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Abstract approved _____ William O. Forster

Manual and automatic solvent extraction techniques were used to concentrate organic material from natural waters. Field and laboratory extractions were compared to determine the most probable method and best solvents for efficient concentration of organic material from estuarine and sea waters.

The large scale extraction processes utilizing extractors made from 55 gallon drums were operated both by manually moving perforated metal discs through the water and solvent for mixing and by bubbling air through the aqueous-solvent mixture. Resulting samples proved large enough for the detection of short-chain fatty acids (carbon length 1 through 10). Identification of organic constituents was by gas chromatography-mass spectroscopy.

Other portions of these samples were separated by column chromatography with subsequent analysis by infrared, visible, ultraviolet, and mass spectroscopy. The proximity of the Hanford

Atomic Product Operations to the collecting area caused some of the organics to be labeled with trace amounts of radionuclides.

A counter-current pulse column extractor was used at sea aboard the Research Vessel YAQUINA. The organic solvent, hexone, used in this extraction was distilled before and after the extraction with various fractions of solvent being kept for control. The extraction was carried out under varying chemical and physical conditions. The hexone was back-extracted into hydrochloric acid, sodium bicarbonate, or sodium hydroxide before analysis by flame-ionization gas chromatography and mass spectroscopy. Other portions of the product hexone were analyzed for total solid matter recovered and carbon, hydrogen, and nitrogen content.

Small intermediate scale extractions were carried out in the laboratory using several solvents. Resulting organic material was analyzed by tandem gas chromatography-mass spectroscopy. Where possible, organic derivatives were prepared to aid in the identification of the recovered organic material.

The advantages and disadvantages of large scale extractions and small laboratory procedures were discussed.

Techniques of Solvent Extraction of Organic Material from Natural Waters

by

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INTRODUCTION

The purpose of this investigation is to develop solvent extraction techniques for the removal of organic matter from natural waters. Both shipboard and laboratory methods for the extraction of organic matter in amounts suitable for qualitative chemical analysis will be described.

The chemical composition of dissolved organic matter in sea water (that which passes through a 0.45μ filter) is among the least known subjects in oceanography. Knowledge of the 1) vertical and horizontal distribution of dissolved organic matter, 2) its variation in water masses, 3) changes with season, and 4) effects of biological activity, is minimum. The total dissolved organic carbon is of the order of 1-10 mg per liter and organic nitrogen is 0.03 to 0.4 mg per liter. This low concentration together with the high concentration of inorganic matter makes analysis very difficult.

The importance of organic matter is paramount to both the chemist and biologist. Organics are an integral part of the chemistry of the oceans since they may be involved in complexes, increasing the solubility of calcium carbonate and phosphate in sea water. It has been shown by Smith, Tatsumoto, and Hood (1960) that amino

acids and proteins may form carbamino carboxylic acid complexes with CO_2. They have indirectly shown these complexes to be present in sea water and have indicated their importance to photosynthesis. It is known that substances exist which lower surface tension. Adams (1937) and Hardman (1941) determined that these materials are not suspended matter.

It is known that organic substances and transition metals in sea water can be concentrated in the biota. Some organics and some of the transition metals, separately or combined, may be required for the growth of organisms. The purpose of elemental constituents has been reviewed and investigated by numerous groups but the subject is relatively untouched in oceanography. Rona, Hood, Muse and Buglio (1962) indicate that small portions of manganese and zinc are complexed in some manner with organics in sea water. According to Fogg (1953) extracellular products of blue-green algae are known to form complexes with inorganic ions. It seems probable that organic substances are not important as actual nutrients for these algae, but serve to regulate their ionic environment.

Organic compounds containing iron (Laevastu and Thompson, 1958) and boron (Gast and Thompson, 1958 and Noakes and Hood, 1961) are assumed to be present since higher concentrations were found in sea waters that had been strongly oxidized than in untreated

sea water samples. It must be noted that, at present, no direct evidence exists that organics are specifically chelated with the metals in the aquatic environment. However, organisms possess the ability to produce organic-metal chelates via metabolic processes and release them to the environment either by excretion or degradation after death. In addition, metabolism and oxidation of detrital and terrestrial runoff substances are likely sources of some of the organic matter dissolved in sea water. Duursma (1960), however states that the dissolved organics originate from dead phytoplankton and detritus and not from living cell excretions.

Various reports can be found on the nature of dissolved organic compounds in sea water, but relative percentages of organic classes such as proteins, lipids, vitamins and carbohydrates for a single water mass are unknown. Independent reviews by Cooper (1965) and Duursma (1965) tabulate known information concerning organic matter in sea water.

The small quantity of organics in sea water leaves the chemical oceanographer with the alternative of developing more sensitive equipment than is presently in use, or of processing large volume samples. The latter was selected for expediency. Of the several chemical

procedures available, solvent extraction appeared to be a plausible but expensive procedure to use in the aquatic environment.

Solvent extraction is not new to oceanography, but past work has been with small samples (five gallons or less). Slowey, Jeffrey and Hood (1962), and Williams (1961) isolated lipids from samples taken from the Gulf of Mexico and the Pacific Ocean, respectively. These samples were extracted with ethyl acetate and chloroform. Subsequent separation and analyses by gas chromatography led to the identification of saturated and unsaturated fatty acids of chain lengths varying from 10 to 22 carbons. Koyama and Thompson (1957) identified acetic, lactic and glycolic acids after extraction from Pacific Ocean water. A few hydrocarbons were identified in the organic solvent extracts of sea water by Slowey et al. (1962), who used thin layer chromatography (TLC) to separate certain fractions eluted from a silicic acid column prepared according to directions of Hirsch and Ahrens (1958).

Actual analyses of samples pose many difficulties due to the low concentration and high numbers of compound classes. Work by sanitation engineers has been of immense aid in group separation. Mueller et al. (1958) identified organic acids in water by chromatographic methods. River water was characterized for organic materials affecting taste and odor by Ryckman et al. (1961).

EXTRACTION PROCEDURES

Instrumentation and methods of sample collection became major concerns once solvent extraction was chosen as the means of organic matter concentration. Procedures used at different collection sites will be described separately.

Field Extraction of Estuary Water

Surface water from the Columbia River estuary at Astoria, Oregon was pumped through a 10.5 inch membrane filter (0.45μ) with glass fiber pre-filter (Gelman Instrument Company, Ann Arbor, Michigan) with a submersible Jacuzzi pump. The water was extracted in three specially prepared 55-gallon drums. Each drum had one end cut out and a crossbar welded onto the drum at the cut-out end. A copper tube was welded perpendicular to the bar to serve as a guide for the mixer handle. The mixer was made of 14-gauge sheet metal with many 3/4-inch diameter perforations. Three such pieces of sheet metal, having a diameter of one and one-half inches less than the drum, were attached to a 3/8-inch aluminum rod which served as a handle. The plates were spaced six inches apart. Stirring and mixing were thus achieved by manually moving the perforated discs up and down. This apparatus (Figure 1) is similar to that used by Jeffrey and Hood (1963).

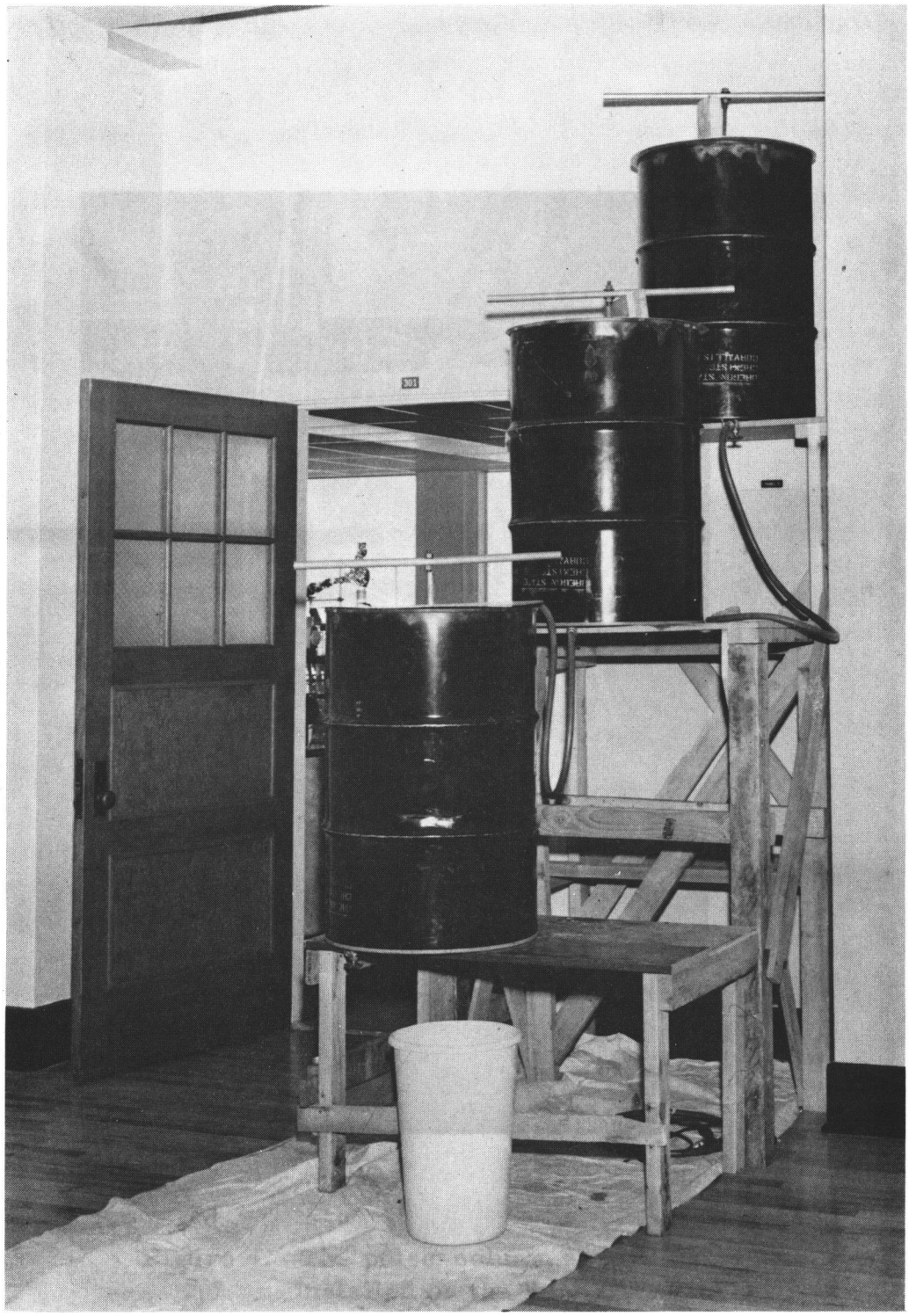

Figure 1. Apparatus used in estuarine water for solvent extraction of dissolved organics.

Each 151 liter batch of water was extracted three times with 19 liters of reagent-grade petroleum ether, which had been scrubbed twice with concentrated sulfuric acid and distilled water before redistillation (the forerun and residue distillates were used as a blank). The filtered water, after adjustment to a pH of three with glacial acetic acid and addition of the solvent, was agitated by a minimum of 30 strokes of the mixer. After allowing the layers to separate, the water was drained into another drum for a second extraction. After the third extraction, the water was discarded. A total of 2041 liters of river water was extracted in this manner.

The petroleum ether was collected and redistilled in two high speed condensors. Distillation was carried out at a temperature of 45 ° C at a pressure of 745-750 mm Hg.

Field Extraction of Sea Water

Large Volume Tanks

Two extractions of sea water were made, using two different methods at two locations. The initial experiment, with large volume tanks, was aboard the USCG lightship COLUMBIA, anchored 15 km offshore from Astoria, Oregon. The sampling location was in the channel of the Columbia River where the salinity was 26.0‰ ± 2.0‰ during sampling. The extraction apparatus for this experiment

consisted of two towers fabricated from three 55-gallon drums welded end-to-end with a cone welded to one end. A one-inch pipe and valve extending from the cone served as a drain and attachment for an air hose. A one hp submersible pump with Lexan impellers (Jacuzzi Pump Company) was used to obtain water from a depth of approximately three meters. After filling the towers with filtered sea water (0.45μ membrane) and adjusting the pH to 3.5 with glacial acetic acid, air was turned on vigorously agitating the water and petroleum ether (which had been scrubbed with concentrated sulfuric acid and redistilled). Ratio of water to solvent was 140 to 15. The mixture was agitated for one hour. Although this procedure was faster than hand mixing, approximately one-half of the solvent was lost through evaporation.

A total of 4385 liters of sea water was extracted by the above process with the solvent recycled after distillation. A final volume of four liters of the extract was returned to shore facilities for detailed analysis.

Pulse Column

For improved efficiency of extraction, a pulse column, similar to one designed at the Hanford Laboratories (1961), was adapted for use at sea aboard the Research Vessel YAQUINA. Of the simple liquid-liquid counter-current extractors, the pulse column has

proven to be an efficient device for the separation of trans-uranium elements. An important factor in its application to the extraction of organic compounds is the choice of solvent. The ideal solvent would have high extraction coefficients for these various molecules (these coefficients are presently unknown because the organics in sea water are unknown), optimum vapor pressure, ease of purification, stability and cost. Based on these criteria, hexone (methyl isobutyl ketone) was chosen. This decision was supported by previous experience with hexone in solvent extraction by the Hanford group. Reagent grade hexone (817 liters) was redistilled at a pressure of 260 mm Hg. A portion of the redistilled solvent was chromatographed for reference on an "Aerograph Model A-700" gas chromatograph, as were portions of the forerun and residue (which were collected and analyzed separately).

Because of the low flash point of hexone (30° C), explosion-proof equipment was used throughout. Vent lines connected all feed and receiving vessels and an additional exhaust fan was provided in the laboratory.

The sea water to be extracted was taken in through a sea-cock about two meters below the surface, forward of the ship's waste disposal. The sea-cock was opened as needed while the ship was underway. Extractions were carried out over an 82 hour period during which time the vessel covered the route shown in Figure 2.

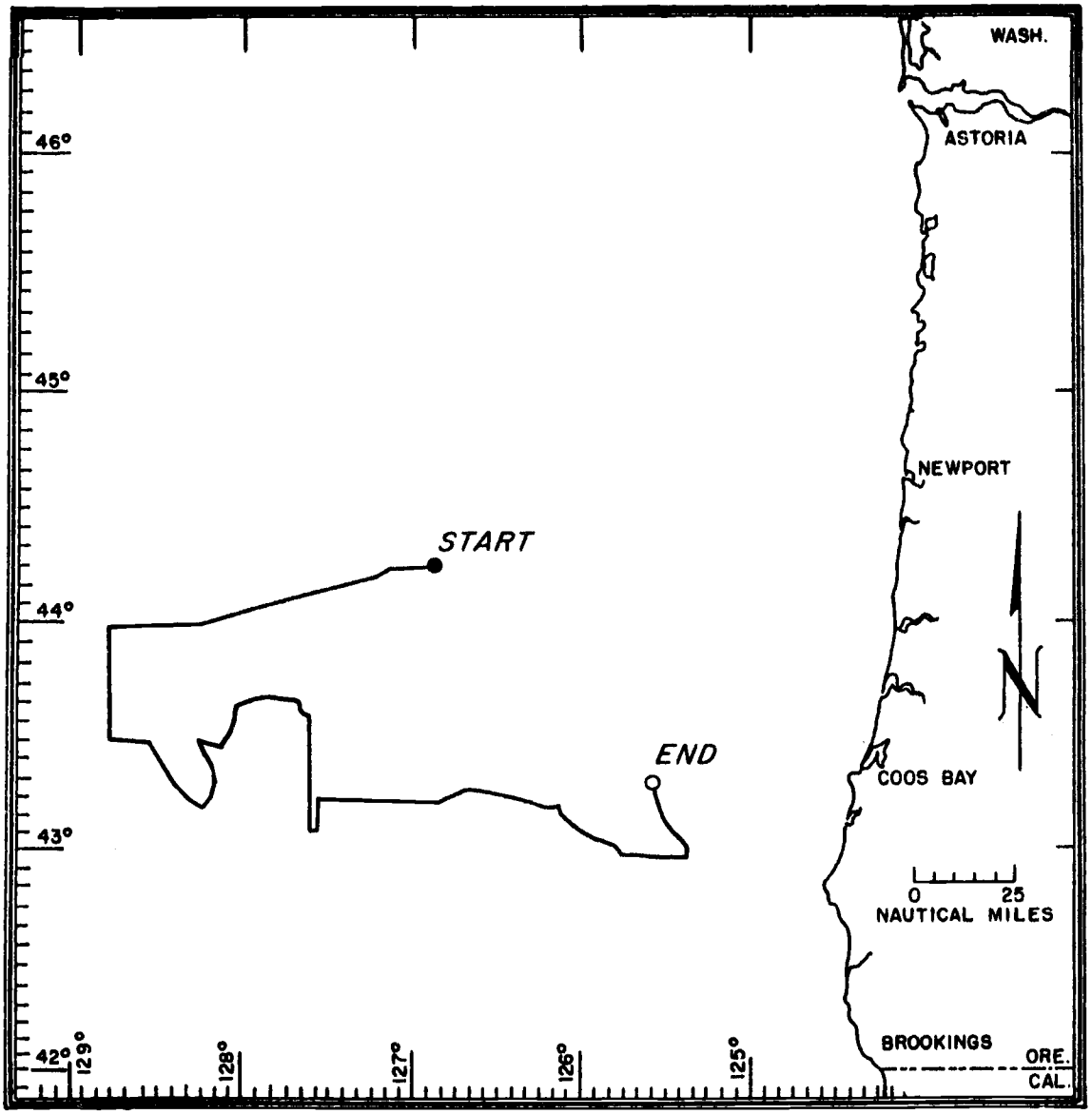

Figure 2. Track-line of R/V YAQUINA during extraction period.

