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DETERMINATION OF TOTAL HYDROCARBONS IN SEA WATER AT THE MICROGRAM LEVEL WITH A FLOW CALORIMETER

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SUMMARY

One litre of water is extracted with 10 ml of 1,2,2-trichlorotrifluoroethane and the extract is then concentrated to 100 μl. A 50-μl volume is injected into a high-performance liquid chromatographic system with a flow calorimeter that measures the absorbance of hydrocarbons on porous glass beads. A short silica gel column in the system removes non-hydrocarbon material. A chromatogram is obtained within 3 min and only one peak has to be evaluated. The minimum detectable amount is 4.0 ± 3.4 μg/l. These values can be improved by extracting larger volumes. The equipment used is fairly inexpensive and can readily be taken to sea.

INTRODUCTION

In order to make a meaningful study of the distribution of hydrocarbons in an aquatic environment under normal conditions, one is often faced with the problem of making a large number of determinations on a relatively small sample volume (under 5 l) containing compounds that are present in only low concentrations[1]. It was therefore desirable to develop a method that was capable of fulfilling these requirements. In addition, it was hoped to make the method sufficiently simple to be used at sea, to decrease the time between sampling and measuring and thereby to help reduce the complications brought about by storage.

Infrared detectors have been used for measuring quantitatively the hydrocarbon content in water[2–4]. However, they usually have a lower sensitivity of only 0.05 mg (ref. 3), and great care must be taken to separate the hydrocarbons from other non-polar compounds, as the detector measures all the C–H bonds present regardless of any other functional groups that might possibly be attached to the molecule.

Sensitive methods are available for the measurement of aromatic hydrocarbons based on their absorption of ultraviolet (UV) light[5] or on measuring their fluorescence in the UV region after having been excited[6–8]. These measurements, however, do not show the amount of non-aromatic hydrocarbons present, which quantitatively are far more abundant in natural waters[1,9]. Another disadvantage

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of these optical methods is that the response per unit weight varies from compound to compound, making it necessary to report all experimental values with respect to a more or less arbitrary standard.

Gas–liquid chromatography (GLC) has often been used in the analysis of mineral oil present in polluted water[2,4,10] and gives a high resolution of the individual hydrocarbon compounds. However, in water not directly concerned with oil pollution, it is difficult to obtain quantitative values, as the small amount of the hydrocarbon material present would produce individual peaks that are too weak to measure accurately. Also, the extracted samples have to be purified by liquid chromatography in order to separate the hydrocarbons from other compounds, before they can be analyzed by means of GLC. Another difficulty is that a single chromatogram takes about a half an hour to run and requires some time to evaluate and may involve sophisticated electronic equipment such as integrators.

In this laboratory, a method has been used[11] that consists in purifying the extract on a thin-layer chromatographic (TLC) plate, scratching off the part containing the hydrocarbons, eluting them, and then determining their amounts in a CHN analyzer. This method, however, was found to be too cumbersome to be used for rapid determinations and contained too many steps, each of which were prone to contamination.

EXPERIMENTAL

The layout of the high-performance liquid chromatographic (HPLC) system used is shown in Fig. 1. All of the major parts were obtained from Varian Aerograph (Walnut Creek, Calif., U.S.A.). The solvent delivery system is a pressurized solvent container made of stainless steel. A small helium bomb provides the pressure, which forces the solvent through the system. The tubing is also made of stainless steel with an O.D. of 1/8 in. and an I.D. of 1.8 mm. The injector is of the septum type with a silicone rubber/PTFE laminated septum. The flow calorimeter is an adsorption detector and is placed in a well insulated water-bath containing about 12 l of water.

