previously described.

c) Take the residue from the headspace-sampling flask and analyze by the coupled-column LC procedure.

d) Mark the best tangential baseline on the chromatogram and measure the peak heights (area) from this baseline.

e) Calculate the response factor by dividing each peak height (or areas) by the weight of that compound added.

3. RESULTS AND DISCUSSION

3.1 METHODOLOGY

In designing an analytical method for a hydrocarbon baseline study requiring determinations of single components at the µg/kg (ppb) level, the criterion of prime importance is the development of a contamination-free procedure that minimizes possible losses of sample. Such a procedure would entail as few sample transfers, as few solvents, and as few concentration steps as possible. The headspace sampling and LC techniques described herein meet this criterion and in addition offer several other advantages: 1. The time involved in the work-up of samples is short, a matter of hours. 2. Compounds having a wide range of molecular weights can be analyzed. 3. Samples are handled in a closed system, which provides a hydrocarbon-free nitrogen atmosphere from the time of transfer to the headspace sampling flask until the volatile components are trapped on the TENAX-GC pre-column. 4. The non-volatile components are trapped on an LC pre-column, a concentration step involving neither solvent extractions, nor solvents other than hydrocarbon-free water. 5. There is added sensitivity, since the entire sample is analyzed instead of a small aliquot.

Also of prime importance is the early introduction of an internal standard for purposes of quantitation. A mixture of several aromatic hydrocarbons is used to correct for the varying recoveries of compounds over the molecular weight range analyzed. The internal standard is added to water samples in the field at the time of collection, and to sediment and tissue samples at the earliest practical stage, in the laminar flow hood before sample work-up begins. This early addition of the internal standard contrasts with some earlier procedures [2,4] where the internal standard is added after sample work-up, just prior to final analysis.

By comparing data on recoveries of the internal standard from water by headspace sampling/gas chromatography and by coupled-column liquid chromatography, it is evident that the LC technique becomes more effective as the headspace sampling becomes less efficient. Headspace sampling is especially suited for the series of 1-3 condensed ring aromatic hydrocarbons. In the event of an oil spill, such compounds have been suggested as being of greatest immediate toxicity for marine organisms [14,15]. The coupled-column liquid chromatographic technique is especially suited for the 3-6 condensed ring polynuclear aromatic hydrocarbons. These compounds are of considerable interest due to their suspected carcinogenicity [16].

Glass capillary rather than conventionally packed gas chromatographic columns were used, since the capillary columns provide superior separation of single components. The use of thermal focusing with liquid nitrogen during the transfer of volatile components to the capillary column further improves the chromatographic resolution by keeping the injection profile reasonably narrow. Originally XAD-2 resin was used as the trapping agent, but its thermal stability was found to be inferior to that of TENAX-GC for thermal desorption of trace organics. Figure 11 shows chromatograms obtained by flash heating XAD-2 to 275 °C and TENAX to 375 °C. High resolution gas chromatography with low blanks is of extreme importance in gas chromatography-mass spectrometry with a goal of single compound identification.

Reverse phase coupled-column LC was chosen as the analytical method for the less volatile compounds in water, since it presents an efficient concentration technique

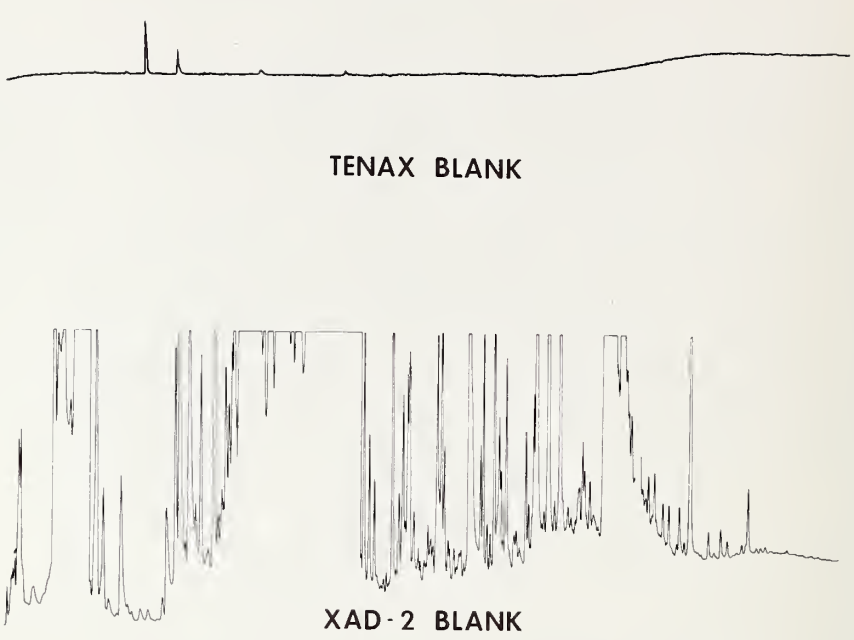


Figure 11. Comparison of flashing blanks of XAD-2 and TENAX-GC adsorbents.

that does not use organic solvents. The use of elution focusing (stripping the sample from the pre-column onto the analytical column without loss of resolution) allows one to extract large volumes of sample with the relatively inexpensive pre-column and then rely on the more expensive analytical column only for the analysis of the compounds of interest. Reverse phase LC affords an advantageous isolation of the non-polar hydrocarbons onto the pre-column with freedom from polar compound interferences. Finally, coupled-column analysis is non-destructive and the eluted compounds are available for spectrometric examination.

Tasks remaining once the high resolution chromatographic techniques and sample preparation scheme were developed, were to prove that the water used was in fact hydrocarbon-free and that only minimal contamination of the sample would occur during handling. Water blanks were determined by pumping separate 10-liter portions of hydrocarbon-free water through TENAX-GC and Bondapak C18 packed columns dosed with the internal standards, and then measuring the blanks by the respective chromatographic techniques. System blanks were obtained with 600 ml of hydrocarbon-free water plus the internal standard taken through the headspace sampling and coupled-column procedure. Chromatographic blanks were obtained by analyzing clean TENAX-GC and Bondapak C18 pre-columns by their respective chromatographic techniques. Results obtained for the various blanks are summarized in Table 1. Since the water blank is essentially equivalent to the chromatographic system blank, it can be concluded that the water blank is negligible.

Originally the headspace analysis technique was designed to trap only those very volatile compounds most likely to be lost in sample handling (e.g., benzene, toluene). Following headspace sampling, the residual sediment (or tissue) sample was filtered through a clean, dry and weighed paper Soxhlet thimble. The residue was Soxhlet-extracted with hydrocarbon-free water for 16 hours, followed by Soxhlet extraction with methanol for four hours, and finally methylene chloride for 20 hours. The water from the Soxhlet extraction (or a water sample after headspace sampling) was extracted with methylene chloride and all the organic extracts were combined and evaporated to near dryness by vacuum distillation. These concentrated extracts were analyzed by gas chromatography. The solid residue in the Soxhlet thimble was used for a dry-weight determination.

Subsequently, it was determined that by varying certain operational parameters various compounds not usually considered as being volatile could be removed from the sample during the headspace analysis procedure, and the higher aromatic compounds could be removed by the coupled-column procedure. Therefore, the Soxhlet extraction step was deleted from the overall analysis procedure.

Table 1. Trace organic levels (μg) determined in water, system and chromatographic blanks

	Headspace sampling system (μg)	Coupled-column liquid chromatography (μg)
Water blank	<0.5	<0.5
System blank	2.9 ± 1.3	<0.5
Chromatographic blank	1.1 ± 0.8	<0.5

3.2 PRINCE WILLIAM SOUND/NORTHEASTERN GULF OF ALASKA BASELINE STUDY

Data have been obtained over several seasons from the eight sites in the Prince William Sound and the six sites along the Northeastern Gulf of Alaska. Physical data for the water at these sites are contained in Table 2. Table 3 summarizes the hydrocarbon data obtained over a period of two years for sediment from the sites. Table 4 similarly summarizes the data for water samples collected for the first time on the September 1974 sampling trip. Figure 12 contains chromatograms of sediment samples taken at Hinchinbrook Island and Old Valdez. These chromatograms demonstrate the sensitivity of the headspace sampling technique and the appearance of hydrocarbon contamination, respectively. The only site consistently low in maximum hydrocarbon content is Hinchinbrook Island at the opening of the Sound. The data presented in Tables 3 and 4 represent the range of compounds isolated by headspace sampling only. The coupled-column liquid chromatographic technique now developed extends the molecular weight range substantially.

Table 2. Physical data taken at various sampling sites.

Site	Temperature °C	pH	Conductivity mA/Vcm (mmho/cm)	Salinity %
Hinchinbrook				
Fall 73	10.3	8.3	32.9	
Spring 74	6.9	7.7	29.2	
Fall 74	7.7	7.4	27.2	25.9
Mineral Creek				
Summer 73	8.4	8.1	7.6	
Fall 73	6.8	8.25	15.2	
Spring 74	5.5	7.9	27.2	
Dayville Mud Flats				
Summer 73	10.2	8.0	14.3	
Fall 74	7.5	7.1	5.4	4.6
Wells Bay Low Beach				
Fall 73	11.5	8.3	21.7	
Spring 74*	4.1	6.9	3.8	3.8
Wells Bay High Beach				
Spring 74	9.1	7.8	22.0	20.6
Bligh Island				
Fall 73	----	8.3	30.8	
Siwash Bay				
Spring 74	6.9	6.8	8.7	
Fall 74	7.4	7.2	29.0	32.0
Old Valdez				
Fall 74	4.3	7.3	1.6	1.3
Cape Yakataga				
Fall 74	8.0	---	20.2	12.6
Katalla River				
fall 74	10.0	---	0.7	0.4
Anchor Cove				
Fall 74	8.0	7.1	28.2	26.9
MacLeod Harbor				
Fall 74	8.5	7.4	33.1	31.0
Middleton Is.				
Fall 74	7.2	7.4	28.5	27.2
Squirrel Bay				
Fall 74	7.4	7.2	29.0	32.0

* Measurements taken in intertidal river.