The route was determined by a group of geological oceanographers aboard the R/V YAQUINA, and the deviations in the ship's course were not of significance to the extraction experiment.

Constant monitoring of the water with a CTI instrument (conductance-temperature indicator) indicated a salinity of 31.65-32.60‰ ± 0.5‰ and a water temperature of 11° C ± 0.5° C. Temperature of the extraction system was 14° C. The water was pumped (Jacuzzi, one hp submersible) through a 0.45μ membrane filter and glass fiber prefilter as before, and introduced into the pulse column for extraction. The pulse column schematic is shown in Figure 3, and Figures 4 and 5 show the equipment in place aboard the R/V YAQUINA. Flow rates and operating conditions are found in Table 1. Tests made at sea with the apparatus described were completely satisfactory.

The actual extractions at sea were carried out under varied conditions (Table 2).

On return to shore the product solvent was redistilled at a pressure of 70 mm Hg (65° C) using water at 4° C to cool the condenser and dry ice trap to collect volatile materials.

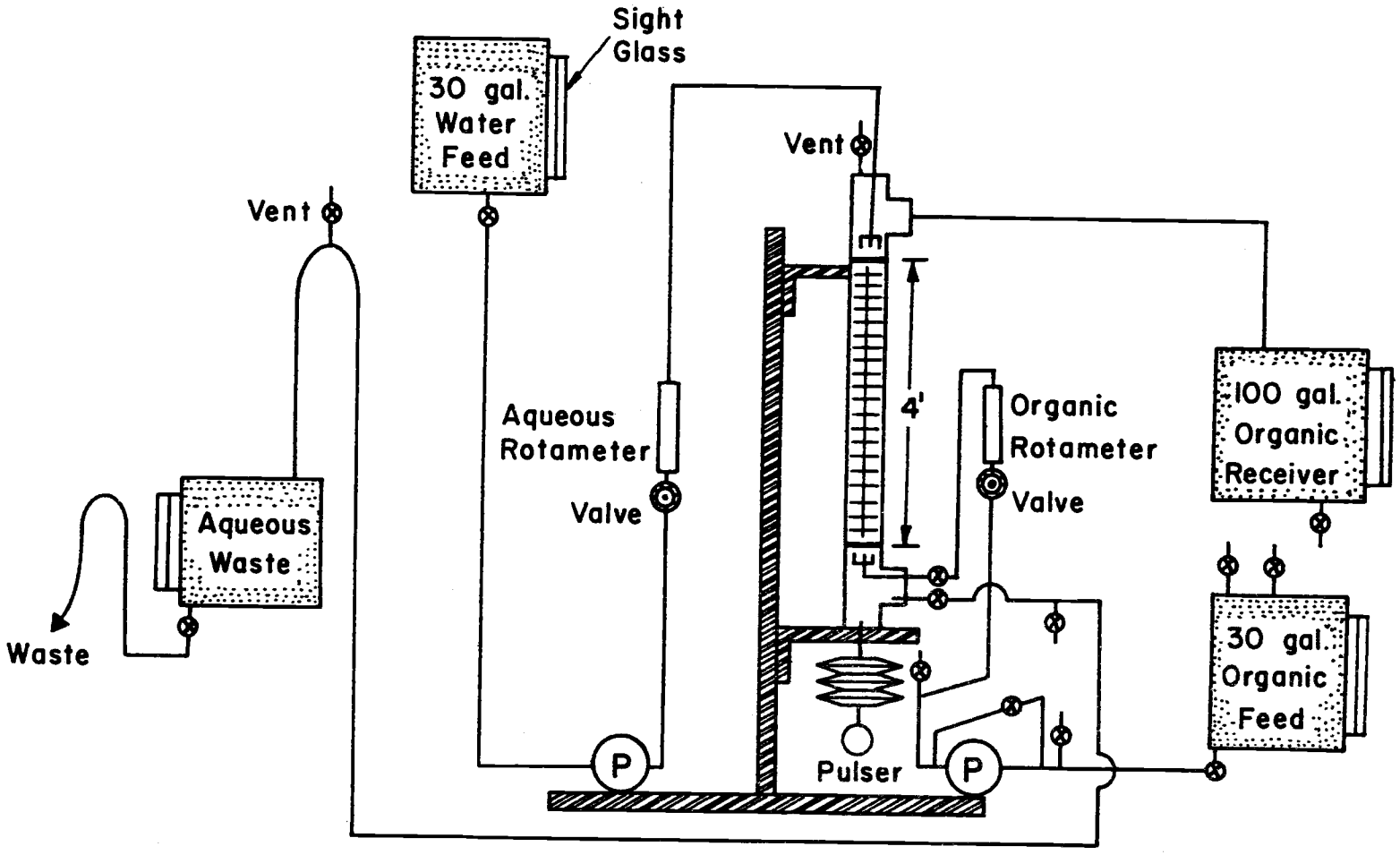

Figure 3. Schematic of pulse-column.

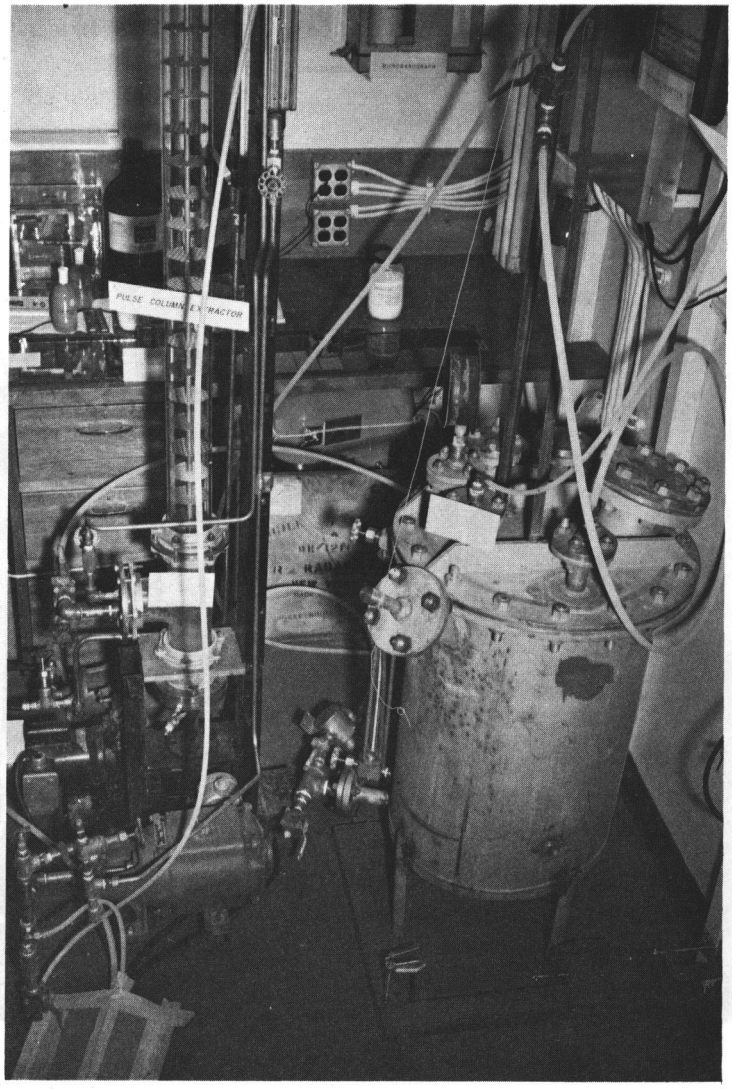

Figure 4. The pulse-column apparatus as installed on the R/V YAQUINA.

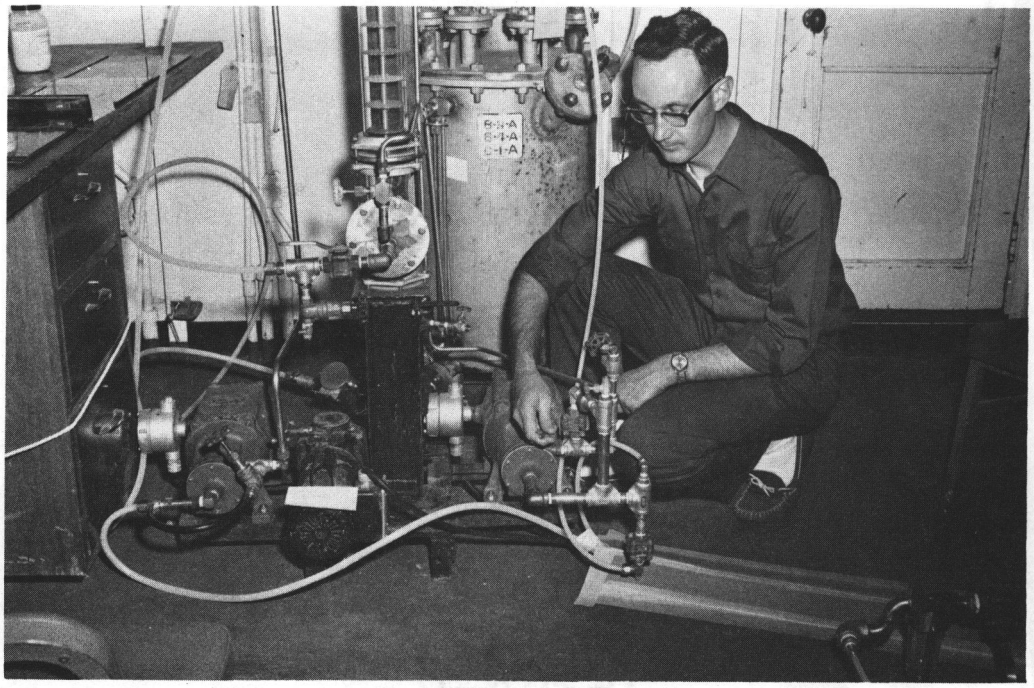

Figure 5. The base of the pulse-column apparatus.

Table 1. Operating conditions of pulse column.

Column size	three-inch diameter--four-foot length with two one-foot T joints
Cartridge	two-inch spacings, fluorothene sieve plates 3/16-inch hole, 23% free area separated by five stainless steel nozzle plates 3/16-inch holes, 23% free area
Flow rates	aqueous--3000-3760 ml/min solvent--300-350 ml/min
Pulse	frequency--64 to 80 cps with 80 cps majority of time amplitude--one to one and one-half inches
Holdup	five aqueous to one organic solvent

Both aqueous continuous and organic continuous operation were tried. The latter appeared to give a better dispersion and was used during the experiments.

Table 2. Solvent data for pulse column extraction.

Experiment no.	Volume of sea water (liters)	pH of water	Volume of solvent used (liters)	Volume of solvent recovered (liters)	% Recovery of solvent
1	4600	8.2	379	265	70
2	6297	3.5(a)	341(b)	189	55.5
3	700	3.5(a)	389	300	77
4	511	3.5(a)	57(c)	15(d)	26.5

(a) pH adjusted with HCl
(b) Solvent recycled
(c) Solvent continually recycled with flooding (lack of phase separation)
(d) This solvent is assumed to be in near equilibrium with the sea water

Laboratory Extractions of Sea Water

The more ideal environmental conditions of the shore laboratory and use of small volumes (one to two gallons) would facilitate and expedite the analyses of the major organic constituents in sea water. In anticipation of achieving these improvements and reducing collection expenses, a small scale extractor was set up (Figure 6). Various spectrograde reagents were used with this apparatus to determine if other major organic constituents were present which were not extracted by petroleum ether.

The solvent was continually distilled and refluxed. The condensed vapor was collected in a small internal tube with a small funnel on the end. The pressure head of solvent, due to the difference in density of aqueous and solvent phases, forced the redistilled solvent to pass through the fritted lower end of the tube up through the water. The increased level of solvent at the top of the system would then cause it to flow into the distilling flask. A magnetic stirring bar kept the water-solvent system constantly mixed.

In each case, the water was collected 15 miles off Newport, Oregon, and was at a salinity of 32‰ ± 0.5‰. The first extraction was carried out using anhydrous diethyl ether. Reagent grade solvent was purified by washing with concentrated sulfuric acid and distilled water. The reagent was redistilled before use, discarding the

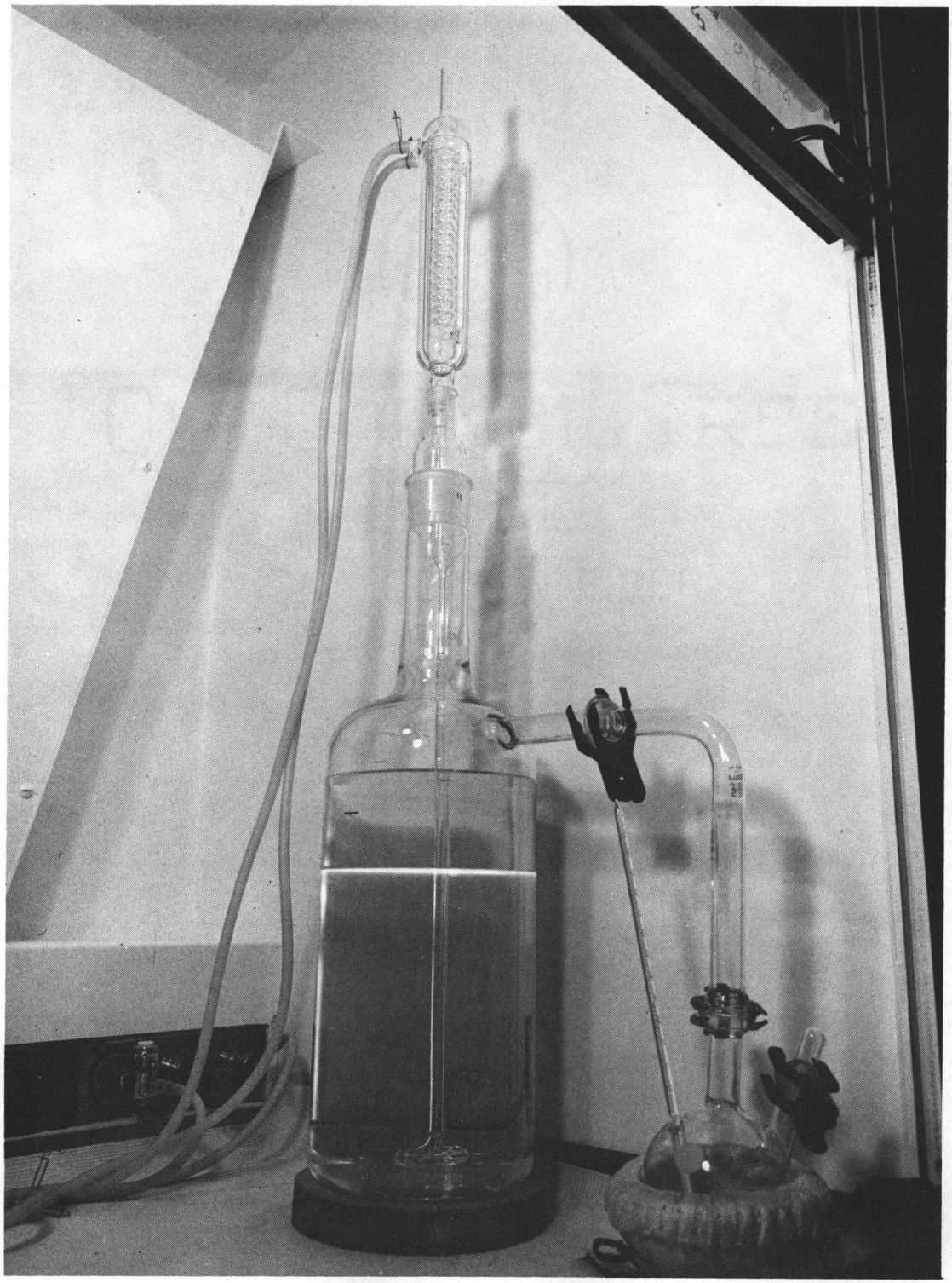

Figure 6. Small scale extractor.

forerun and residue redistillate. Two liters of solvent were used in the 36 hour extraction. Filtered (0.45μ) sea water (4800 ml) was adjusted to a pH of 3.5 with 1.5 ml of acetic acid before extraction.