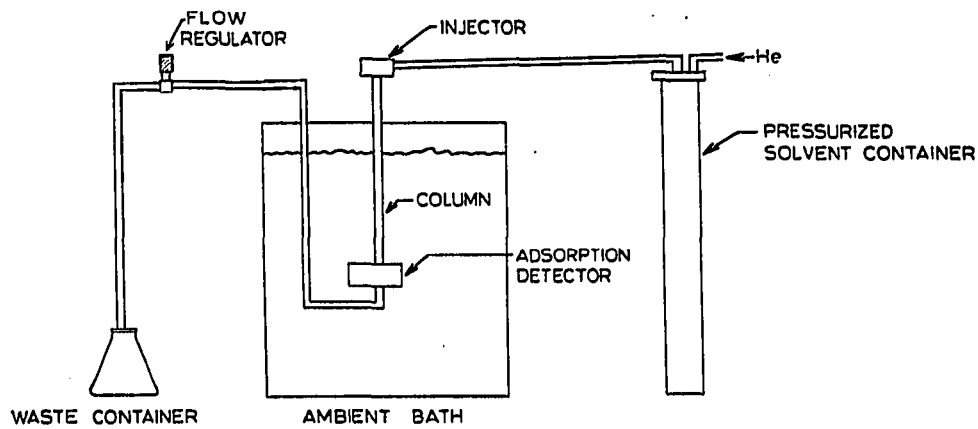

Fig. 1. Layout of the HPLC system. The tubing has an O.D. of 1/8 in. and the ambient bath a volume of about 12 l.

The detector is shown in detail in Fig. 2, and it consists of two thermistors placed within the solvent stream. The upper thermistor is embedded within a non-adsorbing material or is not embedded at all, while the lower thermistor is embedded in an adsorbing material. Any compounds transported by the solvent through the detector that are capable of adsorbing on this material do so and produce a heat of adsorption, which in turn changes the resistance in the lower thermistor. An electrical imbalance occurs and is registered on a 0–1 mV potentiometric strip-chart recorder. Under ordinary conditions, desorption of the compound from the adsorbent follows, resulting in a loss of heat around the lower thermistor, and a negative peak results after the positive peak (Fig. 3).

In order to test the characteristics of the detector and to investigate various solvent–adsorbent combinations, a column 5 cm long with an I.D. of 1.8 mm was filled with non-porous glass and attached between the detector and the injector. It was found that the detector was very sensitive to changes in the flow-rate, and a flow regulator was therefore built into the system. The detector was also sensitive to changes in pressure and the injection of a sample often resulted in a pressure front, which produced a sharp deflection on the recorder. This was avoided by placing the flow regulator after the detector, so that a pressure is maintained on the entire system and the effects resulting from the additional pressure due to an injection are proportionally not as great.

The solvent used has to be non-polar with a low boiling point, as the samples are obtained by extracting the hydrocarbons, which are non-polar, from the water. These extracts must then be concentrated rapidly with the lowest possible loss of the hydrocarbon material. At first, *n*-hexane was used as the solvent with *n*-tetracosane as the material to be determined. The following materials were tried as

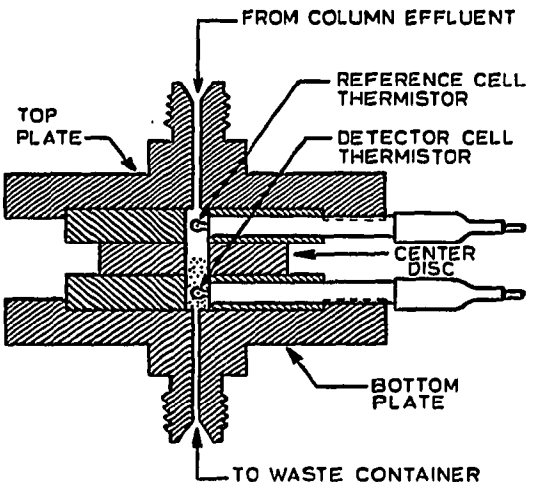

Fig. 2. Diagram of the flow calorimeter used. It is an adsorption detector, with the detector cell thermistor embedded in a material that is capable of adsorbing hydrocarbons.