Table 3. Hydrocarbon content of sediment samples as expressed in percentages by molecular weight region

Site/Season	Total maximum hydrocarbon Content (µg/kg wet weight) Obtained by Headspace Sampling	Percent of total Hydrocarbons by Molecular Weight Region			
		Mesitylene	Naphthalene	Propylnaphthalene (or Trimethylnaphthalene)	Phenanthrene
Hinchinbrook Is.					
Summer 73	83 (1)*	35	29	36	
Fall 73	115 (1)	26	26	48	
Spring 74	63 ± 10 (4)	43 ± 9	26 ± 3	31 ± 10	
Fall 74	91 ± 29 (4)	25 ± 10	10 ± 2	10 ± 2	51 ± 11
Mineral Creek					
Summer 73	121	31	34	34	
Fall 73	147 ± 20 (4)	29 ± 4	28 ± 1	42 ± 4	
Spring 74	190 ± 2 (2)	32 ± 6	26 ± 2	42 ± 6	
Dayville Mud Flats					
Summer 73	254 ± 12 (3)	28 ± 2	30 ± 2	42 ± 1	
Fall 73	153 ± 23 (3)	30 ± 8	26 ± 3	44 ± 7	
Spring 74	147 ± 51 (1)	33 ± 15	26 ± 3	41 ± 13	
Fall 74	167 ± 26 (3)	6 ± 3	3 ± 1	11 ± 5	80 ± 8
Wells Bay Low Beach					
Fall 73	148 ± 18 (2)	47 ± 3	32 ± 1	20 ± 3	
Spring 74	123 ± 3 (2)	51 ± 6	32 ± 6	16 ± 0	
Wells Bay High Beach					
Summer 73	255 ± 47 (2)	37 ± 1	26 ± 4	36 ± 3	
Spring 74	175 ± 38 (2)	48 ± 0	25 ± 1	26 ± 1	
Old Valdez					
Spring 74	91 ± 8 (2)	26 ± 4	18 ± 1	56 ± 4	
Fall 74	167 ± 8 (2)	12 ± 4	14 ± 5	74 ± 9** value for combined region	
Orca Bay					
Spring 74	288 ± 65 (3)	52 ± 5	24 ± 4	23 ± 2	
Bligh Island					
Summer 73	73 (1)	25	18	57	
Fall 73	171 ± 9 (2)	31 ± 1	22 ± 4	46 ± 3	
Spring 74	249 ± 12 (5)	43 ± 3	26 ± 4	30 ± 4	
Siwash Bay					
Spring 74	105 ± 12 (3)	29 ± 9	26 ± 3	45 ± 6	
	311 ± 8 (2)	45 ± 4	36 ± 1	20 ± 6	
Fall 74	61 ± 6 (3)	53 ± 2	20 ± 5	27 ± 8** value for combined region	
Cape Yakataga					
Fall 74	72 ± 16 (3)	26 ± 10	13 ± 5	12 ± 2	48 ± 17
	367 (1)	37	18	8	37
Katalla River					
Fall 74	566 ± 143 (3)	28 ± 8	31 ± 2	40 ± 14** value for combined region	
MacLeod Harbor					
Fall 74	124 ± 49 (5)	22 ± 4	23 ± 3	34 ± 5	21 ± 5
Middleton Island					
Fall 74	155 ± 45 (4)	32 ± 6	36 ± 8	14 ± 1	18 ± 2
Squirrel Bay					
Fall 74	956 ± 63 (2)***	11 ± 4	4 ± 3	84 ± 6** value for combined region	

* ( ) denotes the number of samples analyzed.

** Phenanthrene internal standard not recovered so component amounts included in propylnaphthalene (or trimethylnaphthalene) molecular weight region.

*** Abnormally high value for maximum hydrocarbon content not consistent with low values obtained from water analyses. This was a very adsorptive sediment requiring 5x the amount of internal standard to be seen in the gas chromatogram. This site visually appears to be quite pristine. It is under continuing investigation.

Table 4. Water analyses - fall 1974 sampling

Site	Total Maximum Hydrocarbon Content (µg/kg) Obtained by Headspace Sampling	Percent of Total Hydrocarbons by Molecular Weight Region			
		Mesitylene	Naphthalene	Propyl-Naphthalene	Phenanthrene
Dayville Mud Flats					
Surface	2.7 ± 1.0 (3)*	36 ± 20	21 ± 3	44 ± 19	**
10 meter	1.6 ± 0.2 (2)	38 ± 1	42 ± 2	20 ± 1	**
Old Valdez					
Surface	14.9 (1)	27	29	44	**
10 meter	0.95 ± 0.38 (3)	20 ± 6	37 ± 8	44 ± 9	**
Cape Yakataga					
Surface	11.3 ± 0.9 (3)	21 ± 10	32 ± 1	47 ± 10	**
Katalla River					
Surface	3.4 ± 1.3 (3)	21 ± 8	23 ± 3	54 ± 11	**
Anchor Cove					
Surface	8.0 ±0.1 (2)	21 ± 1	8 ± 3	8 ± 1	63 ± 4
10 meter	3.6 ± 0.4 (2)	55 ± 16	10 ± 1	14 ± 4	20 ± 11
Hinchinbrook Is.					
Surface	5.3 ± 1.8	46 ± 33	19 ± 19	27 ± 32	11 ± 2
MacLeod Harbor					
Surface	7.3 ± 0.1 (2)	53 ± 4	8 ± 2	7 ±2	31 ± 1
10 meter	30.5 (1)	57	23	6	14
	3.9 ± 1.2 (3)	76 ± 6	10 ± 3	12 ± 5	**
Middleton Island					
Surface	5.4 (1)	26	16	22	36
Siwash Bay					
Surface	3.0 (1)	41	45	13	**
10 meter	1.9 ± 0.1 (3)	40 ± 10	7 ± 2	30 ± 9	22 ± 8
Squirrel Bay					
Surface	4.5 ± 1.1 (3)	26 ± 10	8 ± 4	42 ± 18	23 ± 5
10 meter	5.1 (1)	44	4	9	44

* ( ) denotes number of samples analyzed.
** Phenanthrene not included in internal standard.

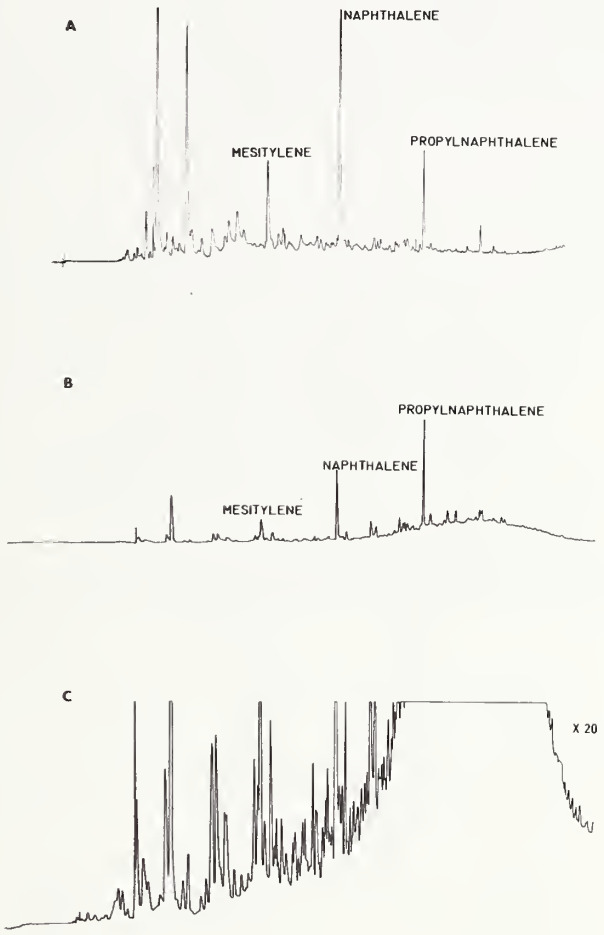


Figure 12. Chromatograms of sediment samples taken from two intertidal zone sites. (A) Hinchinbrook Island - open beach site. (B) Old Valdez - site with apparent trace level hydrocarbon content. (C) Chromatogram B expanded 20-fold. Labeled peaks represent internal standard components added at the µg/kg level.

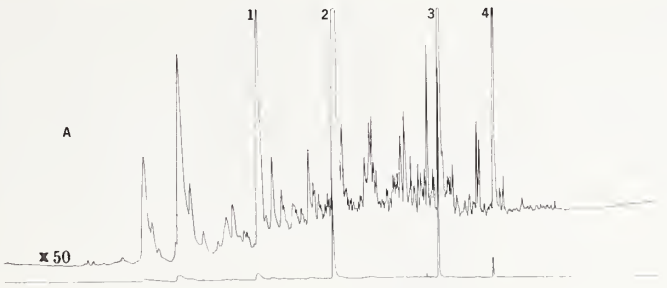
The variability in the values reported in Tables 3 and 4 is the standard deviation of replicate values from the mean of the replicate values. However, the number of samples analyzed for each site does not necessarily allow a strict statistical analysis of the data (see below). These values were accumulated over the course of two years during which minor refinements in methodology were being made. This may account for a small increase in the variability observed.