Spectrograde cyclohexane was the second solvent used. Again the water was filtered (0.45μ) and 8100 ml of sea water (pH 8.1) were extracted with 650 ml of solvent for 282 hours.

Finally 8100 ml of sea water (adjusted to a pH of a 3.5 with two ml of concentrated HCl) were extracted with spectro-grade quality pentane (purified with oleum and potassium permanganate). In each of the three extractions the solvent was redistilled until the volume was reduced to approximately five ml. Thereafter, each fraction was analyzed with a tandem gas chromatograph-mass spectrograph. Where necessary, the autoprep GC was used to separate fractions for analysis by mass spectroscopy and IR.

CHEMICAL PROCEDURES

Radioanalysis

The estuarine concentrate (two liters) was mixed with approximately five grams of PVP (polyvinylpyrrolidine), a powder used by the brewing industry to remove phenolic groups causing undesirable taste (Woof, 1962). The PVP and solvent were filtered through a 0.45μ metricel filter. The filter and PVP were placed in a 12 ml plastic tube and counted in a five-inch well NaI (Tl) scintillator crystal. In an attempt to identify two unknown peaks observed in the gamma-ray spectrum, the sample was counted periodically for the next nine months. Eventually the tube was crushed with a hydraulic press into a pancake of about one and one-half inches diameter and five mm thick. The sample was then counted in a Packard multidimensional anticoincidence counter.[1]

Another radioanalysis was made on a portion of the sample taken on the USCG lightship COLUMBIA. A thick black material, removed from the distillation flask after the petroleum ether redistillation, was collected separately and stored in a polyethylene bottle. Later the material was partially dissolved with acetic acid and diethyl

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[1]Equipment built and operated by R. W. Perkins for Battelle Memorial Institute, Pacific Northwest Laboratories, Richland, Washington.

ether. The sample size of ether extract was reduced from 50 ml to three ml. This material was counted in a glass tube in the five-inch well crystal. The sample was dissolved in chloroform for infrared (IR), and in cyclohexane and dioxane for ultra-violet (UV) and visible spectrometry. The cells used were 0.2 mm NaCl for IR and one mm, five mm, and ten mm cells for UV and visible spectrometry.

Fatty Acid Analysis

Gas Chromatography

The estuarine sample was collected and preliminary examination was carried out in the Food Technology Department at Oregon State University. A 25 ml portion of the concentrate was reduced to dryness in a vacuum rotary evaporator. Diethyl ether (four ml of anhydrous reagent) and three ml of boron trifluoride-methyl alcohol (BF_3-MeOH) were added and warmed with a heating mantle. The mixture was refluxed for approximately five minutes, then washed with 30 ml of petroleum ether and 20 ml distilled water according to the instructions of Metcalfe and Schmitz (1961). The layers were separated, reduced to approximately one ml, and a 0.5µl sample was injected into a Wilkens Model B-600 gas chromatograph. A 3% DEGS on chromosorb PAW column at 160° C was used in this and subsequent experiments at Oregon State

University.

Another 25 ml portion of the sample was back-extracted into 100 ml of 15% sodium bicarbonate ($NaHCO_3$) aqueous solution. The pH was lowered to three with hydrochloric acid (HCl) and the sample was extracted with 50 ml of reagent grade anhydrous ether. Sample volume was reduced and esterified as described above. A 0.7µl portion was injected into the gas chromatograph under the same conditions as in the preceding experiment.

Strong acids were isolated by back-extraction into aqueous solution, then extracted into ether for further chemical procedures.

Portions (100 ml) of the estuarine and sea water extracts were back-extracted into 100 ml of 5% aqueous $NaHCO_3$. The layers were separated into a 500 ml separatory funnel. The pH of the aqueous $NaHCO_3$ extract was adjusted to three with HCl before addition of 50 ml of anhydrous diethyl ether. After a few minutes of vigorous shaking, the aqueous layer was drained off and the ether portion reduced in volume to five ml under a stream of nitrogen.

The sample was esterified with BF_3-MeOH reagent since the small chain fatty acid esters are relatively volatile and could easily be lost during preparation. It was then transferred to a separatory funnel containing 20 ml water. After shaking, the ester layer was collected and the water removed with anhydrous sodium sulphate (Na_2SO_4). Prepared samples were then chromatographed in a

Wilkens Model 700 auto-prep gas chromatograph using a column of 20% STAP on chromosorb PAW. The column temperature was manually programmed from 50° C to 190° C. After separate and mixed standards were chromatogrammed under identical conditions for comparison of retention times, the instrument was set to collect each separate peak for further analysis. The collection tubes were immersed in dry ice, and the gas chromatograph was manually operated for three cycles utilizing 20μl of sample on each cycle. The sides of the collection tubes were rinsed down with 0.5 ml of reagent grade chloroform.

Mass Spectroscopy

The collected fractions were analyzed on a CEC Model 21-130 mass spectrograph operated at 68 volts of electron energy and the injection port at 100° C. The resulting spectra were corrected for the presence of the solvent ($CHCl_3$) and trace amounts of water by subtracting the peaks resulting from these two contaminants.

Other portions of the estuarine and sea samples were treated to prepare methyl esters. These samples were injected into the F&M gas chromatograph, operated in conjunction with the CEC Model 21-130 mass spectrometer. Each peak was identified in the mass spectrometer.

Infrared Spectroscopy

A portion of the methylated sample was analyzed by IR to substantiate the presence of esters. The esterified samples (both direct and back-extracted) were evaporated to dryness under nitrogen and taken up with reagent-grade chloroform. Before putting the samples in the liquid cell holders, Na_2SO_4 was added to remove residual water. The two samples were then analyzed in the range of 2-16μ.

Adsorption Chromatography-Silicic Acid Column

Elution Process

A silicic acid column was prepared according to directions of Hirsch and Ahrens (1958). A similar column was used by Jeffrey et al. (1962) to separate solvent extracted sea water into classes of compounds. Separation occurs because different classes of organic compounds are partitioned in the silicic acid column and can be individually eluted with solvents of increasing polarity.

The silicic acid (suitable for chromatography use) was washed with absolute methanol; after settling, the methanol and suspended silicic acid were removed. This procedure was repeated with methanol and then twice with diethyl ether. The silicic acid was then dried in air, and packed in a 17 cm by three cm bed in a glass column. Elution was as shown in Table 3.

Table 3. Elution of silicic acid column (scheme of Hirsch and Ahrens).

Fraction	Solvent	Compounds eluted
I	50 ml 1% diethyl ether in petroleum ether	Paraffinic hydrocarbons
II	75 ml of 1% diethyl ether in petroleum ether	Squalene, beta-carotene unsaturated hydrocarbons
III	225 ml 1% diethyl ether in petroleum ether and 60 petroleum ether	Sterol esters, alpha-tocopherol
IV	240 ml 4% diethyl ether in petroleum and 200 ml 8% ethyl ether in petroleum	Triglycerides, free fatty acids, fatty alcohols
V	450 ml 8% diethyl ether in petroleum ether and 50 ml 25% diethyl ether in petroleum ether	Unesterified steroids, vitamin D_3, vitamin A alcohol
VI	200 ml 25% diethyl ether in petroleum ether	Diglycerides, tributyrin
VII	300 ml diethyl ether	Monoglycerides, vitamin A acetate, lithocholic acid, chimyl alcohol
VIII	400 ml absolute methanol	Phospholipids, monoacetin

Spectroscopy

The various fractions of the two samples were analyzed by IR, UV, and visible spectrophotometry. The solvent was evaporated over a steam bath under dry nitrogen to inhibit oxidation.

Chloroform was used as solvent for IR analysis. The UV and visible spectra were produced with cyclohexane as the solvent.

Thin Layer Chromatography

The different fractions were also separated by thin layer chromatography. These plates were prepared by slurrying 40 grams of silica gel G (in distilled H_2O) which was spread on glass plates with a variable spreader set at one mm. The plates were air dried and placed in an oven at 60° C for one hour. The various solvent systems used for developing were acetic acid-hydrochloric acid-water (1:1:1), sprayed with 2% ferric chloride and 2', 7' dichlorofluoroescene; and toluene:ethyl acetate (19:1) sprayed with sulfuric acid and 2', 7' dichlorofluoroescene.

CHN Analysis

In addition, several fractions were analyzed for carbon, hydrogen and nitrogen with a F&M Model 180 instrument. Direct mass spectroscopic analysis was also attempted.

Hexone Separations

Total Organic Material Extracted

On return to laboratory facilities at Oregon State University,

300 ml portions of the various product hexone (hexone used for extraction) samples were evaporated to determine the quantity of organic matter recovered by extraction. This material was analyzed for CHN content.

Distillations

The remaining product was placed in the 12 liter distilling flask. A 500 ml flask at the receiving end of the condensor was rotated so that the first 300-400 ml could be collected separately. The complete distillation was at a pressure of 70 mm Hg (65° C) with 40° C water used to cool the condensors. Each distilled fraction from the different experiments was coded for subsequent identification (Table 4).

A Podbielniak Series 3475 high temperature distillation apparatus was used in an attempt to fractionate the cold trap material. Since the instrument will only accommodate small volumes, the fractional distillation of other fractions was not attempted.

The sample POW-B (CT) was allowed to equilibrate in the distillation apparatus with the first fraction appearing at about 56° C. A possibility existed that this fraction was acetone (a known contaminate in the original hexone); therefore, the sample was analyzed with a Varian Associates Model A60 Nuclear Magnetic Resonance Spectrometer. This fraction was also chromatographed.

Table 4. Code system for distilled product solvent.

Basic (pH=8.2) conditions	Code	Acidic (pH-3.5) conditions	Code
One cycle solvent		Two cycle solvent	
Final distillate	POW-B (FD)	Final distillate	POW-A-2 (FD)
First distillate	POW-B (1-D)	First distillate	POW-A-2 (1-D)
Cold trap distillate	POW-B (CT)	Cold trap distillate	POW-A-2 (CT)
Interface material	POW-B-I (FD)	One cycle solvent	
	POW-B-I (1-D)	Final distillate	POW-A-1 (FD)
	POW-B-I (1-D)	First distillate	POW-A-1 (1-D)
	POW-B-I (CT)	Cold trap distillate	POW-A-1 (CT)
		Cycled solvent until flooded	
		Final distillate	POW-A-F (FD)
		First distillate	POW-A-F (1-D)
		Cold trap distillate	POW-A-F (CT)
		Interface material (from Two cycle solvent)	
		Final distillate	POW-A-I (FD)
		First distillate	POW-A-I (1-D)
		Cold trap distillate	POW-A-I (CT)
		Interface material (from Flooded solvent	
		Final distillate	POW-A-IS (FD)

Back Extractions

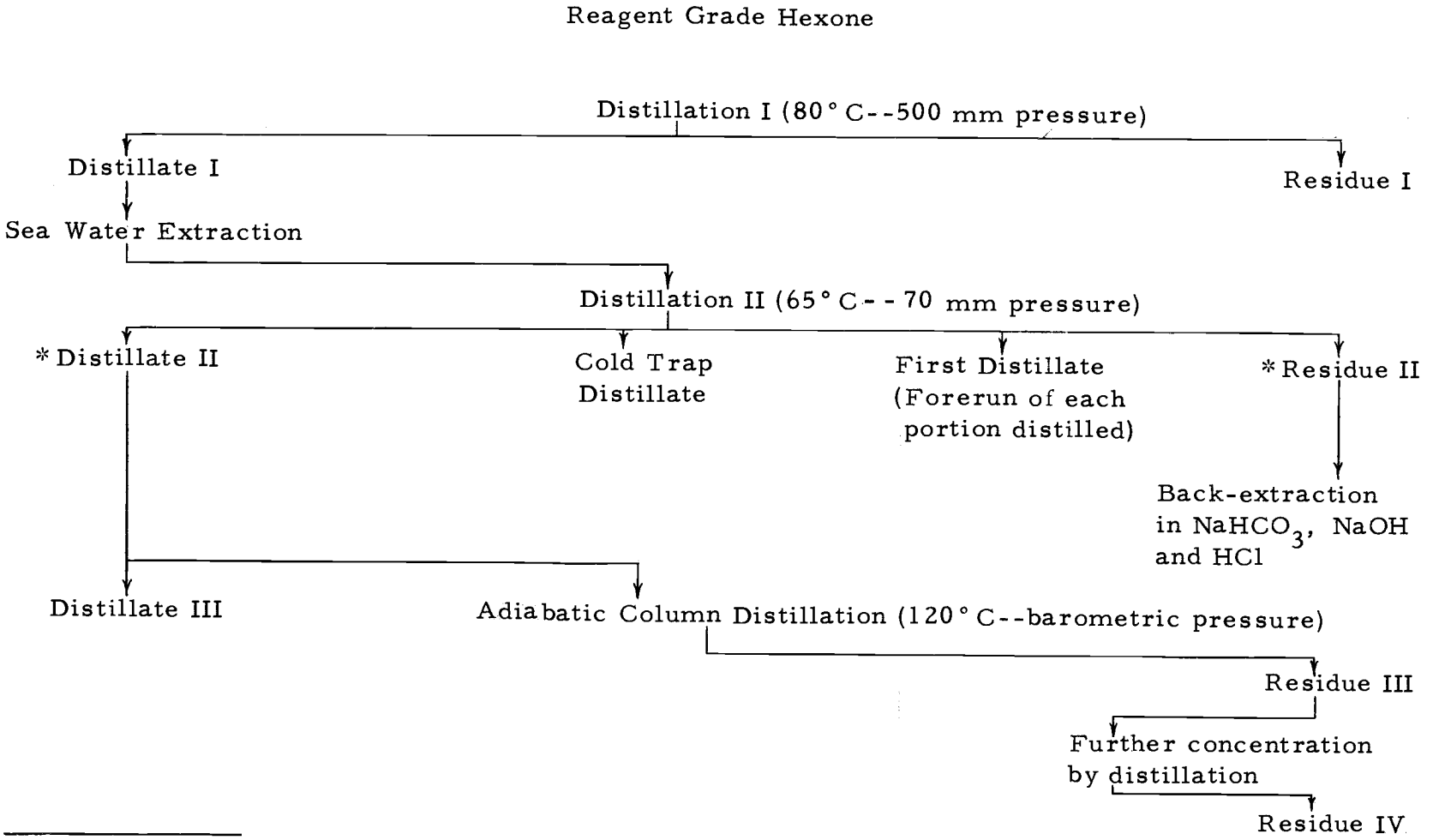
Other fractions of the product solvent were back-extracted into aqueous 10% $NaHCO_3$, aqueous 5% NaOH and aqueous 5% HCl. Subsequently, the $NaHCO_3$ and NaOH back-extracts were adjusted to a pH of 3.5 with HCl and then extracted with reagent grade diethyl ether, chloroform, and, in some instances, cyclohexane. The aqueous HCl back-extract was adjusted to a pH of 9.5 with KOH before extraction with the same solvents as noted above (see appendix).

The samples collected in ether and $CHCl_3$ were evaporated on a steam bath under a stream of dry nitrogen. A spectrum of each was chromatographed on the Aerograph A-700 with a four-foot by 3/8 inch 5% SE-30 PAW column on programmed temperature. Samples with spectra having the most distinct peaks (i.e. the greatest difference in retention times) were selected for analysis by mass spectrometry at Monsanto Chemical Company, St. Louis, Missouri.