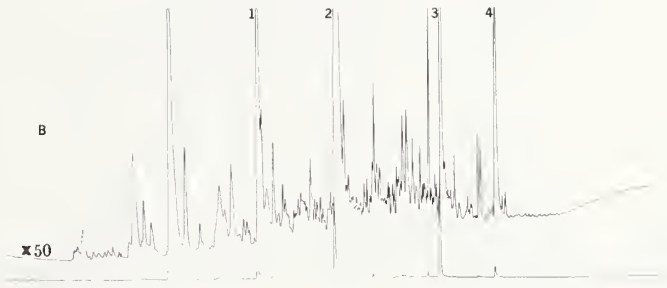
The internal standard used for quantitation consisted of a mixture of aromatic hydrocarbons. Since aliphatic hydrocarbons can be more efficiently outgassed than aromatic hydrocarbons [17], the values in Tables 3 and 4 represent a maximum hydrocarbon content over the molecular weight range analyzed. It should be noted that the internal standard utilized in the more recent analyses differs from that used in the prior ones by the addition of phenanthrene to the standard. This addition of phenanthrene to the standard is believed to provide a more realistic evaluation of the recoveries of the compounds in the phenanthrene molecular weight region. Comparison of the Fall 1974 results with those for samples previously collected and analyzed (Table 3) indicates that the addition of phenanthrene has little effect on the value for the total maximum hydrocarbon content. In several instances the phenanthrene standard peak was not observed on an individual gas chromatogram of a sample (denoted by ** in the tables) and the organic compound peaks within the phenanthrene region were included in the propyl/trimethylnaphthalene region. This treatment can cause the calculated concentrations for these two molecular weight regions to be incorrectly biased. A single percentage value calculated using the combined results from these two regions is therefore presented as more accurately reflecting the correct percentage.

Some seasonal variability in hydrocarbon content is observed in the various sites. However, no reproducible pattern is discernible. High values do not necessarily represent petroleum pollution but could be of biological origin.

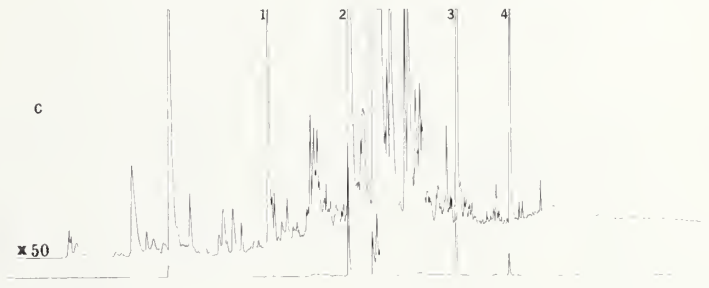
Two values are presented for sediment from Siwash Bay, Spring 1974 and Cape Yakataga, Fall 1974. In the first case different sets of sediment samples, although visually similar, had varying hydrocarbon values. In the second case (Cape Yakataga) three of the results from a single sample bottle showed a maximum hydrocarbon content of 72 ± 28 µg/kg (wet weight) while the fourth analysis from the same bottle indicated 367 µg/kg. Since the sediment was well mixed in a clean dish prior to analysis, one must assume that this inhomogeneity is microscopic in nature, i.e., a contaminant was associated with a single (or very few) grains of the sand in the sample. Inasmuch as the site at Cape Yakataga is near known oil seeps, hydrocarbon contamination of the sediment might occur in the form of discrete balls of tar or globules of weathered oil. It is worth noting (see Table 3) that the percentages of total hydrocarbon found in each molecular weight region of the 367 µg/kg sample agree (within the standard deviations) with those of the other three Cape Yakataga samples.

The Middleton Island sediment samples further illustrate the large standard deviations which can be found in the sediment analyses. The sediment samples, although collected within an area of 600 cm^2 on the beach at the eastern tip of the island, are inherently inhomogeneous and this leads to the largest source of variability in the determination. The sediments analyzed were collected and brought back to the laboratory frozen in two separate bottles. The contents of each bottle were well mixed prior to analysis and provided enough sample to allow duplicate determinations; the four resulting gas chromatograms are represented in Figures 13 A-D. The overall chromatographic pattern, i.e., elution profile as a function of time, is similar in all





Figures 13A, B. Gas chromatograms of Middleton Island sediment samples taken from the first bottle. Peaks labeled 1-4 are from the internal standard compounds mesitylene, naphthalene, trimethylnaphthalene and phenanthrene, respectively.



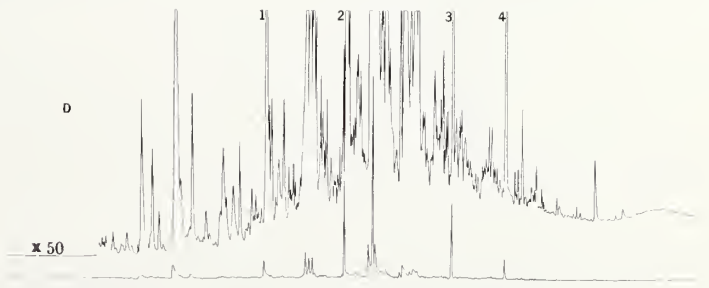


Figure 13C, D. Gas chromatograms of Middleton Island sediment samples taken from the second bottle.

these chromatograms and unique to the Middleton Island sediment samples. Presented in Figures 14 A-D are histograms of concentration vs. time corresponding to the gas chromatograms in Figure 13. These histograms graphically point out the microscopic and macroscopic inhomogeneity of the samples collected. There is a gross single species concentration difference between the first set of histograms (Figures 14 A, B) and the second set (Figures 14 C, D) indicating that the samples contained in the two bottles are not identical. Furthermore, histograms of samples from the same bottle still show noticeable single species concentration differences. This demonstrates that the sediment samples from a single bottle may not be homogeneous even though the bottle contents were mixed prior to analysis.

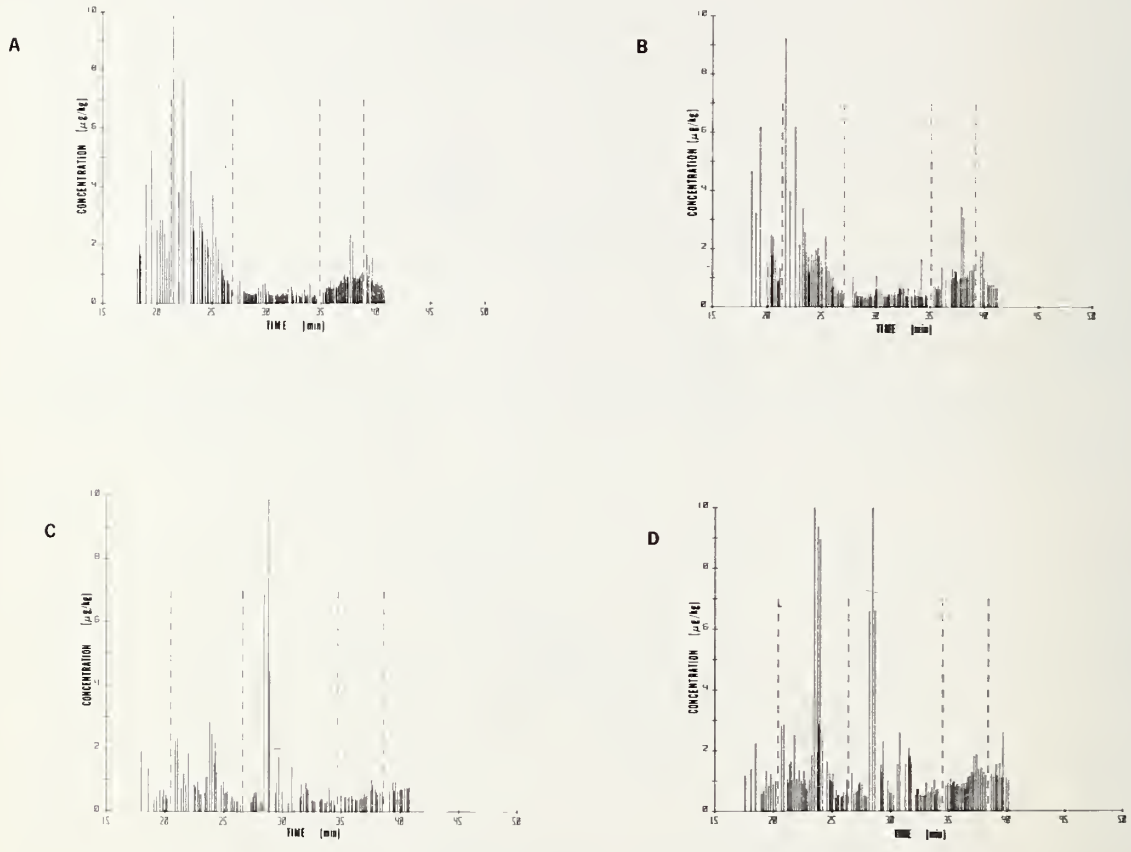


Figure 14. Concentration vs time histograms of Middleton Island sediment samples. (A and B) bottle 1, (C and D) bottle 2. Peak heights in chromatograms 13A-D have been reduced relative to the internal standard peak heights and plotted as single species concentrations.

Included in this report are results of detailed GC-MS analyses of sediments from the Katalla River and Old Valdez. The gas chromatograms obtained from both of these sediments indicated the presence of a "petroleum hump," a broad peak due to unresolved clusters of large numbers of individual components. The total ion chromatograms (computer-generated gas chromatograms) for these two sediments are presented in Figures 15A and 16A, respectively. Figures 15B and 16B contain the m/e 43 single ion records

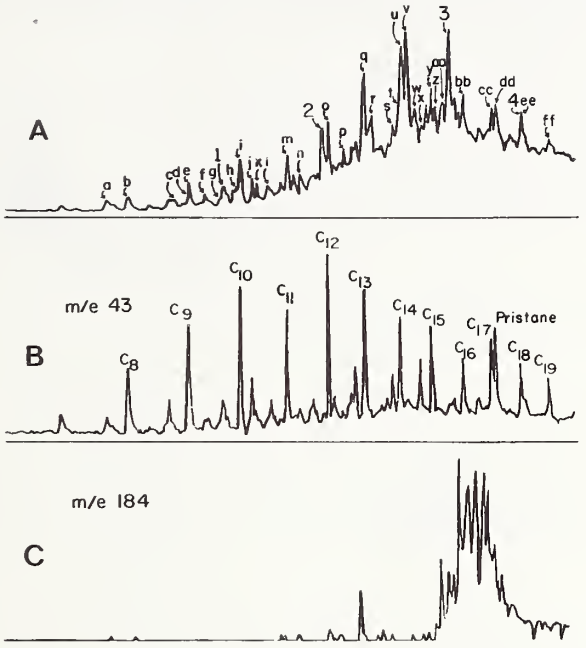


Figure 15. GC-MS analysis of Katalla River sediment: (A) total ion chromatogram, (B) m/e 43 single ion record, (C) m/e 184 single ion record indicating presence of C_4-naphthalenes. C_x = normal saturated alkane containing x carbon atoms. Ø-C_x = benzene substituted with x carbon atoms (e.g. Ø-C_3 could be trimethyl, propyl-, isopropylbenzene, etc.) Peaks labeled 1,2,3,4 are internal standards: mesitylene, naphthalene, trimethylnaphthalene, and phenanthrene, respectively. Identifications followed by "?" are not definite due to incompletely resolved spectra.

a = Ø-C_1
b = C_8
c,d = Ø-C_2
e = C_9
f = Cyclohexane-C_3
g,h = Ø-C_3
i = C_{10}
j,l = Ø-C_4
k - Cyclohexane-C_4
m = C_{11}
n = Cyclohexane-C_5
o = C_{12}
p = Cyclohexane-C_6
q = C_{13}
r = Ø-C_6
s,x = naphthalene-Et (?)
t = C_{14} (?)
u,v,w = naphthalene-$(CH_3)_2$
y = C_{15}
z,aa = naphthalene-C_3
bb = C_{16}
cc = C_{17}
dd = pristane
ee = C_{18}
ff = C_{19}

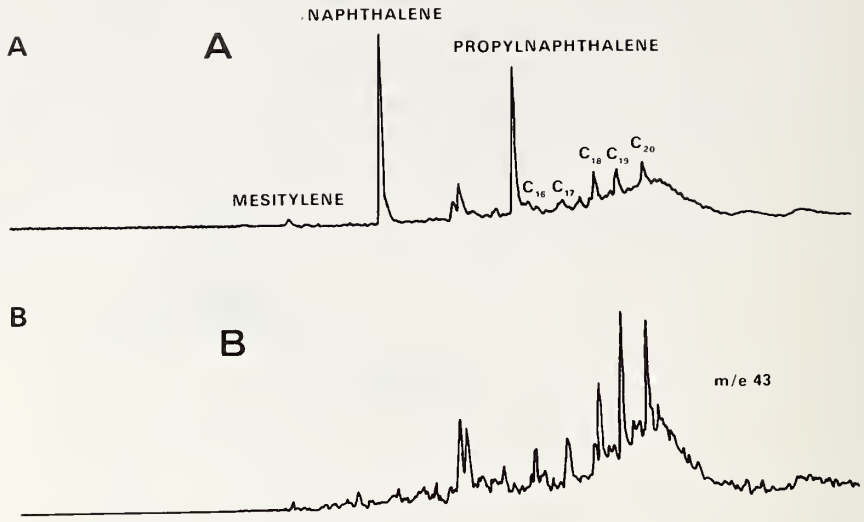


Figure 16. GC-MS analysis of Old Valdez sediment. (A) Total ion chromatogram. (B) m/e 43 single ion record. The aromatic hydrocarbon peaks labeled are internal standards.

for these two samples. (Single ion records are computer-generated mass specific gas chromatograms used in locating particular compounds or classes of compounds containing an indicative m/e peak in their mass spectra.) A peak at m/e 43 is indicative of aliphatic hydrocarbons and in Figures 15 and 16 these single ion records help identify the homologous series which one could not have visually identified in the complex gas chromatograms obtained from these sites.

The GC-MS analyses of the sediments from the Katalla River confirm that the major constituents are all hydrocarbons. Due to the large number of aromatic hydrocarbons and to the absence of any even/odd preference in the aliphatic series, the presence of petroleum is indicated [18]. This is an expected result since the Katalla River site is downstream from known oil seeps. The sediments from Old Valdez were collected at a site subject to an oil spill caused by the 1964 earthquake. Although the GC-MS analyses of these sediments indicate much lower levels of hydrocarbons than in the Katalla River sediment, trace hydrocarbon contamination is still present ten years after the spill. The aliphatic nature and petroleum origin of these hydrocarbons is further suggested by the m/e 43 single ion record and the mass spectrometric identification of the major peaks as straight-chain alkanes again without any even/odd preference. Another indication of the age of the hydrocarbons in the Old Valdez sediment is the shift of the oil hump to higher molecular weights than in the Katalla River chromatogram.

One final note of interest concerns the complexity of the petroleum hump, which contains a large number of unresolved hydrocarbon peaks. By the use of single ion records some compounds can be easily resolved. An example is shown in Figure 15C which contains the single ion record for m/e 184, the molecular ion for C_4-substituted

naphthalenes. Similar single ion records can be generated for other aromatic families.

The coupled-column LC procedure has been applied to water samples following headspace analysis (figs. 17-19). To the usual mixture of aromatic compounds included in the internal standard, benzpyrene was added as representative of the larger condensed ring hydrocarbons. During headspace analysis compounds more volatile than phenanthrene are substantially removed from the sample. As a result, the internal standards remaining in the sample to characterize the high molecular weight UV absorbing polynuclear aromatics are phenanthrene (only ∿20% removed during headspace sampling) and benzpyrene.

Water samples taken from the Katalla River (fig. 17) and Old Valdez (fig. 18) sites show low but measurable levels of UV absorbing (254 nm) components. On the other hand, water samples collected at Squirrel Bay (fig. 19) are clean in comparison. These results are as expected, since the Katalla River is a known oil seepage area, Old Valdez was the site of an oil spill in the 1964 earthquake and Squirrel Bay appears to be a pristine area.

Figure 17. Coupled-column liquid chromatographic analysis of Katalla River water. Peaks labeled 1 and 2 are the internal standard compounds phenanthrene and benzpyrene.

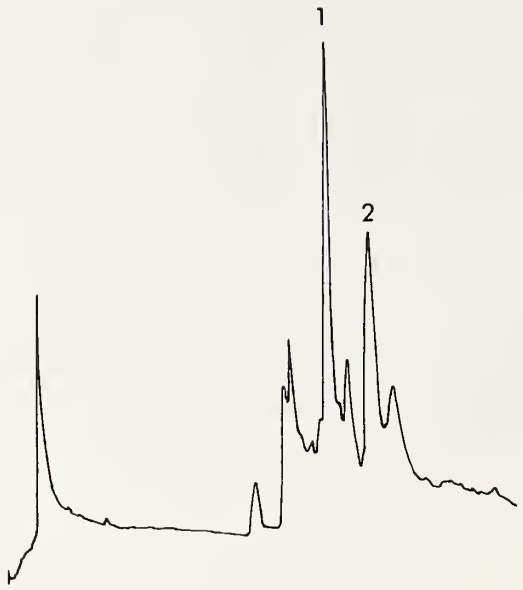


Figure 18. Coupled-column liquid chromatographic analysis of Old Valdez sea water. Peaks labeled 1 and 2 are the internal standard compounds phenanthrene and benzpyrene.

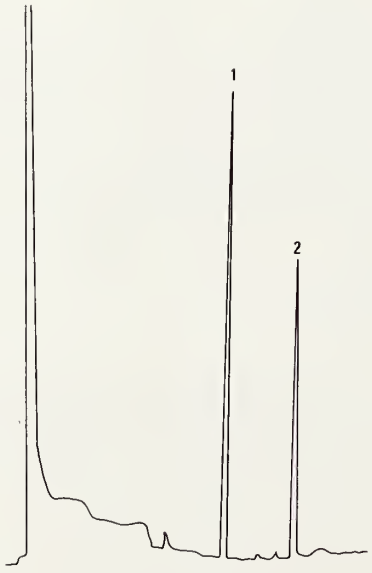


Figure 19. Coupled-column liquid chromatographic analysis of Squirrel Bay sea water. Peaks labeled 1 and 2 are the internal standard compounds phenanthrene and benzpyrene.