Quality control of the solvent is essential; thus, several distillations were performed and the residues examined (Figure 7). Each of these residues and distillates was analyzed by gas chromatography.

Analysis of Laboratory Extracts

The diethyl ether and cyclohexane collected after extraction



* A carbon, hydrogen and nitrogen analysis was performed on Distillate II and on Residue II.

Figure 7. Flow diagram of hexone distillations as monitored by flame ionization gas chromatography.

was reduced by distillation and analyzed both by mass spectroscopy directly and with the tandem gas chromatograph-mass spectrometer (GC-MS) system. Pentane (Eastman Company, batch P1501) was purified with oleum and permanganate before extracting sea water. The extract was analyzed with an F&M 810 gas chromatograph coupled to a CEC 21-130 mass spectrometer utilizing methods described in Monsanto CRD-64 Special Report No. 1601. Further separation of the extract was accomplished by adsorption chromatography on silica gel (ASTM-method D1319-58T), resulting in a paraffin-olefin fraction and an aromatic fraction.

These samples were run at a lower temperature--programming rate and a lower starting temperature with a ten-foot by 1/4-inch column of 18% carbowax-1% $AgNO_3$ on 60/80 chromosorb W as well as a column of 20% SE-30.

RESULTS AND DISCUSSION

The two extractions using petroleum ether (estuarine water and sea water) by large volume tanks were treated identically; therefore, the results of the analyses will be combined to allow comparisons.

Estuarine and Large Volume Tanks

Radioanalysis

The PVP extract of the estuary water sample was first analyzed for radioactivity in September, 1964. Peaks number 2 and 4 (Figure 8) were identified as chromium-51 and zinc-65, respectively. The other two peaks were periodically analyzed for the next nine months. Half-life studies tentatively showed peaks 1 and 3 to be due to hafnium-175 and rubidium-83; although the source of these two isotopes is unknown. This PVP sample was subsequently analyzed (July, 1965) on a multi-dimensional gamma-ray spectrometer at Battelle Northwest Laboratories by R. W. Perkins. Zinc-65, thorium-232, and cobalt-60 were identified. Due to an accident to the author, no analyses were attempted from September, 1964 to July, 1965. By this time chromium-51 had decayed and the absolute identity of the peaks was not possible.

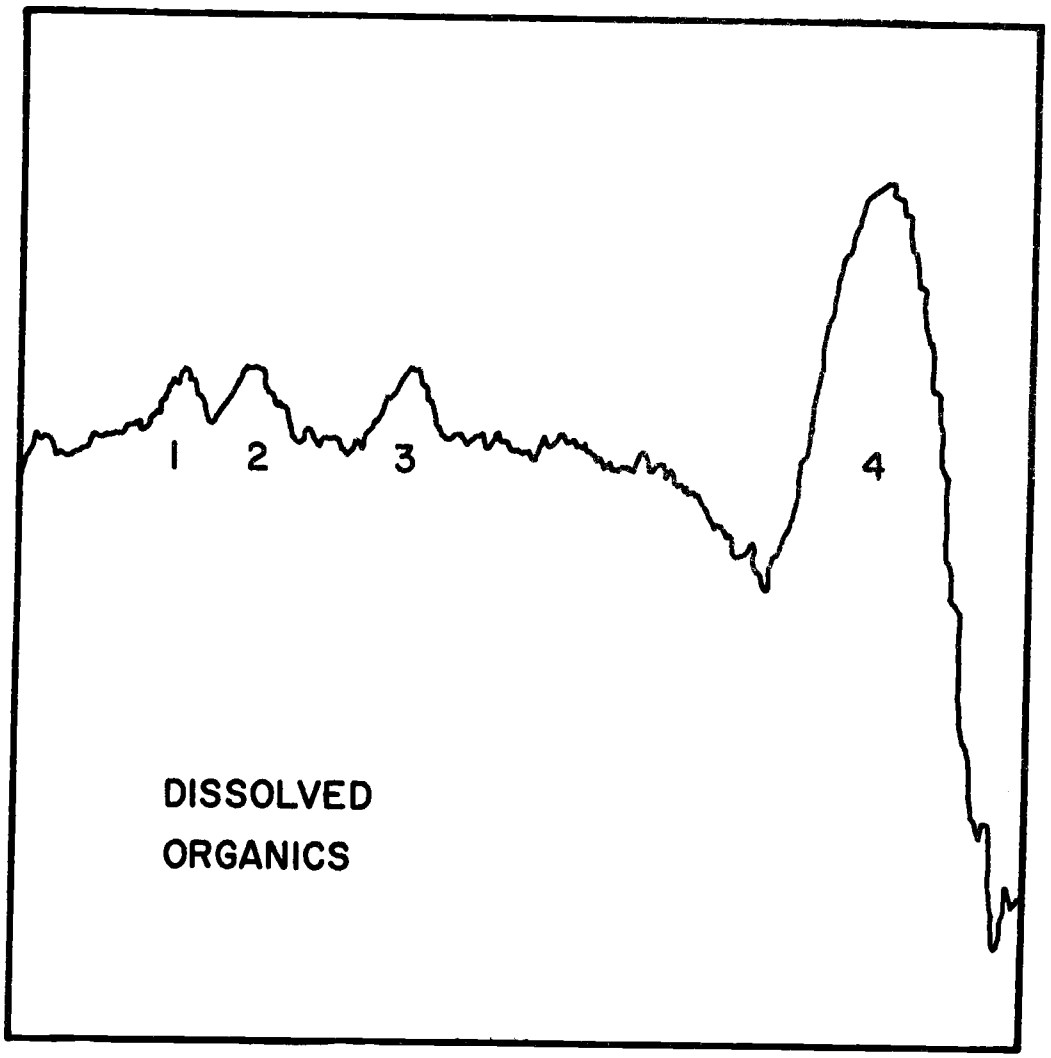


Figure 8. Spectrum showing chromium-51 (2) and zinc-65 (4) in dissolved organics from the Columbia River.

The solid residue left in the distilling flask from petroleum ether redistillation was analyzed in an anti-coincidence gamma-ray spectrometer and cobalt-60 was identified in both estuarine and sea water samples.

The identification of the organic matter was not as conclusive as the above. The ultra-violet spectra of the two samples gave no indication of the identity of the organic matter. The visible spectra (Figures 9 and 10) are typical of a porphyrin, although it must be noted that a combination of other organic compounds could give similar spectra. Since vitamin B_{12} (a porphyrin) contains cobalt, the appearance of ^{60}Co in the gamma-ray spectrum tends to support the conclusion that porphyrin may be present in this residue.

Porphyrin rings are widely distributed in nature and their presence in water seems reasonable. Porphyrins serve in oxygen transport systems, oxygen storage systems, respiratory chains of most cells, and as prosthetic groups of certain enzymes. Chlorophyll and related pigments of green plants are modifications of the porphyrin nucleus.

Fatty Acid Analysis by Gas Chromatography

Carbon Chain Length of One to Ten

The fractions collected from the Wilkens Model A700 Autoprep

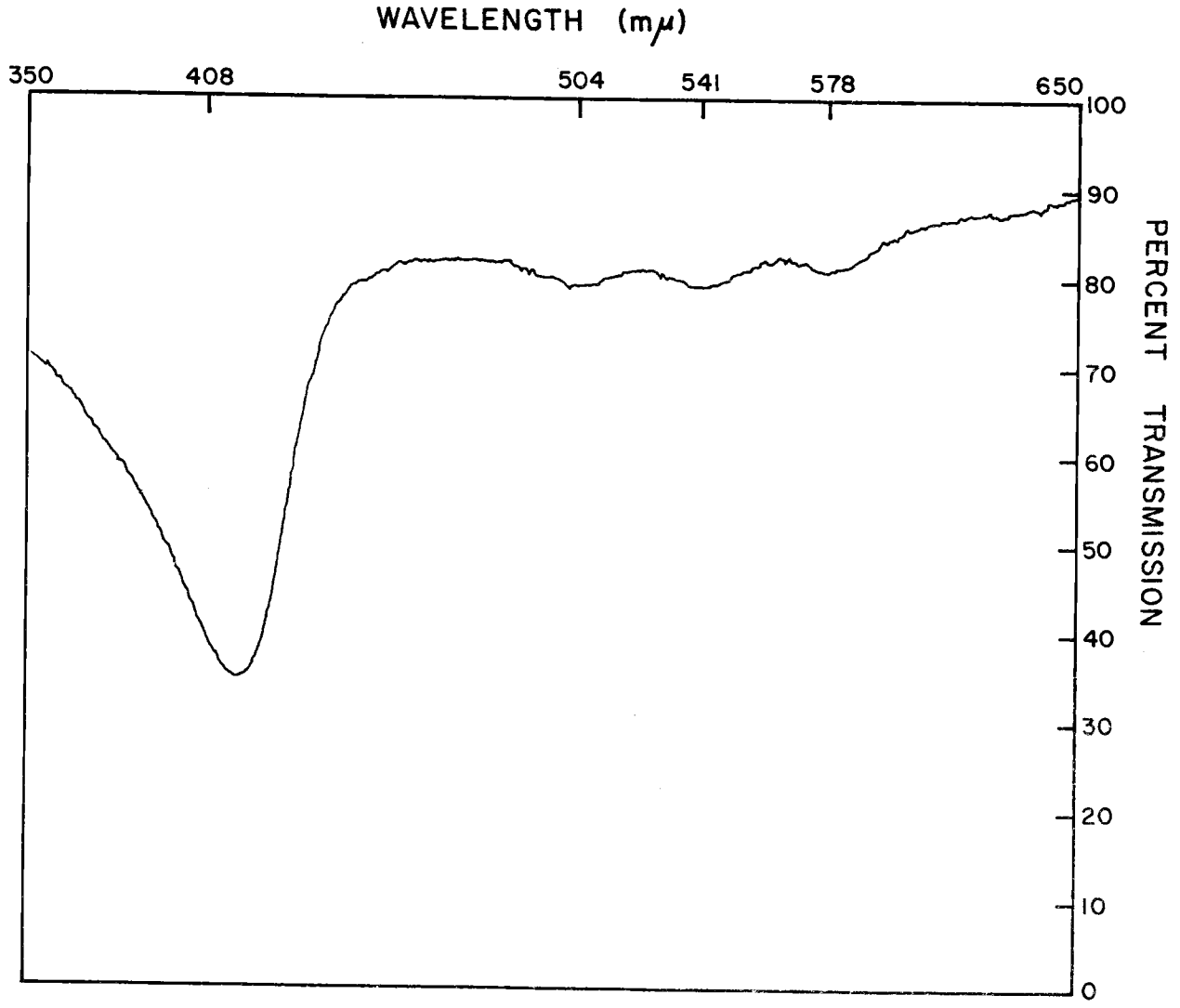


Figure 9. Visible spectrum of sea water interface material.

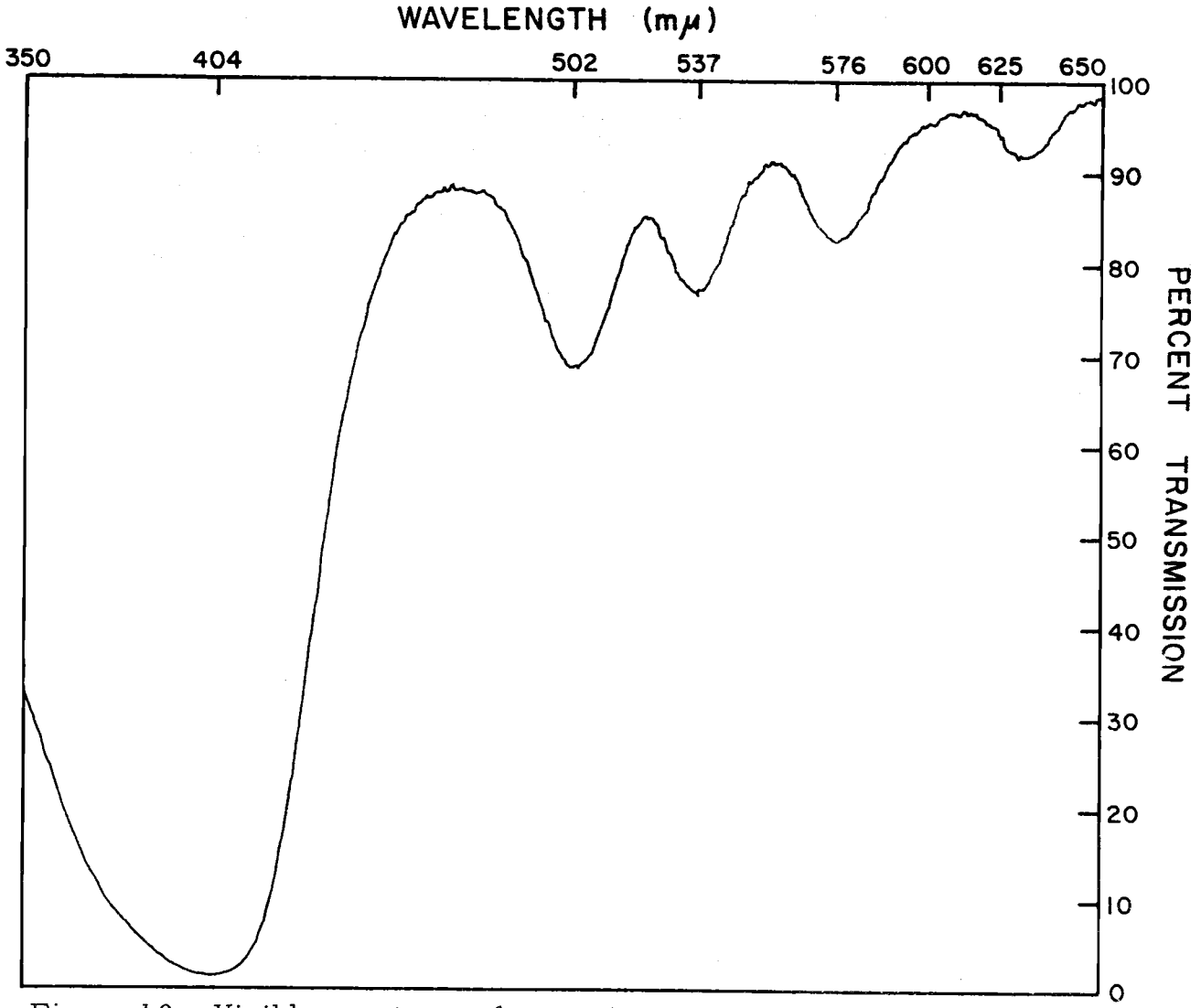


Figure 10. Visible spectrum of estuarine water interface material.

GC were analyzed on a CEC Model 21-130 mass spectrometer with resulting spectra corrected for the presence of the solvent ($CHCl_3$) and trace amounts of water.

Subsequently, similarly prepared samples were introduced directly into the mass spectrometer from a F&M Model 500 gas chromatograph using a column of 20% STAP on Chromosorb P-AW. Identical results were obtained (Table 5).

Table 5. Mass spectral analysis of GC Prep.

RW	SW
Methyl acetate	Methyl formate
Methyl butyrate	Methyl acetate
Methyl hexanoate	Methyl propionate
Methyl octanoate	Methyl butyrate
Methyl nonanoate	Methyl hexanoate
Methyl decanoate	Methyl octanoate
	Methyl nonanoate
	Methyl decanoate

The forerun and residue of the redistilled solvent were treated and analyzed in the same manner as the extract samples. None of the compounds identified in the water samples was present in these solvent fractions. On a subsequent trip to the USCG lightship

COLUMBIA, a test was made to eliminate the possibility of contamination by the air supply. Air was bubbled through glass wool and petroleum ether which was washed again after the experiment. Both the glass wool washings and the petroleum ether were reduced in volume and identically esterified. No fatty acid esters were found in these samples.

The fatty acid esters found in Table 5 were saturated. It must also be noted that since acetic acid was used to adjust the pH of the water prior to extraction, acetic acid would be carried through each step of the procedure.

Fatty acids with odd numbers of carbon atoms are rare in nature; therefore, explanation of the presence of C_3 and C_9 is difficult. It is also noted that more short-chain fatty acids were present in sea water than estuary water. This seems contrary to expectation since estuary water should contain more organic matter from degraded vegetation and subsequent dissolution. Aquatic life is rich in both of the collection areas; therefore, little difference would be expected as a result of release of metabolic entities..