4. CURRENT RESEARCH - MARINE TISSUE ANALYSIS

Work is progressing on the development of an analytical method for the determination of petroleum hydrocarbons in the various tissue samples obtained in Prince William Sound. Initial efforts are being conducted with Mytilus samples collected in 1973 and 1974. The analytical method being developed employs dynamic headspace sampling to remove organic components present in the homogenized tissue sample followed by GC quantitation of the components. This technique avoids much of the sample handling necessary in digestion/ extraction methods and permits the determination of trace (microgram per kilogram) levels of the organic components. Since most of the organic compounds being removed from the tissue homogenate are biological in origin, a substantial part of the methods development is being concentrated on a means for effective removal of the biogenic compounds from the adsorbent column used in headspace sampling prior to gas chromatography. Effective clean-up of the adsorbent column should permit a reduction of the biogenic background in the chromatogram (see fig. 20), and allow greater sensitivity (sub-microgram per kilogram) for individual components than now possible. Presently GC-MS is being used to identify the major components removed from the tissue. This information will facilitate the development of a specific sample clean-up scheme. High speed liquid chromatography is being investigated for its application to this clean-up process.

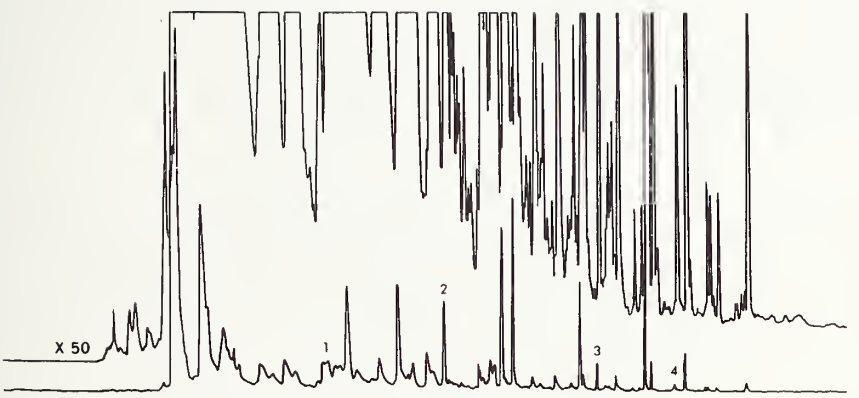


Figure 20. Gas chromatogram of headspace sampled Mytilus tissue without any chemical clean-up of the TENAX-GC pre-column. Numbered peaks correspond to the internal standard compounds (1 = mesitylene, 2 = naphthalene, 3 = trimethylnaphthalene and 4 = phenanthrene) each added at the ∿80 µg/kg level.

Another area of concern is the relative recoveries of the internal standard compounds used for quantitation and the hydrocarbons incorporated in the tissue sample. Good quantitation is dependent upon the internal standard added at the beginning of the sample work-up. It is therefore imperative to know if the internal standard components are recovered to the same extent as these components would be if incorporated in the tissue matrix. Studies utilizing Mytilus and oyster samples fed ^{14}C-labeled aromatic hydrocarbons are being used to compare recoveries of these hydrocarbons. The tissue

samples are homogenized and subjected to headspace analysis with the amounts of ^{14}C-labeled compound on the TENAX-GC pre-column and remaining in the sampling flask water and tissue solids being determined by liquid scintillation counting.

Experiments performed in this laboratory indicate that 78 ± 12% of ^{14}C-naphthalene incorporated into Mytilus tissue can be recovered via conventional headspace sampling and collected on a TENAX-GC packed pre-column. Live mussels were collected and exposed to sea water containing the ^{14}C-naphthalene in an aquarium. The mussels were then shucked and frozen until analyzed. Following headspace sampling the collected ^{14}C-naphthalene was stripped off the TENAX column with 10 ml of hexane. A 2 ml aliquot of the hexane was then evaluated by liquid scintillation counting.

The headspace-sampled homogenate remaining in the flask was centrifuged and the water and solids separated. Combustion of the solid residue and subsequent liquid scintillation counting of the $^{14}CO_2$ produced accounted for an additional 16 ± 4% of the ^{14}C-activity. Combustion of the entire tissue residue avoids problems of inhomogeneity of the tissue and the problem of color quenching in solubilized tissue aliquots. The water from the centrifuged flask homogenate was extracted three times with toluene and its activity also evaluated by liquid scintillation counting. The flask water accounted for 12 ± 5% of the total ^{14}C-activity.

Further experiments in this laboratory using non-labeled naphthalene, trimethylnaphthalene and phenanthrene added as internal standards to the tissue homogenate have been conducted. Following headspace-sampling and capillary column GC calculations indicate that 61 ± 20% of the naphthalene, 31 ± 22% of the trimethylnaphthalene and 16 ± 14% of the phenanthrene can be recovered. These results were obtained by alternating on-column injections of known amounts of the three internal standards with the GC runs of the tissue samples and comparing peak heights of the respective standards.

Indications are, therefore, that at least in the case of naphthalene, an internal standard added to a homogenate of mussel tissue can be recovered to the same extent as naphthalene incorporated into the live mussel. Further work is continuing with other aromatic compounds used as internal standards and will also consider several aliphatic compounds of interest.

The authors wish to express their appreciation to Mrs. Donna L. Kline for her efforts in typing this manuscript and preparing the camera-ready copy for publication.

5. APPENDIX A

PREPARATION OF HYDROCARBON-FREE WATER AND ORGANIC SOLVENTS

Introduction

Fossil hydrocarbon contaminants seem to be present in most solvents, even those of reagent grade quality. These solvents must be purified to a level that at least makes the blank tolerable. Generally this can be achieved by distillation, one, two or three times, depending upon the level of initial impurity of the solvent and the effectiveness of distillation in removing the undesired impurities. Column chromatography can also be useful in this regard.

Water

In the work of this report the principal "solvent" used is water. Technology is readily available and well developed for the preparation of highly pure water (even though the usual objective is removal of inorganic constituents and not hydrocarbon contaminants as is required in this work). Organic constituents can be removed from the water by distillation from alkaline potassium permanganate followed by passage of the distilled water through a column packed with XAD-2 macro-reticular copolymer.

Equipment

1) Glass Stills

Glass stills for the distillation of water are constructed from the following components.

a) Still pot -- five-liter, two-necked, round-bottom flask with ℔ 24/40 joints.

b) Distillation column -- 30 mm o.d. x 90 cm glass tubing with ℔ 24/40 joints, pinched at the bottom to support the packing of four mm ceramic Berl saddles.

c) Still Head -- ℔ 24/40 ground joints; the top of the still head is a ℔ 10/30 joint to accommodate a thermometer.

d) Adapter Tube -- glass 105° angle with ℔ 24/40 joints. This connects the still head to the condenser.

e) Condenser -- Graham type, jacket length 300 mm.

f) Adapter -- glass with side arm to relieve pressure. This connects the condenser to the receiver.

g) U-Tube -- stainless steel tubing 10 cm x 0.6 cm (1/4 in) o.d. bent into a U-shape and packed with TENAX-GC held in place with cleaned glass wool at each end of the tube. This connects to the side arm of the adapter f) with a Teflon sleeve and prevents absorption of room vapors by the distillate.

h) Receiver -- two-liter round-bottom flask with a ℔ 24/40 joint.

i) Heating Mantle -- used with Variac to control heating rate.

2) Ceramic Berl Saddles

Clean four millimeter ceramic Berl saddles are used for packing the distillation column.

3) Water Storage Bottles

Clean empty sulfuric acid bottles (9 lb bottles) are used for storing the

distilled and hydrocarbon-free water.

4) XAD-2 Copolymer Column Chromatographic System

Water obtained by distillation is further purified by passage through a chromatographic column packed with XAD-2 adsorbent (see fig. 21 for details).

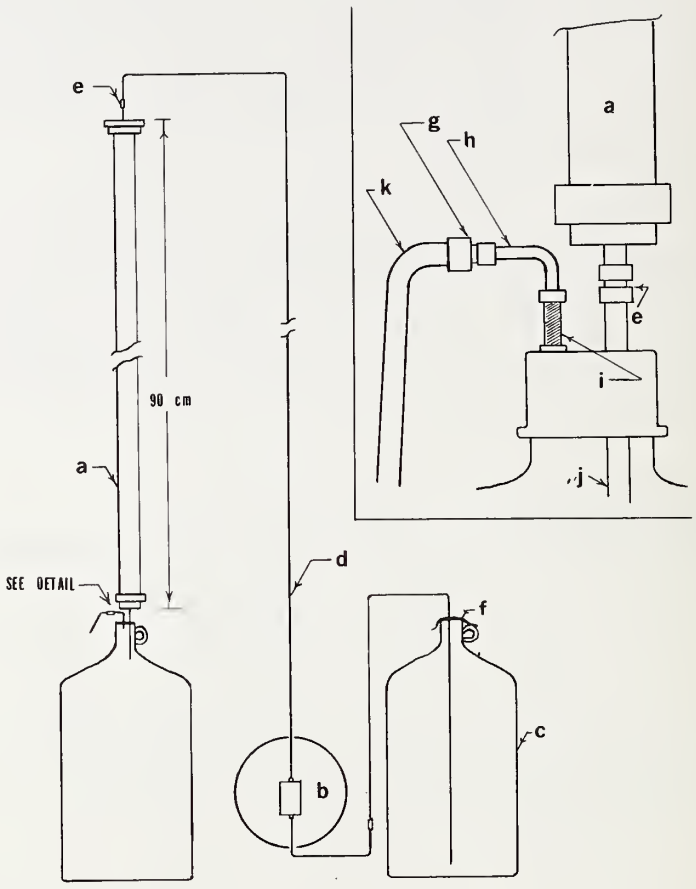


Figure 21. Column Chromatography System for Water Purification. (a) stainless steel column packed with XAD-2 copolymer, (b) Milton-Roy Mini-Pump, (c) water supply bottle (one-gallon size), (d) stainless steel tubing (1/4 in) (e) Swagelok union, (f) aluminum foil, (g) Swagelok reducing union, (h) stainless steel tubing (1/8 in o.d.), (i) bulkhead connector, (j) stainless steel drip tube (1/4 in), (k) stainless steel tubing (1/4 in) packed with TENAX-GC.

a) A stainless steel, preparatory chromatography column (2.54 cm o.d. x 1 m) available from DuPont is used to contain the copolymer. A 10 µm stainless steel filter at the exit end of the column retains the packing.

b) The system uses a Milton-Roy Minipump (Model 196-89) capacity 460 ml/hr maximum and 6900 kPa (1,000 psi) maximum or equivalent for pumping the water.

c) Stainless steel tubing (1/4 in) is used along with stainless steel Swagelok fittings to connect the various components of the pumping system.

d) A plastic screw cap with two holes (>0.6 cm in diameter) fits on the receiver bottle. One hole in the cap permits entry of the drip tube from the chromato-

graphic column. The second hole is fitted with a 15 cm stainless steel (1/4 in) vent tube packed with TENAX-GC polymer to trap hydrocarbons that might diffuse into the receiver bottle. A Swagelok stainless steel bulkhead union is used to make the connection.

5) Soxhlet Extractor

An extra-large Sochlet extractor (64 mm i.d. x 185 mm usable depth) is used for cleaning the XAD-2 copolymer prior to its use in water purification. A heating mantle and Variac are used with the Soxhlet extractor.

6) Ultrasonic Cleaner

This is used for cleaning the Berl saddles.

Reagents

Reagents used in cleaning the Berl saddles and XAD-2 copolymer:

1) methylene chloride -- reagent grade for cleaning Berl saddles.
2) acetone -- reagent grade for cleaning Berl saddles.
3) concentrated sulfuric acid -- used warm for cleaning Berl saddles and glass still pots.
4) distilled water (from in-house source) -- used for cleaning Berl saddles and water storage bottles.
5) glass wool -- used for wrapping distillation column.
6) potassium permanganate -- used in distillation pot for hydrocarbon-free water.
7) potassium hydroxide -- used in distillation pot for hydrocarbon-free water.
8) sodium oxalate -- used with sulfuric acid to prepare a cleaning solution for the still pots.
9) XAD-2 copolymer (Rohm and Haas Co., Philadelphia, Pa.)
10) acetonitrile -- reagent grade used for cleaning the XAD-2 copolymer.
11) methanol -- reagent grade used for cleaning the XAD-2 copolymer.

Procedure for Distillation of Water

1) Clean the Berl saddles as outlined below.

a) Soak the saddles in reagent grade methylene chloride with ultrasonic agitation for 30 minutes.

b) Decant the methylene chloride and repeat sonication with reagent grade acetone as the solvent.

c) Rinse the saddles several times with distilled water.

d) Soak the saddles for 24 hours in warm concentrated sulfuric acid, stirring periodically.

e) Rinse saddles with eight changes of distilled water agitating ultrasonically for 5 minutes at each rinse.

f) Dry saddles in oven at 110 °C.

2) Pack the still column with portions of the cleaned saddles by adding volumes that increase the height of the packing by three cm at a time. Tap the column gently between additions to settle the packing.

3) Assemble the still after packing the distillation column.

a) Affix a wire joint clip at each joint.

b) Wrap a five mm thickness of glass wool around the distillation column to keep the column at constant temperature.

4) Use water storage bottles that are cleaned as described below.

a) Immerse each bottle in the hot (110 °C) concentrated sulfuric acid bath for at least 30 minutes.

b) Rinse bottles six times with distilled water, 250 ml portions each time.

c) Completely fill the bottles with distilled water and empty.

d) Line the screw caps with aluminum foil that has been washed with methylene chloride and acetone and rinsed with triply-distilled pentane.

e) Before storing alkaline permanganate distilled water, rinse bottle twice with 100 ml portions of the hydrocarbon-free water.

5) Add house distilled water for redistillation through the side arm of the still pot to a level three cm above the top of the heating mantle.

6) Add 10 g of potassium permanganate and four pellets of potassium hydroxide to the pot using a glass powder funnel.

7) Add four clean Berl saddles and cap the side arm port.

8) Heat the water to boiling, setting the power to the heating mantle so that distillation rate is 5 ml/min.

9) Discard the first 500 ml of distillate. (The initial distillation serves to steam clean the system including the packing.)

10) Add 500 ml of water to the pot.

11) Lower the power to the heating mantle so that only gentle boiling occurs.

12) Allow the water to reflux overnight permitting digestion of organic constituents in the water.

13) Raise the heat level so that distillation at 5 ml/ min occurs, and distill 250 ml into a 500 ml flask.

14) Discard this water and replace the receiver with a clean two-liter flask.

15) Distill 1800 ml of water and pour the water into a clean storage bottle.

16) Let the still pot cool for five minutes.

17) Add distilled water through the side arm to the former level.

18) Add two pellets of KOH and two clean Berl saddles and bring the pot to gentle reflux and let stand overnight.

19) Resume the procedure for distillation the following day.

20) Care for the stills on the long term as follows.

a) Keep the still pot at reflux over weekends.

b) Empty the still pot every two weeks and discard the water and boiling stones.

c) Wash the still pot with a solution of five grams of sodium oxalate in 300 ml of water to which is added 15 ml of concentrated sulfuric acid.

d) Flush out this solution at least six times with distilled water and proceed with distillation as before.

Procedure for Further Water Purification by Column Chromatography

1) Clean the XAD-2 copolymer as follows.

a) Assemble the Soxhlet extractor.

b) Pour XAD-2 copolymer into a 150 mm x 57 mm o.d. glass thimble with a fritted-glass bottom (extra coarse porosity). Fill to a level nine cm from the top.

c) Add 500 ml acetonitrile to the boiling flask.

d) Add four clean Berl saddles to the flask as boiling stones.

e) Set the flask into place and turn on the power to a heating mantle.

f) Adjust the reflux ratio so that the solvent drips from the tip of the condenser at the ratio of about one drop every four seconds.

g) Extract the copolymer for 24 hours.

h) Replace the acetonitrile with methanol and extract for another 24 hours.

i) Dry the XAD-2 copolymer in a 500 ml beaker covered with a watchglass in an oven at 110 °C until the copolymer is free flowing. Stir periodically with a glass rod.

j) Use the XAD-2 copolymer immediately to avoid recontamination by room air.

2) Pack the chromatographic column with dry XAD-2 copolymer by adding 40-50 ml of the material each time with tapping between additions.

3) After four or five additions have been made, tamp the packing gently with a clean, ten mm glass rod. Repeat until the column is fully packed.

4) Referring to Figure 21, connect the Minipump to the top of the packed column with stainless steel tubing and Swagelok fittings.

a) Connect to the inlet side of the pump two pieces of 0.6 cm o.d. stainless steel (1/4 in) tubing in series; first connect an elbow 10 cm long and second an inverted "U" of the length and geometry to permit pumping water from a cleaned water storage bottle.

b) Connect a length of stainless steel tubing to the outlet of the column with a Swagelok union to serve as a drip tube.

c) Slip this tube through one of the holes in the receiver bottle cap.

d) Wind Teflon tape around the drip tube where it passes through the cap to seal out room air.

e) Fit the stainless steel TENAX packed vent tube into the second hole in the receiver bottle cap, using a Swagelok bulkhead union to make the connection.

5) Prime the pump.

a) Break the connection at the exit port of the pump.

b) Set the rotary control knob for a pumping rate of 100%.

c) Turn on the pump and drip water from a clean medicine dropper into the exit port until the pump starts to deliver water from the reservoir bottle.

6) Reconnect the column to the exit port.

7) Pump one liter of water through the column to flush the system. Discard the water.

8) Test the column by attaching a new, clean TENAX polymer trap to the column exit and pumping 10 liters of water through it at the rate of 1.5 ml per min.

9) Dry and run the TENAX trap on a gas chromatograph (see section on Gas Chromatographic Analysis). The blank should indicate no contamination above the usual TENAX level.

10) If the water shows no contamination, begin to collect water in cleaned water storage bottles.

11) Cap each bottle with a cleaned aluminum foil lined screw cap.

12) Label bottles indicating the XAD-2 treatment and the date. Use the water within two weeks or discard.

13) Recycle the storage bottles through the concentrated sulfuric acid bath.

14) Keep the column running continuously, never permitting air to get into the system. Reduce the pumping rate for overnight or weekends. Cap the column if long periods of inactivity are anticipated.

Organic Solvents

Organic solvents such as methanol, acetonitrile, methylene chloride and pentane are distilled in glass stills identical to the water stills. Reagent grade methanol, acetonitrile, and methylene chloride and technical grade pentane are used as starting materials. Pentane is distilled three times; the other solvents are distilled only once.

Equipment

1) Glass Stills

Glass stills like those used for the distillation of water are assembled for the distillation of the various organic solvents used in the sampling and analysis procedure.