Carbon Chain Length of Ten to 18

Fatty acids of longer chain length were identified differently. Twenty-five ml of solvent containing the organic material extracted from estuary water and sea water were esterified with BF_3MeOH.

These samples were injected into the gas chromatograph (Autoprep A-700) and retention times were compared with known standards under the same instrumental conditions. Also individual components from five collection cycles of 20μl injection size were collected, diluted with $CHCl_3$ and analyzed on an IR spectrometer (Perkin-Elmer 521) using NaCl cells. Efforts to examine these esters by mass spectroscopy failed because of their low volatility. See Table 6 for results.

Table 6. Methyl esters of long chain (> 10) fatty acids.

Sea Water	River Water
Methyl Undecenoate ($C_{11:1}$)	Methyl Undecenoate ($C_{11:1}$)
Methyl Laurate (C_{12})	Methyl Laurate (C_{12})
Methyl Myristate (C_{14})	Methyl Myristate (C_{14})
Methyl Palmitate (C_{16})	Methyl Palmitate (C_{16})
Methyl Oleate ($C_{18:1}$)	Methyl Oleate ($C_{18:1}$)
Methyl Linoleate ($C_{18:2}$)	Methyl Linoleate ($C_{18:2}$)

In Figure 11, gas chromatograms of the methyl esters identified in the $NaHCO_3$ extract of estuary water and sea water samples are compared with standards.

The longer chain acids have been identified in much smaller water samples. Although this work was not quantitative, evidently

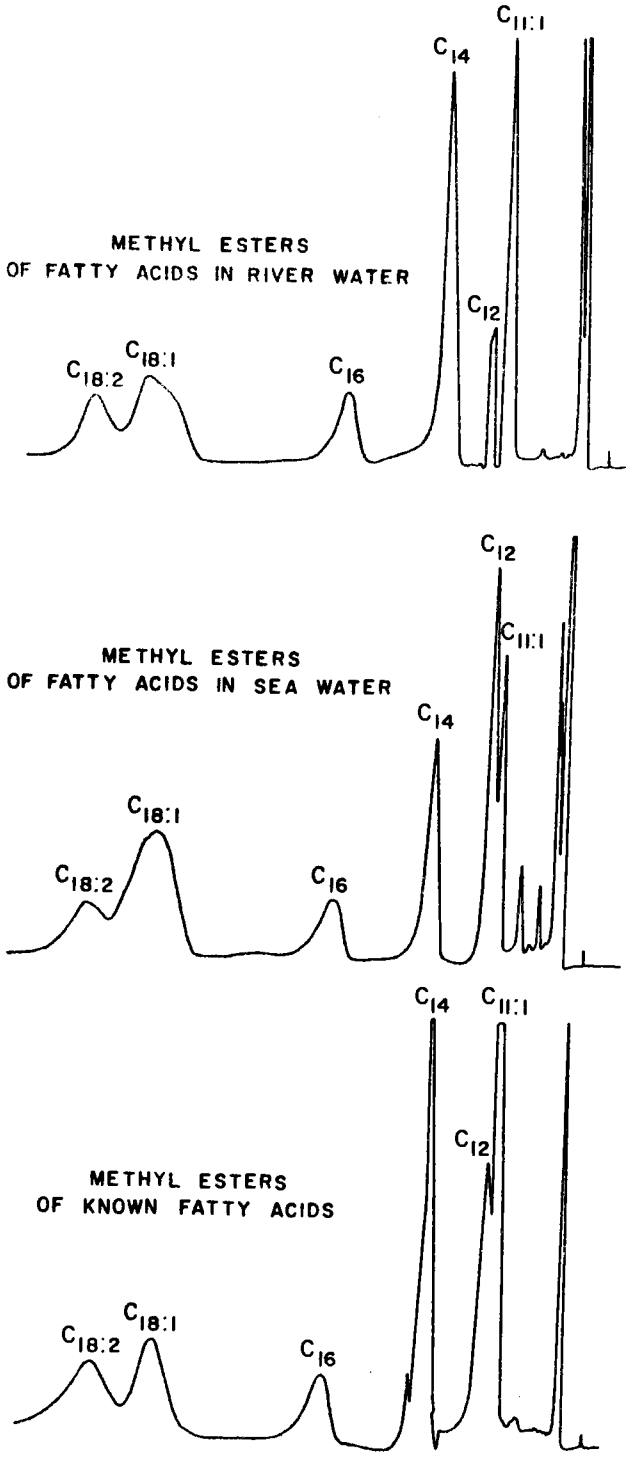


Figure 11. Gas chromatograms of Methyl Esters (10 carbons) of estuarine water extract, sea water extract, and standards.

identification of smaller chain acids requires increased sample size. Either of the extraction devices described in this experiment would be suitable for large volume sampling where high extraction efficiency is not required.

Adsorption Chromatography-Silicic Acid Column

Spectroscopy

The silicic acid column was used for both sea water and estuary water samples. The concentrated petroleum ether was put on the column and eluted with the various solvents. No fraction collector was available; therefore, the eluant for each of the eight groups was collected in one fraction. Also included in this study for comparison were the original petroleum ether extracts of estuary water and sea water. A portion of the solvent extract was extracted into sodium bicarbonate. Finally, the residual petroleum ether (after the two back-extractions) was analyzed. The IR spectra of the estuary and sea water samples are different indicating that similar compounds were not eluted as they should have been; therefore only spectra of the original extract (each was evaporated and taken up with $CHCl_3$), $NaHCO_3$ fraction, NaOH fraction and the residue will be shown (Figures 12, 13, 14, and 15). Possible functional

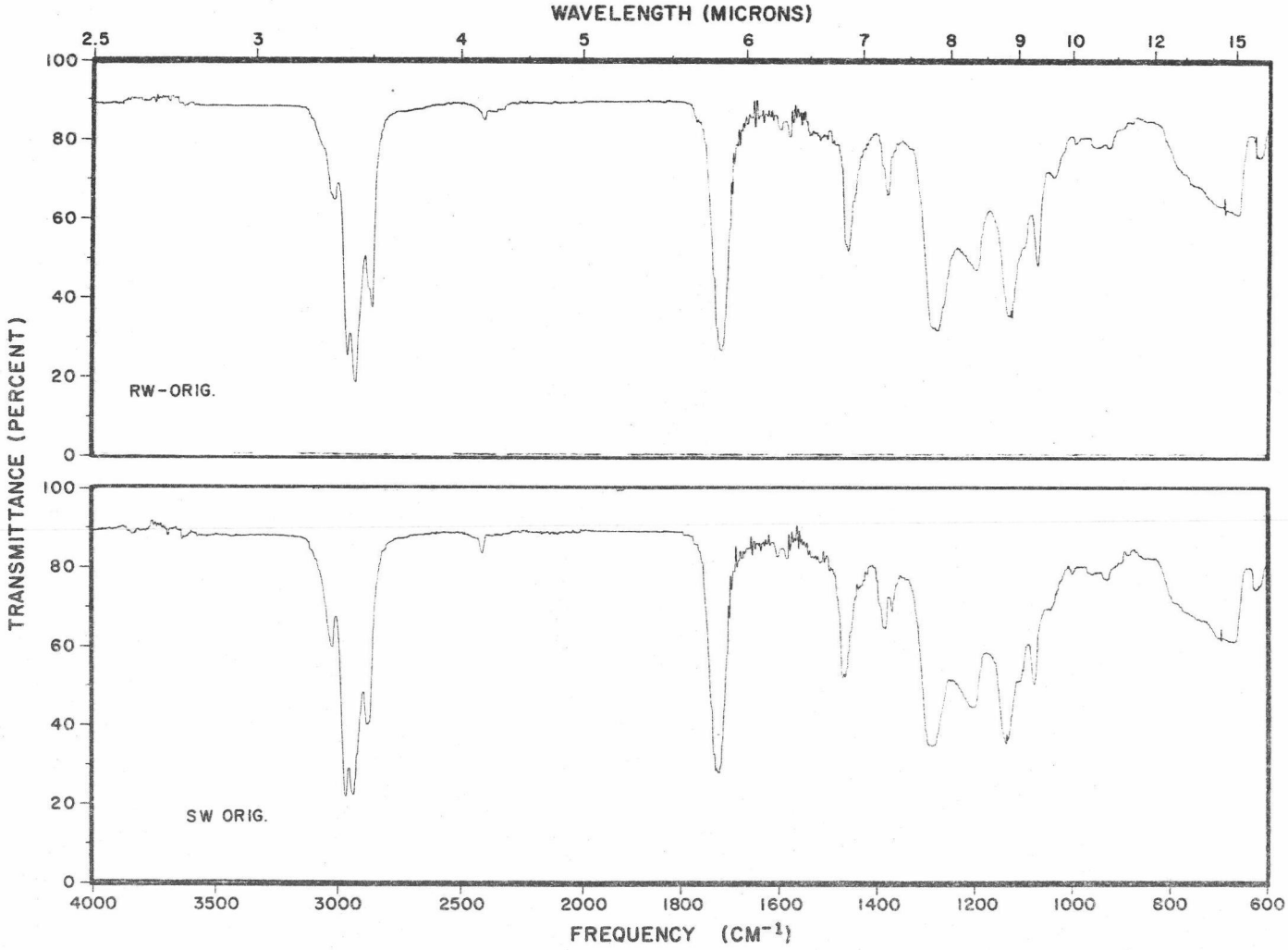


Figure 12. IR spectra of original extract of estuarine water and sea water.

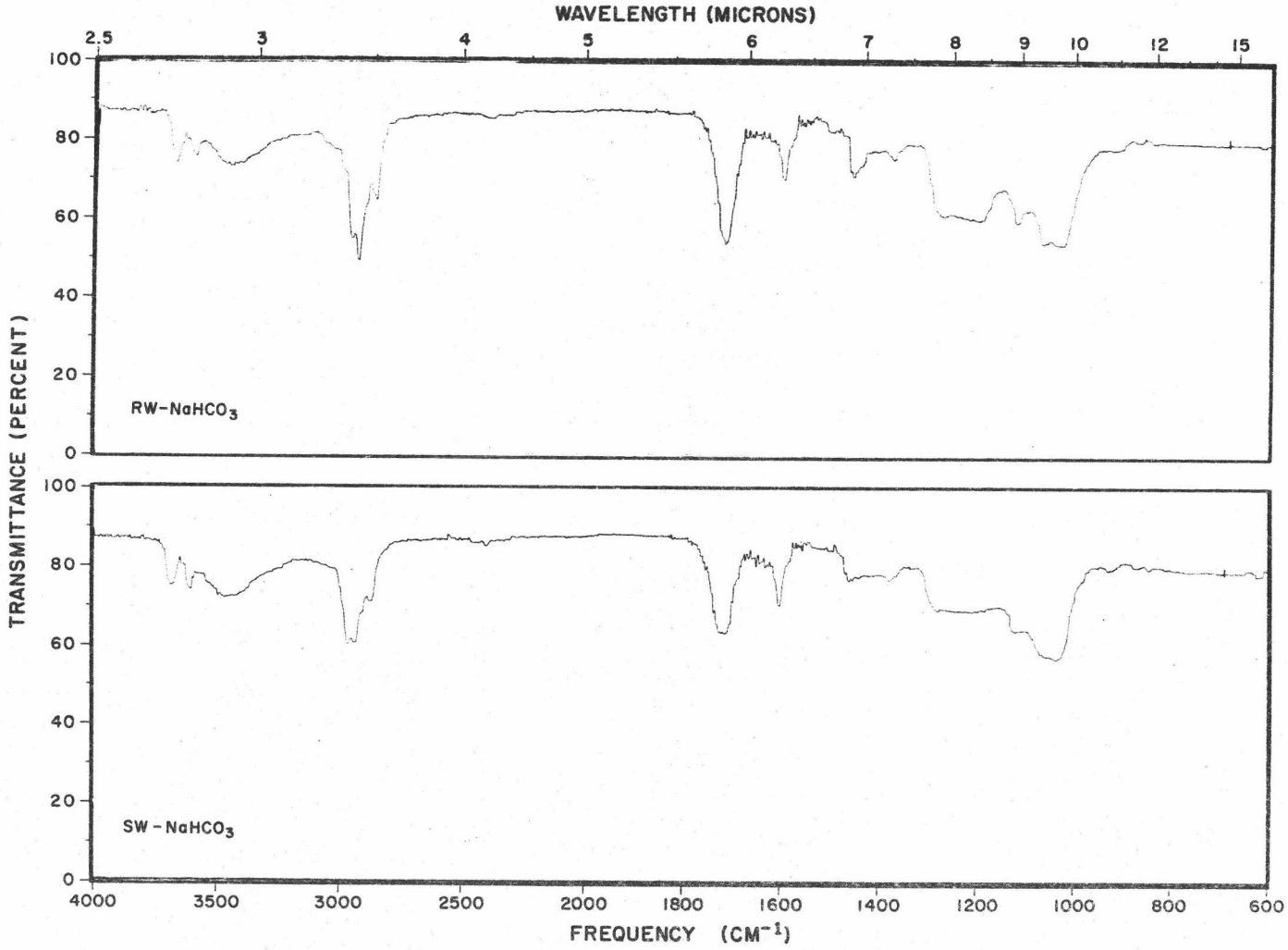


Figure 13. IR spectra of $NaHCO_3$ extract of petroleum ether extract.

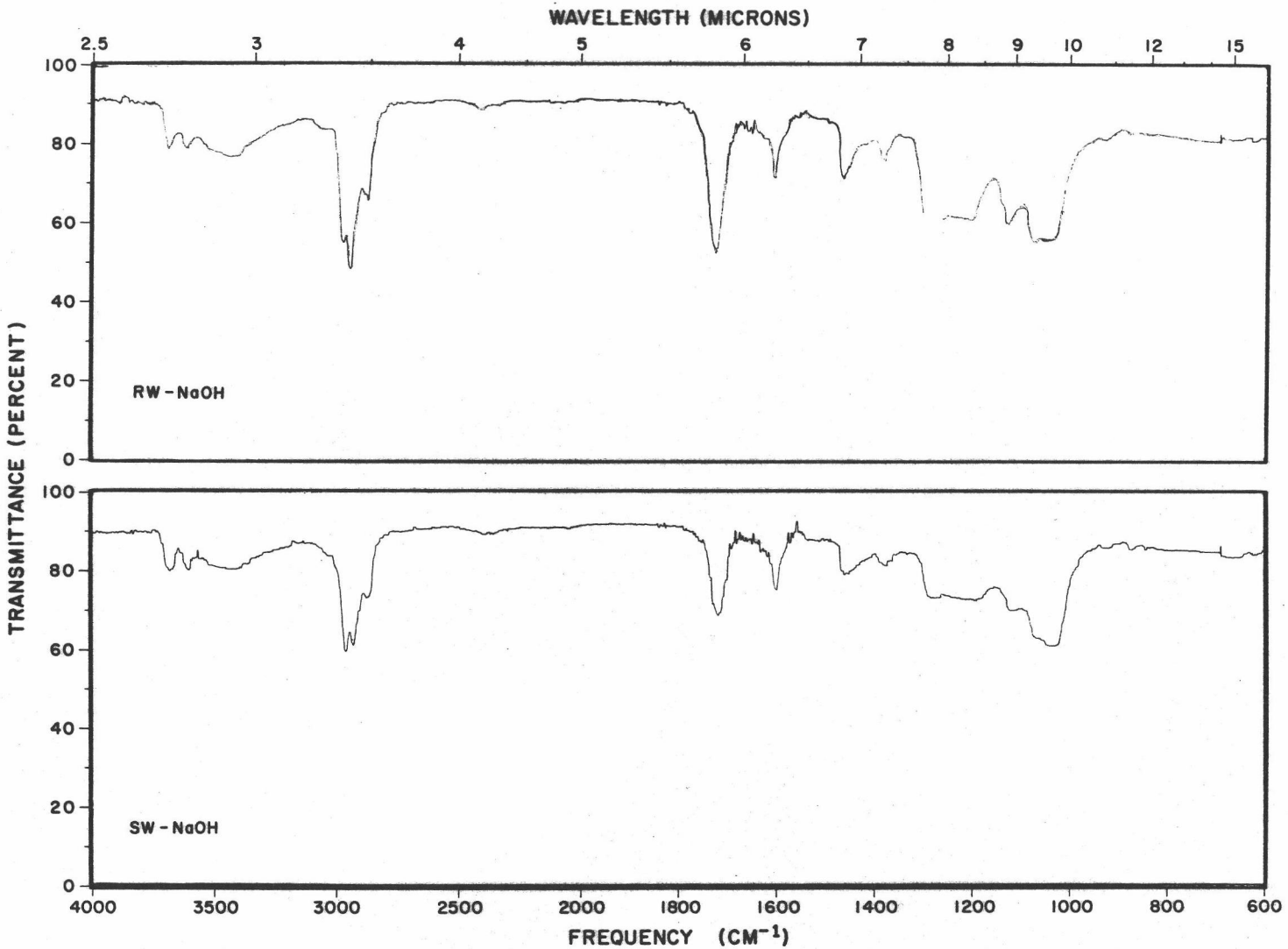


Figure 14. IR spectra of NaOH extract of petroleum ether extract.