2) Solvent Storage Bottles

Brown glass 4 liter bottles (one-gallon size) cleaned in hot concentrated sulfuric acid are used for storing the distilled solvents.

3) Solvent Evaporation System

To check the purity of the distilled solvents a system is constructed to facilitate the evaporation of large quantities of a solvent down to a volume suitable for injection into a gas chromatograph. The system components are

a) A cylinder of compressed nitrogen (water pumped) with a pressure reducing regulator connected to a trap (1.2 cm o.d. x 35 cm aluminum tubing) filled with molecular sieve 5A (20 cm) and activated charcoal (15 cm). Plugs of acetone-pentane washed glass wool are inserted at each end of the tube to keep the packing in place.

b) Copper tubing (120 cm x 0.6 cm o.d.) connected to the exit end of the trap. The tubing is pre-cleaned with methylene chloride, acetone and distilled pentane (in that order), and then flamed (with nitrogen flowing) using a propane torch.

c) Miscellaneous glassware: beakers, glass funnel (125 mm diameter at rim), five ml centrifuge tubes, disposable pipettes.

4) Gas Chromatograph

An instrument with a sensitive flame ionization detector suitable for determining solvent purity is used. This instrument is fitted with a 0.6 cm o.d. x 3 m column packed with 1% OV-101 on 100/120 mesh Chromosorb WHP.

Reagents

1) methanol -- reagent grade for single distillation.

2) acetonitrile -- reagent grade for single distillation.

3) methylene chloride -- reagent grade for single distillation.

4) pentane -- technical grade for triple distillation.

5) carbon disulfide -- reagent grade used for making up samples for gas chromatographic analysis for solvent purity.

Procedure for the Distillation of Solvents and Testing Purity

1) Assemble the distillation equipment in a fume hood so that hazardous concentrations of fumes cannot build up.

2) Collect the first 200 ml of distillate in a 500 ml round-bottom flask and store in a bottle marked "pre-cut/ pot."

3) Collect the next 1800 ml of distillate in a clean two-liter round-bottom flask. Cut off the power to the mantle and wait until the distillation stops.

4) Transfer the distillate to a clean brown storage bottle. Label the bottle appropriately giving the date of distillation.

5) Pour the remainder of the solvent in the pot into the "pre-cut/pot" bottle.

6) Once a month clean the packing in the distillation column by distilling water (to reduce any build-up of residue on the column packing).

a) Distill a total of 1500 ml of water through the system.

b) Distill 250 ml of methanol to remove any remaining water.

c) Resume use for solvent distillation but take a 500 ml pre-cut to assure that the methanol has been removed.

7) To check the purity of the distilled solvents assemble the system for evaporating solvents under a stream of N_2 gas.

8) Pour 400 ml of solvent to be tested into a 600 ml beaker. Place on a hot plate in a fume hood.

9) Invert a glass funnel over the beaker and clamp in place.

10) Warm solvent to about 45 °C.

11) Insert the end of the copper tubing through the stem of the funnel to a point four cm above the surface of the solvent and clamp in place.

12) Slowly adjust the flow of nitrogen to a rate at which slight ripples appear on the surface of the solvent.

13) Volatilize the solvent (periodically lowering the end of the copper tubing to maintain distance from the surface). Evaporate until about two ml of solvent remains in the beaker.

14) Transfer the liquid with a disposable pipette into a 5 ml centrifuge tube.

15) Wash down the sides and bottom of the beaker with four 0.5 ml portions of the solvent under test. Transfer washings to the centrifuge tube.

16) Evaporate solvent to dryness by use of N_2 flow as before.

17) Add 100 µl of reagent grade carbon disulfide.

18) Inject 1 µl and 10 µl of the solution into a gas chromatograph using the three meter column packed with 1% OV-101 on 100/120 Chromosorb WHP.

19) Inject comparable volumes of the carbon disulfide alone as blanks.

20) Inspect the chromatograms for the presence of hydrocarbon contaminants in the distilled solvent.

6. APPENDIX B

DETAILED DRAWINGS OF SEAWATER DROP SAMPLER

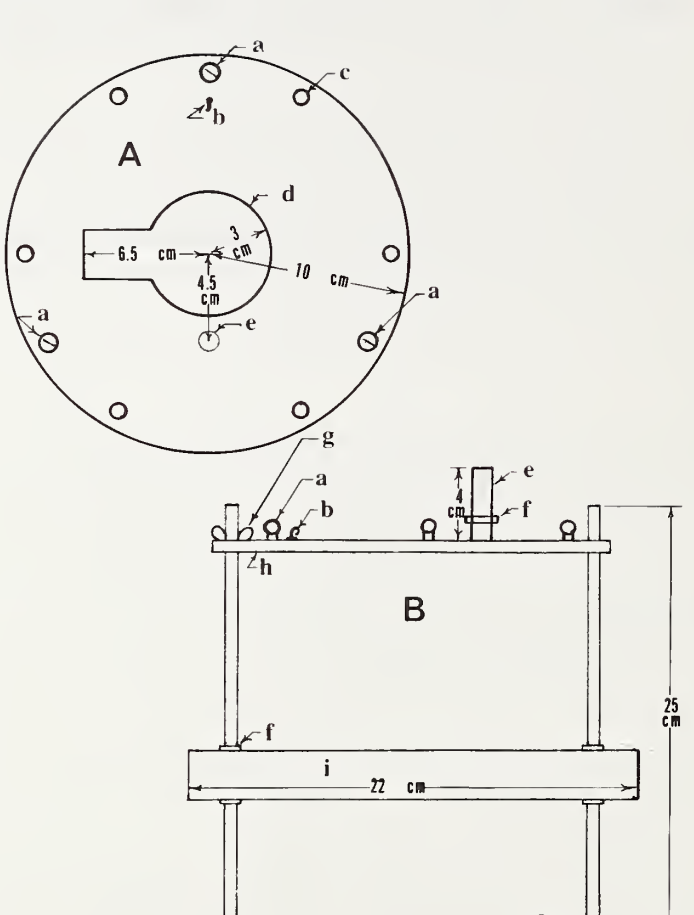


Figure 22. Stainless Steel Drop Sampler. Detailed views of side and top plate. (A) Top plate. (a) Eye hook for attaching chain to top plate; (b) Spring hook; (c) Six holes drilled so that stainless steel threaded rod (1/4 in) passes through; (d) Hole for neck of bottle; (e) Stainless steel threaded rod (3/8 in) for attaching bottle closure unit. (B) Side view. (a) Eye Hook; (b) Spring hook; (c) Six stainless steel threaded rods (1/4 in) welded to bottom plate - Two shown; (e) threaded rod (3/8 in); (f) Nut; (g) Wing nut to clamp top plate; (h) Stainless steel (1/4 in) top plate; (i) Teflon bumper ring (1 in); (j) Stainless steel (1/4 in) bottom plate.

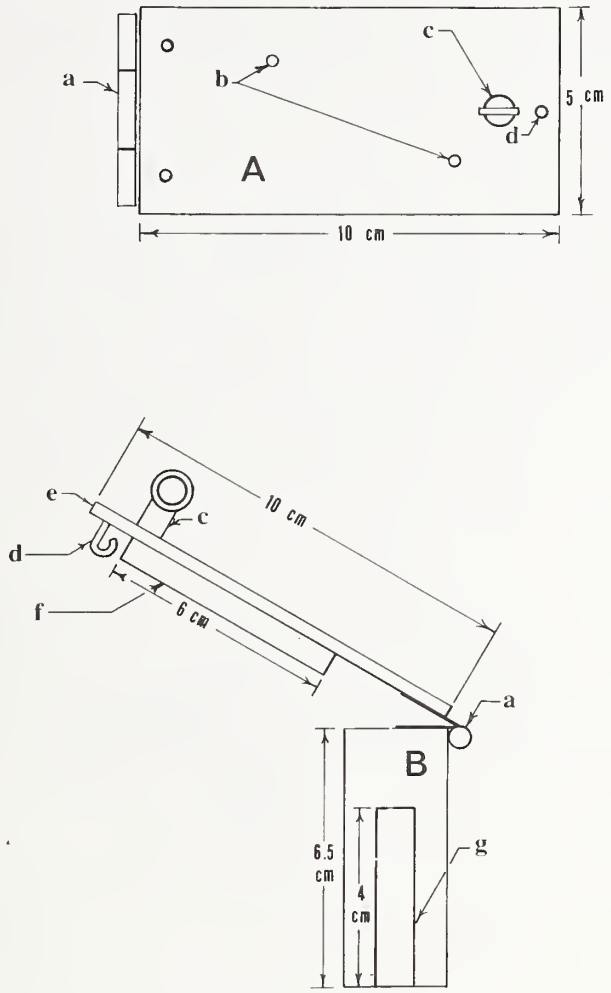


Figure 23. Stainless steel drop sampler collection bottle closure unit. (A) Top view. (a) Hinge; (b) Holes for Teflon gasket; (c) Eye hook; (d) Spring hook. (B) Side view. (a) Hinge; (c) Eye hook for opening closure unit; (d) Spring hook; (e) Stainless steel plate (1/8 in); (f) Teflon gasket; (g) Threaded hole: fits rod (3/8 in) on top plate.

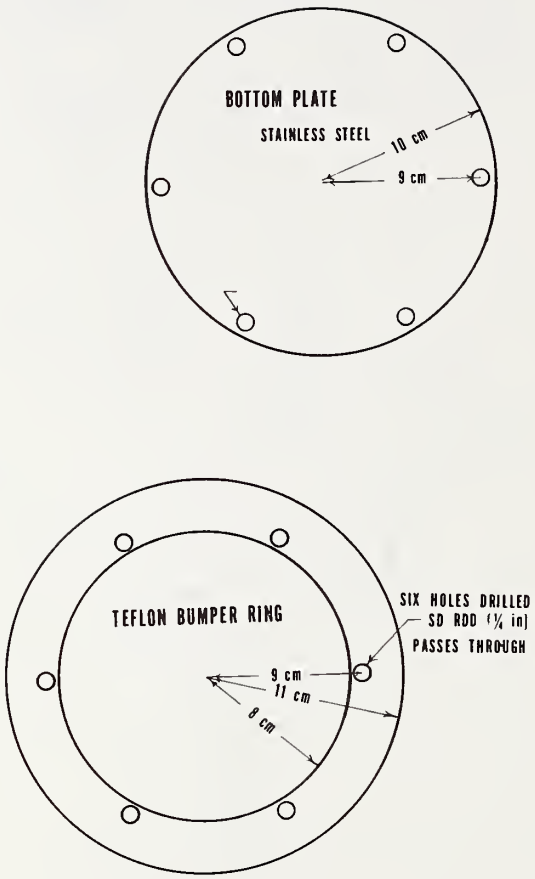


Figure 24. Stainless steel drop sampler. Detailed views of bottom plate and Teflon bumper ring.

7. APPENDIX C

ALTERNATIVE PROCEDURES USED IN HYDROCARBON ANALYSIS: PUMPING OF WATER SAMPLES THROUGH TENAX-GC COLUMNS

Introduction

Pumping water samples through a packed column of TENAX-GC offers an alternative procedure to that of headspace sampling for the determination of trace hydrocarbons. This alternative is feasible, if the water is free of particulate or biological matter. Particulate matter tends to plug up the in-line filters and inhibits pumping of the sample. Biological materials may leak through the filter to the TENAX column potentially causing a biological pyrogram to mask the gas chromatograms of the water sample. In those instances where this alternative method can be used, it can be used on site, and therefore, circumvents the problem of possible sample loss due to breakage during shipping.

Equipment

1) Water Pumping System

A water pumping system consisting of a pump, in-line filter and stainless steel tubing is used to draw the water sample through the TENAX trapping column. This system is constructed from the parts listed below.

a) Milton Roy Controlled Volume Minipump, Model #19689 or equivalent.

b) Nupro in-line filter (7 µm), Swagelok Cat. #SS-4F-7.

c) 0.6 cm o.d. (1/4 in) stainless steel tubing and stainless steel capillary tubing (1/16 in) with an 0.038 cm (.015 in) i.d.

d) Stainless steel Swagelok fittings to permit assembly of the various components of the system..

Procedure for the Pumping of Water Through TENAX Columns

1) Assemble the pumping system as shown in Figure 25.

a) Install a TENAX column on the suction side of the pump.

b) Install an in-line filter up-stream from the TENAX column.

c) Run a suction line from the in-line filter to the sample bottle making sure that the sample inlet is at a higher elevation than the pumping chamber. (It is preferable to have a slight hydrostatic pressure on the pump. This is done by having the pump inlet below the level of liquid in the sample vessel.)

d) Tighten all fittings; the sample suction piping must be absolutely air tight.

2) Purge all air from the pump by operating at full stroke with the discharge to atmosphere until liquid flows freely from the discharge connection. Suction on the discharge end is often helpful. Clean water rather than sample water is used during the priming process.

3) Connect a drainage tube to the discharge side of the pump using capillary tubing. Precise metering of flow is only possible when the pressure on the discharge side of the pump exceeds that of the suction side.

4) Allow the pump to run for at least ten minutes to work out any remaining air.

5) Set the stroke to the desired value by means of the adjusting knob. The scale on the stationary sleeve is calibrated in % full stroke. One liter of water can be sampled in two hours by pumping at 100% of stroke.

6) Once the sample has been pumped through the TENAX column, cap the ends and freeze the column on dry ice for return to the laboratory.

7) Store the TENAX column frozen until it is ready to be dried for gas chromatographic analysis.

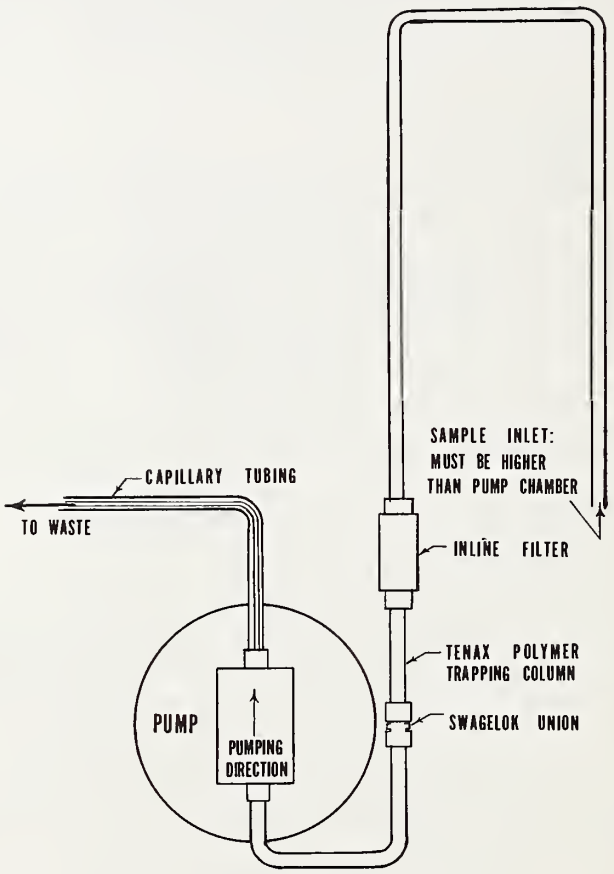


Figure 25. System for pumping water samples through TENAX packed pre-columns.

8. REFERENCES

[1] Brown, R.A., Searl, T.D., Elliott, J.J., Phillips, B.G., Brandon, D.E., and Monaghan, P.H. in Proceedings of the 1973 Joint Conference on Prevention and Control of Oil Spills, pp. 505-519, American Petroleum Institute, Washington, D.C. (1973).

[2] Clark, R.C. Jr., and Finley, J.S., ibid., pp. 161-172.

[3] McAuliffe, C.D., Chem. Tech. 1, 46 (1971).

[4] Warner, J.S., in Marine Pollution Monitoring (Petroleum), pp. 195-196, NBS Special Pub. 409, Washington, D.C. (1974).

[5] Wasik, S.P., and Brown, R.L. in Proceedings of the 1973 Joint Conference on Prevention and Control of Oil Spills, pp. 223-230, American Petroleum Institute, Washington, D.C. (1973).

[6] Farrington, J.W., Teal, J.M., Quinn, J.G., Wade, T., and Burns, K., Bull. of Environ. Cont. and Tox. 10, 129-136 (1973).

[7] Melpolder, F., Warfield, C., Headington, C., Anal. Chem. 25, 1453 (1953).

[8] Grob, K., J. of Chromatog. 84, 255 (1973).

[9] Zlatkis, A., Bertsch, W., Lichtenstein, H., Tishbee, A., Shunbo, F., Liebich, H., Coscia, A., Fleischer, N., Anal. Chem. 45, 763 (1973).

[10] Snyder, L.R., in Modern Practice of Liquid Chromatography, J.J. Kirkland, ed., Wiley-interscience, New York, 1971, pp. 232-235.

[11] Snyder, L.R., J. Chromatog. Sci. 8, 692 (1970).

[12] Smith, V.C., in Proceedings of the 29th International Water Conference, pp. 125, Pittsburgh, Pennsylvania (1968).

[13] German, A.L., and Horning, E.C., J. Chromatog. Sci. 11, 76 (1973).

[14] Wilber, C.G., The Biological Aspects of Water Pollution, Charles C. Thomas, Springfield, Ill., 1969.

[15] Blumer, M., Environmental Affairs 1, 54 (1971).

[16] "Particulate Polycyclic Organic Matter," Committee on Biologic Effects of Atmospheric Pollutants, National Academy of Sciences, Washington, D.C. (1972).

[17] Wasik, S.P., J. Chromatog. Sci., in press.

[18] Bray, E.E., and Evans, E.G., Geochim. Cosmochim. Acta 22, 2 (1961).

9. INDEX

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The low concentration of hydrocarbons anticipated in pollution baseline studies necessitates the development of analytical techniques sensitive at the sub-microgram per kilogram concentration level. The method of analysis developed in this laboratory involves dynamic headspace sampling for volatile hydrocarbon components of the sample followed by coupled-column liquid chromatography for the non-volatile components. These techniques require minimal sample handling, reducing the risk of sample component loss and/or sample contamination. Volatile sample components are separated from the matrix in a closed system and concentrated on a TENAX-GC packed pre-column, free from large amounts of solvent and ready for GC/GC-MS analysis. Non-volatile compounds, such as the benzpyrenes, may be extracted from large volumes of water and concentrated on a Bondapak C18 packed pre-column for coupled-column liquid chromatographic separation and analysis. Results of the application of these techniques are presented and discussed.

17. KEY WORDS (six to twelve entries; alphabetical order; capitalize only the first letter of the first key word unless a proper name; separated by semicolons)

Baseline studies; gas chromatography; gas chromatography-mass spectrometry; hydrocarbons; liquid chromatography; petroleum analysis; trace analysis

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