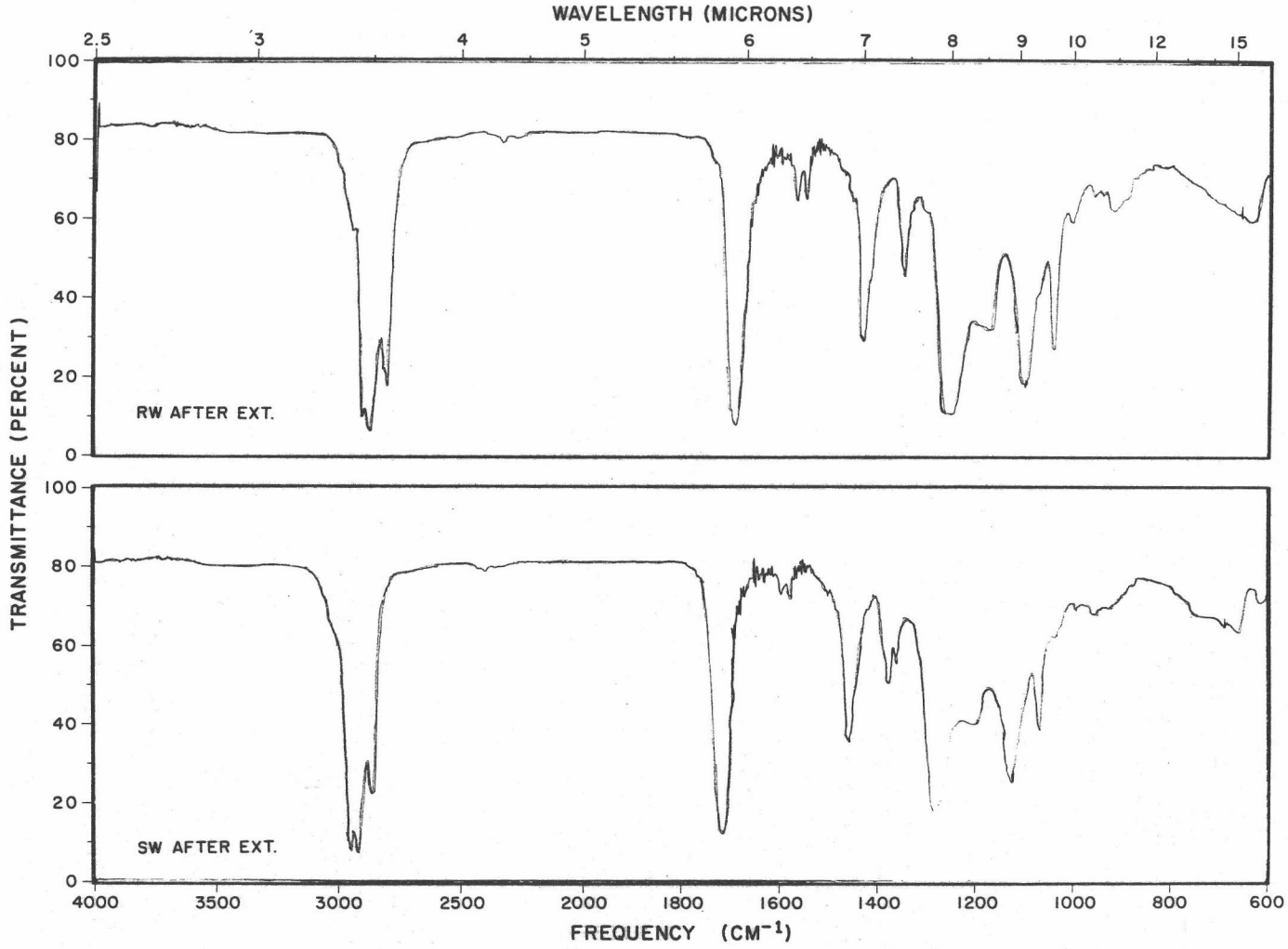


Figure 15. IR spectra of petroleum ether extract after $NaHCO_3$ and NaOH extraction.

groups for the various spectral areas and tabulations of the peaks in the described samples are included in the appendix. The UV analysis was inconclusive as each fraction gave the same spectrum. One could conclude only that no aromatics were present and limited conjugation, if any, existed.

Thin Layer Chromatograms

Thin layer chromatograms were prepared of concentrates from each of the eight fractions (sea water and estuary water) taken from the silicic acid column. As in the IR studies, varying results were noted (see Figures 16 and 17). Fractions I and II are considerably different. Fraction IV of SW was used in preparation of fatty acid esters. Fractions V-VIII show only that there were different compounds or groups of compounds in the two samples (see Figure 17).

It appears that either the unknown compounds vary from estuary water to sea water or that the silicic acid column separation failed. Subsequently, the column was used to separate a known mixture containing the following standards:

1) heptane (I)
2) octene-2 (II)
3) linoleic acid (IV)
4) tributyrin (VI)

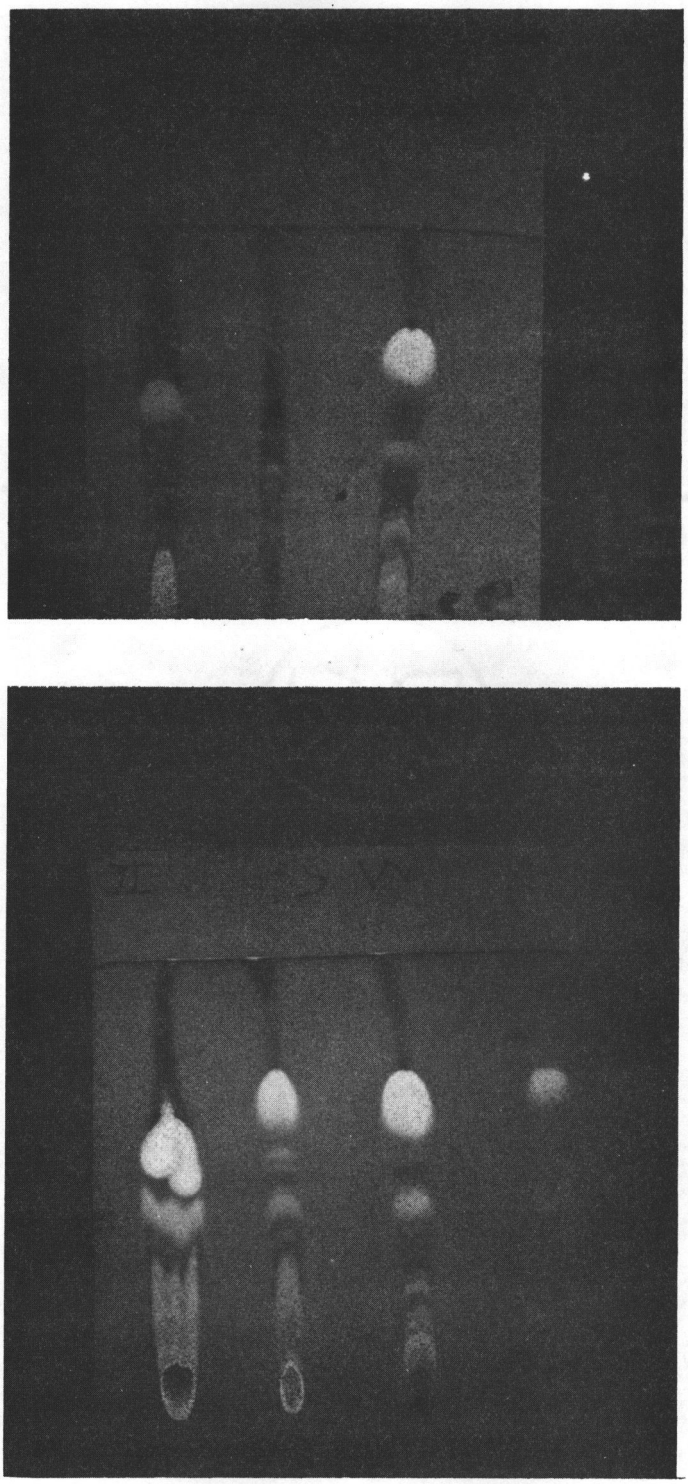


Figure 16. Thin-layer chromatograms of estuarine water, and sea water silicic acid column fractions I, II, III, IV.

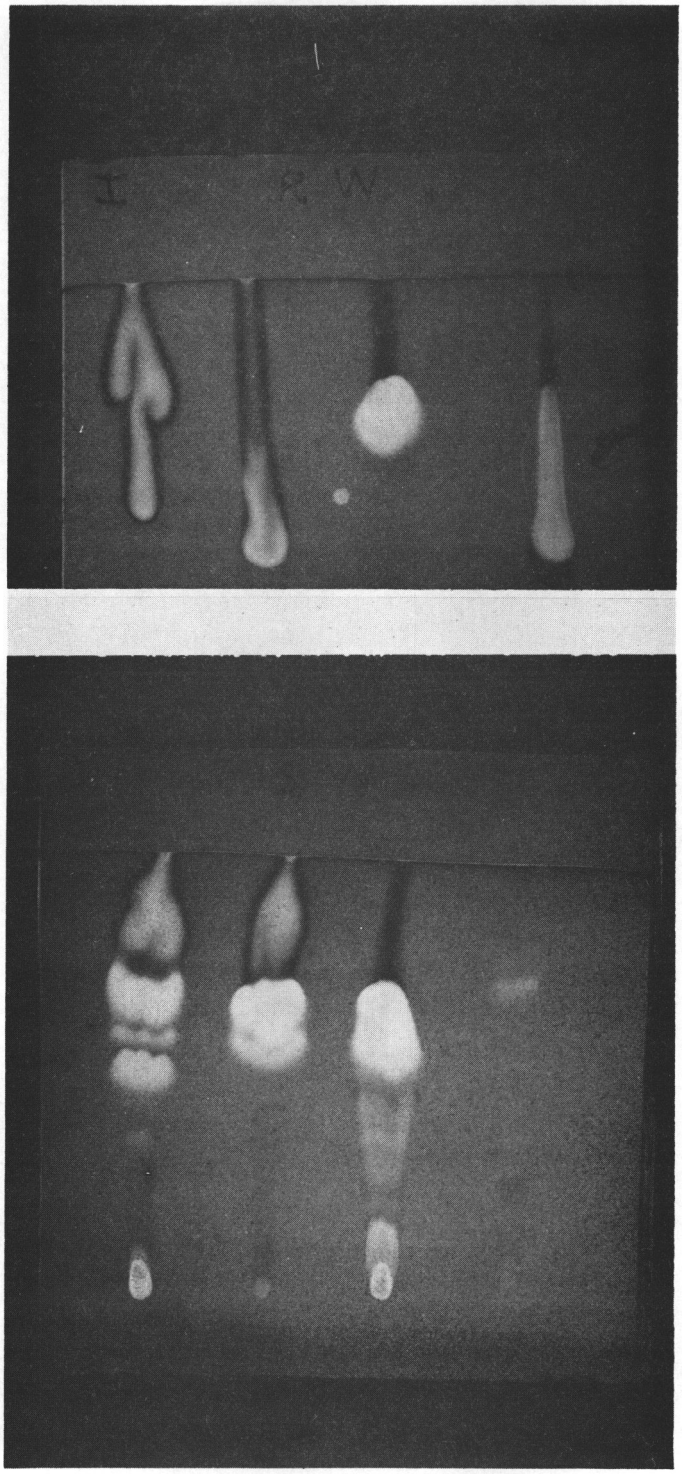


Figure 17. Thin-layer chromatograms of estuarine water and sea water silicic acid column fractions V, VI, VII, VIII.

5) glyceryl monosterate (VII)

6) monoacetin (VIII)

No standards were available for two of the fractions. The fractions collected were chromatogrammed by thin layer chromatography. Fraction I contained two spots; fraction II--three spots; fraction III--one spot; fraction IV--two spots; fraction V--no spots; fraction VI--one spot; fraction VII--one spot; fraction VIII--two spots. Thus, it is concluded that the elutants of the silicic acid column were mixed.

CHN Analysis

Results of CHN analysis were primarily to provide evidence of heterocyclic compounds (Table 7).

Table 7. CHN analysis of petroleum ether extracts (percent).

	C	N	H
Original sea water extract	74.1	--	10.9
Original river water extract	78.1	--	11.1
$NaHCO_3$ ext. of sea water	67.9	--	9.3
$NaHCO_3$ ext. of river water	73.9	--	10.3
NaOH ext. of sea water	67.0	--	9.9
NaOH ext. of river water	71.4	--	10.0
Sea water ext. after $NaHCO_3$ + NaOH ext.	75.5	--	11.5
River water ext. after $NaHCO_3$ + NaOH ext.	76.9	--	10.7

These results show that no nitrogen containing compounds were present. However, since a portion of each sample was pipetted into a combustion boat and allowed to evaporate, the possibility exists that nitrogen may have been lost in the process.

The silicic acid fractions from the original petroleum ether extract were also analysed for CHN content (Table 8).

Table 8. CHN analyses of silicic acid column fractions (percent).

	C	N	H
SW-I	68.4	0.8	10.6
RW-I	69.5	1.0	12.5
SW-II	68.8	1.0	10.5
RW-II	76.4	1.0	10.9
SW-III	70.2	0.6	10.1
RW-III	73.6	0.5	10.4
SW-IV	--	--	--
RW-IV	72.7	0.5	10.3
SW-V	70.3	0.8	9.9
RW-V	72.5	0.8	10.1
SW-VI	--	--	--
RW-VI	75.8	2.0	10.8
SW-VII	68.8	0.7	9.7
RW-VII	72.1	0.8	10.4
SW-VIII	59.5	1.2	8.3
RW-VIII	--	--	--

These results indicate that combustion of the original petroleum ether extract was complete, but insufficient nitrogen was present for conclusive detection. The relative percentages of carbon and hydrogen are the same as in the CHN analysis on the previous page. Carbon percentage seems to always be higher in the river water sample than in the sea water sample. No reason is postulated for this, and in any case, differences are small.

A mass spectroscopic analysis was attempted on these fractions to identify functional groups. Only RW-I and RW-VII provided data:

RW-I--Indications of double bond compounds. Also cyclo compounds

RW-VII--Definite indication of cyclohexane, diphenyl group and phthalate

Hexone Separations

Total Organic Material Extracted

Portions of hexone (300 ml) from each of the four experiments described in Table 2 were placed in an evaporating dish, dried and weighed (Table 9).

A CHN analysis was performed on the product hexone from experiment 1 and 2 (Table 2). The values obtained from extraction of water at basic pH were 67.94%, 10.99% and 4.92%, respectively,

for carbon, hydrogen and nitrogen. The similar values obtained from solvent used to extract acidic water were 68.29%, 12.44% and 4.68%. A sample of residue hexone from the redistillation proved to be void of nitrogen.

Table 9. Weight of organic matter per 300 ml of product hexone.

Experiment number	Mg organic/ 300 ml solvent
1	17.1
2a	29.0
2b	15.0
3	13.3
4	24.7

Gas Chromatography--Flame Ionization

The following figures (18-21) show the spectra resulting from flame ionization detection of the hexone impurities at different stages of the pulse column experiment (see Figure 7 for distillation scheme). Figure 18 shows residue I from the first distillation of the reagent grade hexone prior to extraction of sea water and Figure 19 is a spectrum of the distillate (that was used for extraction of sea water). The spectrum of hexone distillate II following the extraction of sea water (Figure 20) indicates that all of the organic matter was not

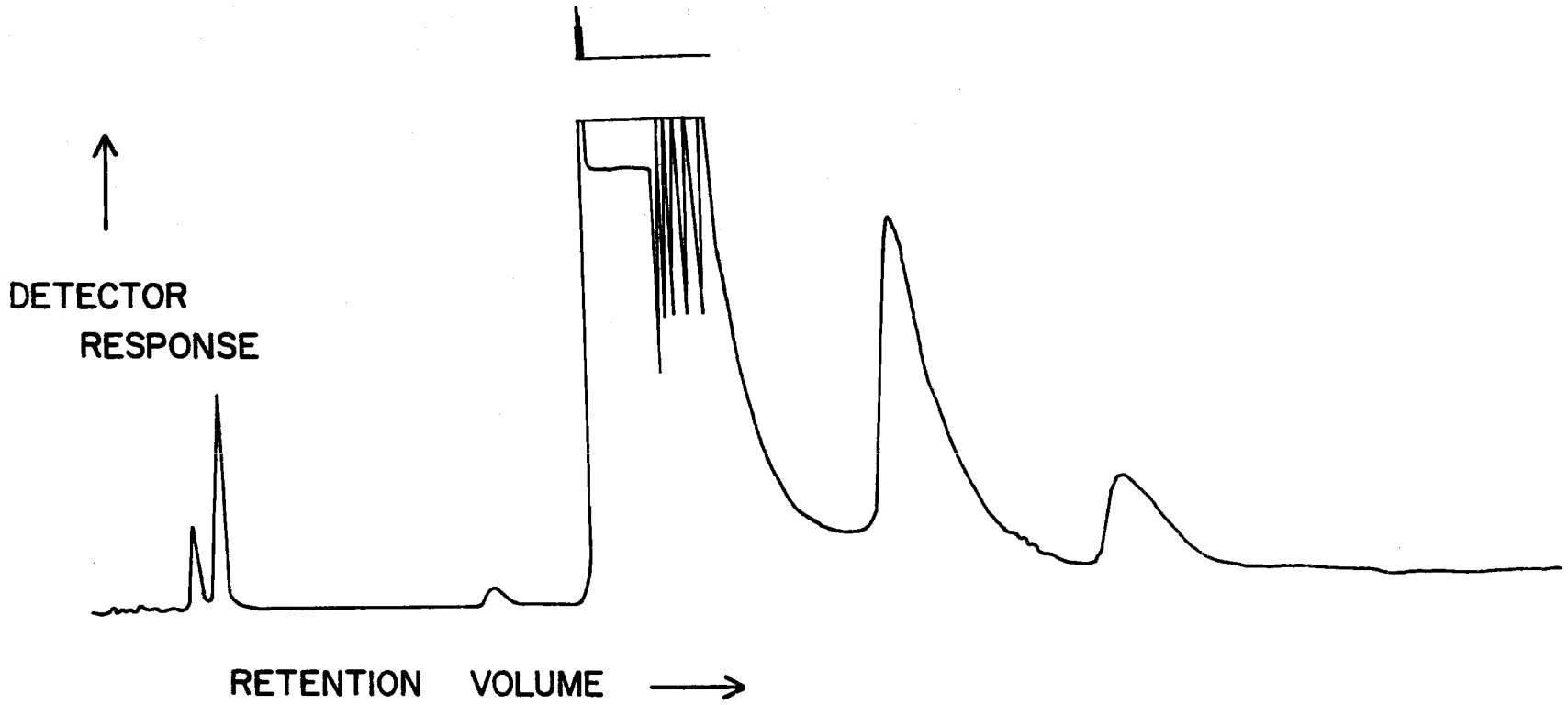


Figure 18. Flame ionization detection of hexone residue from first distillation.

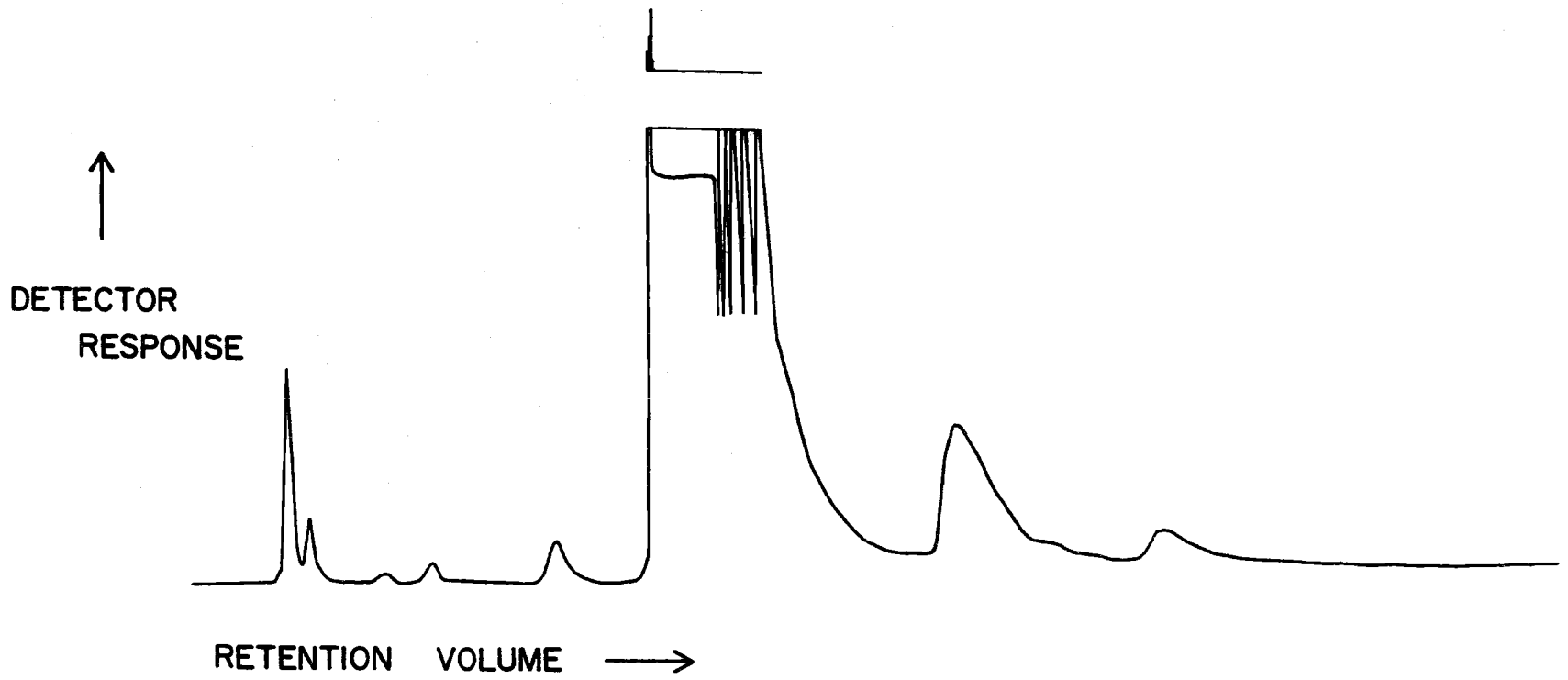


Figure 19. Flame ionization detection of hexone used for extracting sea water.

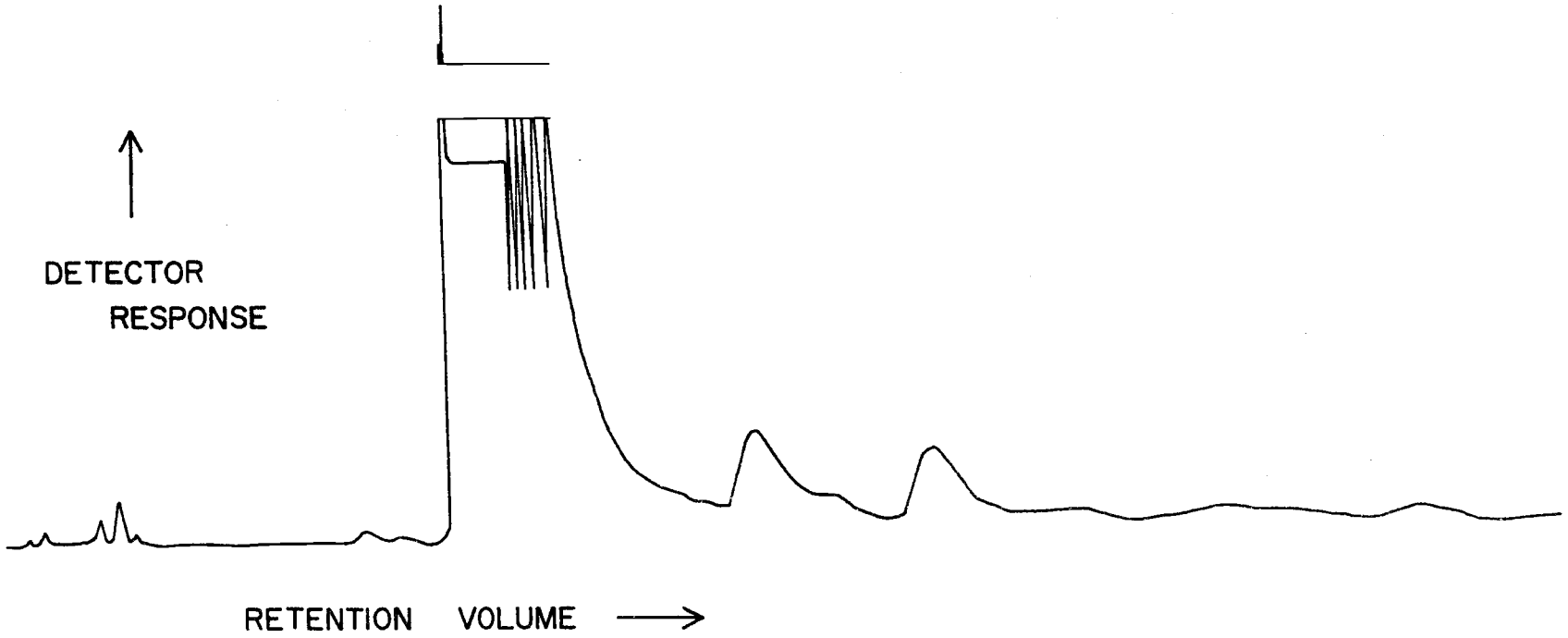


Figure 20. Flame ionization detection of hexone distillate II following sea water extraction.

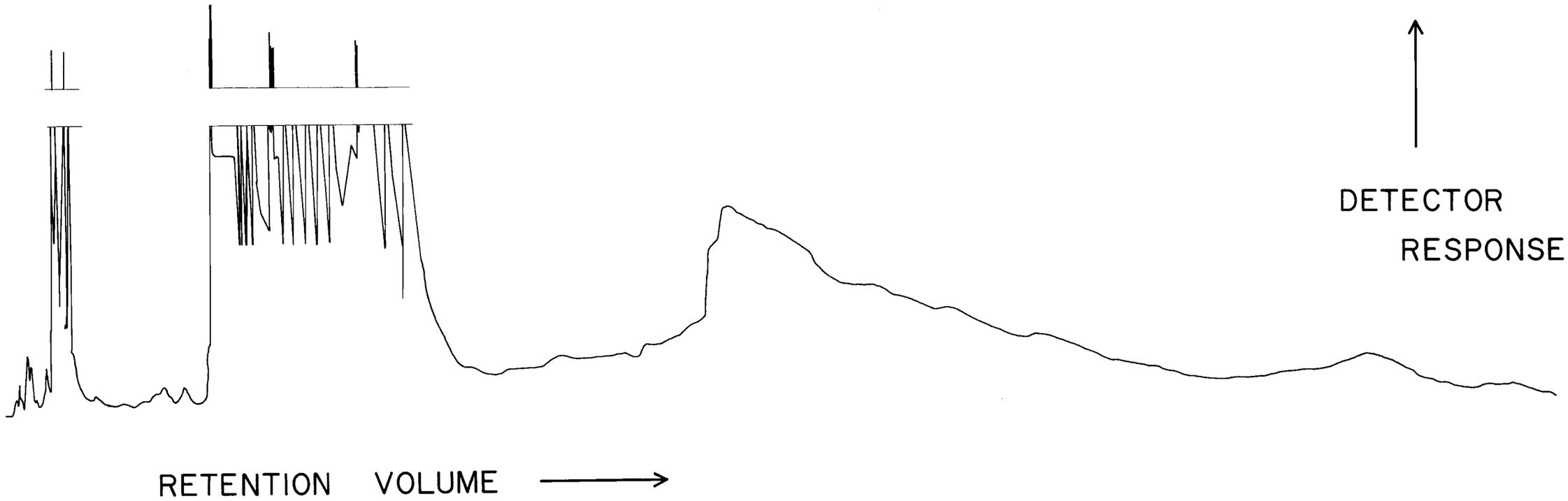


Figure 21. Flame ionization detection. Adibatic distillation of hexone after extraction and distillation.

confined to the residue as desired as several peaks are seen. Further purification of the distillate and concentration of organic matter was attempted by distilling the hexone on an adiabatic column. There are approximately 40 peaks other than hexone in the residue from the adiabatic distillation (Figure 21) indicating considerable organic matter. After extensive heating of the original hexone at 120° C, a GC analysis indicates no degradation occurred.

A portion of samples POW-A and POW-B was analyzed by atomic absorption spectrometry for zinc, cobalt and manganese. All three were detected with zinc being the predominant element. In acid pH, a chloro-complex can be formed with transition elements but this would not be expected to occur at basic pH. Hexone appears to be an excellent solvent for concentrating organic-metal complexes.

High Temperature Distillation Apparatus

The cold trap material from both the basic and acidic water extractions were distilled on the Podbielniak high temperature distillation apparatus. The fraction which distilled over at 56° C was analyzed by NMR and GC. Results were inconclusive because there were at least four compounds in the fraction. Other separation attempts by distillation were equally unsuccessful.

Back Extraction

The mass spectrometric analyses of the $NaHCO_3$, NaOH and HCl back-extracts were difficult because large amounts of hexone were being carried through each chemical procedure. The distillate portion of the saturated hexone interface material was back-extracted into $NaHCO_3$ and analyzed. This portion contained ethyl acetate. Compounds containing four carbons, one hydroxyl and one chlorine were also found in this sample. Other samples analyzed by mass spectroscopy contained oxygenated and chloro-oxygenated compounds although specific compounds were not determined.

Small Scale Extraction

Diethyl ether. The analysis of this extract was limited as acetic acid, which was used to adjust the pH of the sea water, was the only compound identified. It would, of course, be carried through each step of the extraction.

Cyclohexane. Several unknown compounds were separated by GC methods but the vapor pressures were too low to be resolved by the available mass spectrometer. However, a low-intensity fragment ion at m/e 149 was observed on one run, suggesting a phthalic type component.

Pentane. The analysis of this extract was more successful,

as 300-400 mass spectra were recorded and interpreted. However, the complexity of the mixture restricted classification to type and molecular weight. The results of analysis of the paraffin-olefin fraction and aromatic fraction will be found in Tables 10 and 11. Components are listed according to those found in various GC peak areas.

Table 10. Paraffin-olefin fraction of pentane extract (Analysis by mass spectroscopy).

C_7H_{16} [1]	C_7H_{14} [2]	C_6H_{12}				
C_8H_{18}	C_8H_{16}	C_9H_{20}				
C_9H_{20}	C_9H_{18} *	$C_{10}H_{22}$				
C_9H_{16}	$C_{10}H_{20}$ *	C_9H_{18}	$C_{10}H_{22}$			
$C_{10}H_{22}$	$C_{11}H_{24}$	$C_{10}H_{20}$				
$C_{10}H_{20}$	$C_{11}H_{22}$	$C_{10}H_{22}$				
$C_{11}H_{22}$	$C_{10}H_{20}$	$C_{12}H_{22}$				
$C_{11}H_{22}$	$C_{11}H_{20}$ *	$C_{12}H_{22}$	$C_{12}H_{24}$	$C_{12}H_{26}$		
$C_{12}H_{22}$	$C_{11}H_{20}$	$C_{11}H_{20}$	$C_{12}H_{24}$	$C_{13}H_{28}$		
$C_{12}H_{22}$	$C_{11}H_{22}$	$C_{11}H_{20}$	$C_{12}H_{20}$	$C_{13}H_{26}$	$C_{13}H_{24}$	$C_{13}H_{22}$
$C_{12}H_{20}$	$C_{13}H_{24}$	$C_{13}H_{22}$	$C_{13}H_{26}$	$C_{12}H_{22}$	$C_{14}H_{28}$	$C_{14}H_{26}$

Components are listed in decreasing order of their contribution to the total peak area:

* signifies major contribution in addition to first weak peak listed

1 all paraffins in the C_7 through C_{12} range show considerable branching

2 C_nH_{2n} type material may be either cyclic paraffins or olefins (both probably present)

3 appears to be olefinic

Table 10. (Continued)

$C_{13}H_{22}$	$C_{12}H_{20}$	$C_{14}H_{24}$	$C_{14}H_{26}$	$C_{13}H_{24}$		
$C_{13}H_{20}$	$C_{14}H_{22}$	$C_{12}H_{20}$	$C_{13}H_{22}$	$C_{14}H_{24}$	$C_{14}H_{26}$	$C_{14}H_{28}$
$C_{14}H_{24}$	$C_{13}H_{22}$	$C_{14}H_{22}$	$C_{14}H_{26}$			
$C_{15}H_{26}$	$C_{14}H_{24}$	$C_{13}H_{22}$				
C_{15}	C_{14}	unsaturated[3]				
C_{15}	C_{16}	unsaturated[3]				
C_{16}	C_{15}	C_{17}	unsaturated[3]			
C_{18}	C_{17}	C_{19}	unsaturated[3]			
C_{19}	C_{18}	C_{20}	unsaturated[3]			
C_{20}	C_{19}	unsaturated[3]				
C_{20}	C_{21}	unsaturated[3]				
C_{21}	C_{35}	retention times corresponding to n-paraffin				

Table 11. Aromatic fraction of pentane extract (Analysis by mass spectroscopy).

C_7H_8 (Toluene)	C_9H_{20}	
C_9H_{18}		
C_8H_{10} (phenyl-C_2)	C_9H_{18}	
C_8H_{10} (phenyl-C_2)	$C_{10}H_{20}$	
C_9H_{12} (phenyl-C_2)	$C_{10}H_{20}$	
C_9H_{12} (phenyl-C_3)	$C_{10}H_{18}$	
$C_{10}H_{14}$ (phenyl-C_4)	C_9H_{12} (phenyl-C_3)	$C_{11}H_{22}$
$C_{10}H_{18}$	$C_{10}H_{14}$ (phenyl-C_4)	
$C_{11}H_{16}$ (phenyl-C_5)		
$C_{11}H_{16}$ (phenyl-C_5)	$C_{10}H_{14}$ (phenyl-C_4)	
$C_{11}H_{16}$ (phenyl-C_5)		
$C_{11}H_{16}$ (phenyl-C_5)	$C_{12}H_{18}$ (phenyl-C_6)	
$C_{12}H_{16}$	$C_{11}H_{16}$ (phenyl-C_5)	

Table 11. (Continued)

$C_{12}H_{18}$ (phenyl-C_6)	$C_{13}H_{20}$ (phenyl-C_7)	$C_{12}H_{14}$	
$C_{13}H_{20}$ (phenyl-C_7)	$C_{14}H_{22}$ (phenyl-C_8)	$C_{12}H_{16}$	$C_{13}H_{18}$
$C_{13}H_{18}$	$C_{13}H_2O$	$C_{14}H_{22}$ (phenyl-C_8)	
$C_{14}H_{20}$	$C_{15}H_{22}$		

Three groups of unsaturated aromatics (C_{14} to C_{20}) mostly CnH_{2n-8} and CnH_{2n-10} type.
An unknown of molecular weight of 266.

CONCLUSIONS

The pentane blank had no detectable (± 0.2 ppm) impurities. There was further evidence of terpene type structures (lower acyclic type) in the pentane sample but the large number of structures made identification difficult.

Our experience indicates that both small scale and large volume solvent extractions are successful in removing organic material from natural waters. The small scale extraction can be performed with less physical effort and laboratory space requirements, but instrumentation and separation techniques are more demanding due to the limited quantity of organic material extractable from a small sample. Larger amounts of material can be extracted by large volume techniques, but sample collection requires considerably more time and results in many shipboard difficulties.

Either method demands the careful handling of solvents due to their flammability and/or toxicity to personnel. Perhaps the most rigid requirement is the purification of the solvent. Reagent grade chemicals have enough impurities to interfere with analyses, especially those involving tandem gas chromatographic-mass spectrometric analysis. Use of large volumes of organic solvent concentrates the impurities if sufficient precautions are not exercised. The removal of these impurities from large volumes is very difficult

and economically unfeasible.

One alternative to purification requires duplicate analyses of the raw solvent and the extracted sample. Even this may not be sufficient control when using large volumes of solvent, since impurities not evident in the raw solvent may be concentrated in the extraction process.

These results indicate that a small but efficient pulse column extraction would be practical for recovering dissolved organics from sea water. Some effort would be required to permit trouble-free operation at sea, but the inherent efficiency of this method makes its use attractive, considering the low concentration of organics in sea water.

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APPENDIX

APPENDIX

EQUIPMENT SPECIFICATIONS

Gas Chromatography

A. Wilkens Model A-700 autoprep utilizing a 20% STAP on chromosorb PAW, 5% SE-30 chromosorb PAW and a 20% SE-30. (Other columns specifically noted.)

B. Wilkens Model B-600 with a column of 30% DEGS on chromosorb PAW.

C. F&M Model 500, in series with CEC Type 21-130 mass spectrometer. Column noted in each experiment.

D. F& M Model 810, in series with CEC Type 5-124 mass spectrometer.

Thin-Layer Chromatography

Research Specialties kit with variable thickness spreader.

Gamma Ray Spectrometers

A. Nuclear Data 130 AT 510 channel spectrometer.

B. Packard multidimensional anticoincidence counter built by R. W. Perkins, Battelle-Northwest, Richland, Washington.

Ultraviolet and Visible Spectrophotometry

A. Cary, Model 14

B. Beckman DK-2

Nuclear Magnetic Resonance

Varian Associates Model A60.

High Temperature Distillation Apparatus

Podbielniak Series 3475

The following is a tabulation of conditions and limitations for the three mass spectrometers and CHN analyzer used in the analysis of the various samples:

	CEC 21-130	Modified CED 21-201	CEC 5-124
Geometry	cycloidal	6"-60° Nier	cycloidal
Source Temperature	250° regulated	Approx. 200° unregulated	200°
Ionizing Current	50 m amps	50 amp	50 m amps
Ionizing Voltage	68 volts fixed or 0-20 volts varied	70 volts fixed	Low ionization voltage modification
Mass Range	12-230	12-250 (dependent on accelerating voltage)	Approx. 500
Magnet	4800 gauss (permanent)	Varying to 3700 gauss (electromagnet)	6000 gauss
Voltage	Field plate 200-10 volts	Varying accelerating voltage 450-2000 (typically 450/ 800)	Varying
Sweep	Decay of plate voltage (exponentially)	Increasing magnetic current (0.45 ma) non-linear	Increasing magnetic current

	CEC 21-130	Modified CEC 21-201	CEC 5-124
Resolution	1:230	1:200 (degrades with lower accelerating voltage)	1:250
Inlet	Stainless steel block; goldfoil molecular leak 3 liter expansion volume, hypodermic septum inlet 100° C unregulated oven oil diffusion pump; micromanometer	Same as Model 21-130 except temperature varied from 150-225° C	Batch inlet-130° C Continuous inlet-200° C
Sample Size	1-2μl (dependent on vapor pressure) 40-50μl in expanded volume)	4-8μl, approx. 100μl in expanded volume	1-10μl
Output	Faraday cup-electrometer tube amplifier	Same	Same
Recording	5 trace oscillograph	Logrithmic amplifier to a strip chart recorder	5 trace oscillograph
Scan Rate	48 sec/octave (5 min/sample)	180 sec/octave-increased with mass increased mass (25 min/sample)	2.6 sec/octave

CHN Analyzer

The operating conditions of the F&M Model 180 CHN analyzer are as follows:

Carrier Gas	Helium - 35 psig at tank-15 psig at inlet
	Flow - 100 ml/min ± 10 in furnace position, same as in furnace position ± 10 in the bypass position
Temperatures	Furnace - 900° C
	Pre-purifier - 400° C
	Analytical Column 3-120° C
Combustion Time	2 minutes
Balance	Calibrate with 10 mg. Run on 0.1 mg range
Oxygen Source	Ag_2O- MnO_2 - 50/50 powder (Standard F&M mixture).
	Ratio recording mode of operation
Sample Size	0.5-0.8 mg
Data Presentation	Bridge - 200 ma
	Recorder - 16 attenuation for H_2O and N_2
	32 attenuation for CO_2-1/4 inch/min chart speed
Calibration Standards	Dependent upon type of nitrogen bonds expected in sample.
	a) High purity phenylurea for samples that contain only amino type or double bond nitrogen.

b) High purity meta-dinitrobenzene for sample with nitrogen-oxygen bonds.

c) CHN standard cyclohexanone 2, 4-dintrophenyl hydrazone for samples with both amino and nitro nitrogen.

AQUEOUS BACK EXTRACTION AND SOLVENT EXTRACTION CONDITIONS

Sample	Volume of Product Distillate Used (ml)	Aqueous Agent (ml)	Solvent Used for Re-Extraction (ml)
POW-B (CT)	100	200-$NaHCO_3$	100 ml (a) ether + 3 separate ml portions of $CHCl_3$ (b)
POW-B (CT)	100	200-HCl	100 ether + 2-100 $CHCl_3$ (b)
POW-B (CT)	100	100-NaOH	2-100-ether + 100 - $CHCl_3$
POW-A-I (1-T)	500	200-$NaHCO_3$ (c)	
POW-A-1 (FD)	500	2000$NaHCO_3$	2-100 ether + 100 $CHCl_3$ (d)
POW-A-1 (FD)	500	200-NaOH	2-100 ether + 100 $CHCl_3$ (d)
POW-A-1 (FD)	500	2000HCl	100 ether
POW-A-1 (1-D)	2000	350-$NaHCO_3$	
POW-A-1 (1-D)	2000	350-NaOH	2-100 ether + 100 $CHCl_3$ (c)

(a) accidentally spilled
(b) no apparent extraction occurred with cyclohexane
(c) apparently no organic material
(d) did not keep due to apparent lack of organic matter
(e) no apparent back extraction into any of the three reagents

(Continued)

Sample	Volume of Product Distillate Used (ml)	Aqueous Agent (ml)	Solvent Used for Re-Extraction (ml)
POW-A-1 (1-D)	2000	350-HCl	2-100 ether + 100 $CHCl_3$ (c)
POW-A-I (FD)	750	150-$NaHCO_3$	100 ether + 100 $CHCl_3$ (c)
POW-A-I(FD)	750	150-NaOH	2-100 ether (c)
POW-A-I (FD)	750	150-HCl	100 ether + 100 $CHCl_3$ (c)
POW-B (FD)	750	450-$NaHCO_3$	100 ether + 100 $CHCl_3$
POW-B (FD)	750	450 NaOH	100 ether + 100 $CHCl_3$
POW-B (FD)	750	150 HCl	100 ether + 100 $CHCl_3$
POW-A-2 (FD)	1500	250 $NaHCO_3$	2-100 ether + 2-100 $CHCl_3$
POW-A-2 (FD)	1500	250 NaOH	2-100 ether + 2-100 $CHCl_3$
POW-A-2 (FD)	1500	275 HCl	2-100 ether + $CHCl_3$ + 100 cyclo-hexane (d)
POW-A-I (1D)	750 (e)		
POW-A-IS (FD)	750	400 $NaHCO_3$	2-100 ether

(Continued)

Sample	Volume of Product Distillate Used (ml)	Aqueous Agent (ml)	Solvent Used for Re-Extraction (ml)
POW-A-IS (FD)	750	400 NaOH	2-100 ether
POW-B (1-D)	750	400 $NaHCO_3$ (d)	
POW-B (1-D)	750	400 NaOH (d)	
POW-B (1-D)	750	400 HCl (d)	
POW-A-2 (1-D)	1000	400 $NaHCO_3$ (d)	
POW-A-2 (1-D)	1000	400 NaOH (d)	
POW-A-2 (1-D)	1000	400 HCl (d)	

POSSIBLE ASSIGNMENT OF IR ABSORPTION BANDS

Wavelength (μ)	Assignment
2.90 - 3.0	OH stretching of C-OH and NH
3.30 - 3.50	CH stretching
5.72 - 5.80	Carbonyl stretching
6.10	Carbonyl stretching
6.80 - 6.90	CH_2 scissor, aliphatics
7.22 - 7.40	CH_3 symmetrical bending
7.90 - 8.40	band progression in solid fatty acids
8.20	C-O-C (phospholipids)
8.90 - 9.20	C-O-C or kotene
9.50 and 9.60	OH group of glycerides (mono and di)
9.20 - 9.40	P-O-C (phospholipids)
9.50	steroids
10.30	P-O-C
10.30 -10.40	CH bending about trans C=C
10.60 -11.10	OH deformation
13.80 -14.10	long carbon chain (methylene rocking)
14.70	cis double bond

ABSORPTION PEAKS AND RELATIVE INTENSITY OF VARIOUS FRACTIONS

Sample	Peaks and Intensity	Sample	Peaks and Intensity
SW-orig.	3.32 W	RW-orig.	2.34 W
	3.38 S	(PVP added	2.46 W
	3.41 S	and removed)	3.32 W
	3.45 S		3.38 S
	3.48 M		3.40 S
	5.80 S		3.45 S
	6.24 W		3.48 M
	6.33 W		3.50 S
	6.85 S		5.78 S
	7.26 M		6.25 M
	7.34 M		6.33 M
	7.80 S		6.85 S
	8.25 M		7.26 S
	8.80 M		7.32 M
	8.87 M		7.85 S
	9.32 M		8.88 S
	9.63 W		9.32 S
	10.45 W		10.49 W

Sample	Peaks and Intensity	Sample	Peaks and Intensity
SW-$NaHCO_3$	2.72 M	RW-$NaHCO_3$	2.72 W
	2.78 M		2.78 W
	2.94 W		2.95 W
	3.38 S		3.38 M
	3.41 S		3.40 S
	3.50 W		3.50 M
	5.81 M		5.81 S
	6.74 M		6.25 W
	6.93 M		6.87 M
	7.24 S		6.92 M
	7.41 M		7.24 S
	7.73 M		7.41 M
	7.84 M		7.80 M
	8.15 M		8.73 M
	8.32 M		9.00 S
	8.71 S		9.03 S
	9.00 S		9.33 M
	9.05 S		9.63 W
	9.32 S		
	9.61 M		
	9.80 M		
	10.75 W		
	10.93 W		
	11.86 W		
SW-NaOH	2.72 W	RW-NaOH	2.72 W
	2.77 W		2.76 W
	3.38 S		3.38 M
	3.41 S		3.41 S
	3.43 M		3.44 S
	3.50 S		3.50 S
	5.80 S		5.82 S
	5.87 M		6.25 W
	6.25 W		6.33 W
	6.90 W		6.42 W
	7.25 M		6.87 M
	7.80 M		7.26 M
	9.02 S		7.84 S
	9.33 M		8.35 S
			9.33 M

Sample	Peaks and Intensity
SW-after Extract	3.36 S
	3.40 S
	3.43 S
	3.49 S
	5.80 S
	6.25 W
	6.33 W
	6.85 S
	7.26 M
	7.34 M
	7.80 S
	8.86 M
	9.02 W
	9.32 M
	9.65 W
SW-after PVP Extract	3.43 S
	3.49 S
	5.80 S
	6.34 W
	6.84 S
	7.25 M
	7.73 M
	7.38 S
	8.85 M
	9.33 M
	9.62 M
	9.72 M
	15.05 M
RW-after Extract	3.34 W
	3.39 M
	3.44 S
	3.48 S
	3.50 S
	3.53 M
	3.54 S
	5.90 S
	6.25 W
	7.33 W
	6.85 S
	7.26 S
	7.32 M
	7.77 S
	7.84 S
	8.88 S
	9.32 S
	9.65 M
	10.25 M
	10.47 M

GAS CHROMATOGRAPHY OPERATING CONDITIONS

Pressure of Helium	30 PSI
Flow of Helium	228 ml/min.
Temperature:	
Detector	260°
Column	225°
Vaporizor	210°
Output	210°
Column Size	10' x 3/8"
Column Packing	20% STAP* on chromosorb P-AW
Chart Speed	30"/hr.
Attenuation	1 x
Detector:	
Thermal Conductivity	150 ma

*Steroid Analysis Phase