Fig. 3. Peak obtained from the flow calorimeter with an *n*-hexane–graphite system. First a heat of adsorption occurs, which is then followed by desorption, resulting in a negative peak after the positive peak.

adsorbents: porous glass beads, Amberlite XAD-2, silica gel, cellulose powder and cast iron[12], but all of these produced an adsorption that was too weak to be measured satisfactorily. Graphite resulted in a very strong heat of adsorption with the larger hydrocarbons dissolved in *n*-hexane and was used in two studies in this laboratory[13,14]. The use of graphite, however, had two serious limitations: the response per unit weight varied from hydrocarbon to hydrocarbon in a non-linear manner and, in addition, the active sites on the graphite became irreversibly blocked after several extracts had been injected, which resulted in a loss of sensitivity with use, and the adsorbent had to be replaced fairly often.

In order to avoid these problems, the solvent was changed from a low-boiling hydrocarbon to a halogenated hydrocarbon. Any of the non-polar halogen compounds would have been suitable, but as a low boiling point was desired, 1,2,2-trichlorotrifluoroethane (TCF) was chosen. This compound also has the additional advantage that it is transparent to UV light at 254 nm, as measurements on the aromatic hydrocarbons are also made in this laboratory[1,5]. Of the various adsorbents that had been tried before, porous glass beads with a pore size between 3.0 and 4.5 nm were found to be the most suitable. The TCF–glass beads system has a sensitivity that is only about one-tenth of that of the *n*-hexane–graphite system for the longer-chain hydrocarbons, but it has the advantage that the response per

TABLE I

RESPONSE PER UNIT WEIGHT RELATIVE TO NONADECANE

Compound	*No. of carbon atoms in compound*	*Response (%)*	
		5-cm column with non-porous glass beads	*5-cm column with 10% deactivated silica gel*
Nonadecane	19	100	100
Squalane	30	92	93
Octacosane	28	78	76
Tetracosane	24	—*	83
Docosane	22	94	93
Eicosane	20	—	91
Pristane	19	—	90
Octadecane	18	—	107
Heptadecane	17	—	97
Hexadecane	16	101	103
Pentadecane	15	—	97
Undecane	11	93	91
Hexane	6	—	110
β-Carotene	40	—	0
Squalene	30	81	32
1-Docosene	22	—	81
1-Nonadecene	19	82	79
1-Heptadecene	17	79	75
Naphthalene	10	45	29
Stearic acid *n*-decyl ester	28	15	1
Methyl palmitate	17	20	0

* A dash means not tested.

unit weight for the various saturated hydrocarbons is the same regardless of the degree of branching or chain length (Table I), and the adsorbed material is readily desorbed again, causing no blocking of the active sites on the glass beads. The adsorption curve in this system, however, is different in that at first a decrease in temperature is registered, followed by an increase (Fig. 4). Evidently the hydrocarbons displace solvent molecules from the surface of the glass beads, and this desorption is registered by the detector. This desorption also occurred when halogenated hydrocarbons other than TCF, such as carbon tetrachloride or tetrachloroethane, were used as the solvent. This desorption peak was found to be linearly proportional to the amount of hydrocarbons injected.

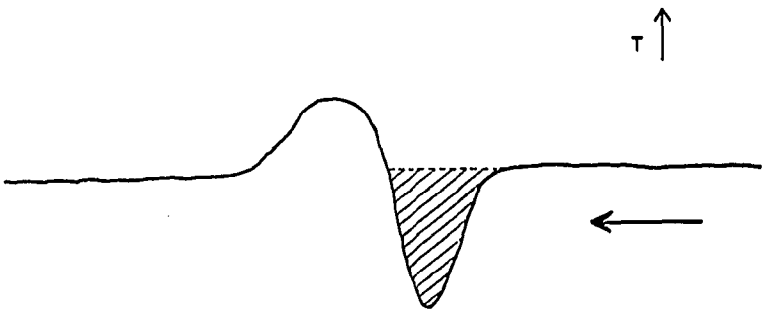

Fig. 4. Peak obtained from the flow calorimeter with a halogenated hydrocarbon–porous glass bead system. A desorption occurs before the adsorption. The area indicated with hatched lines was proportional to the amount injected on the column.

It is important to make certain that only the hydrocarbon material is measured and not other compounds. It can be seen from Table I that the glass beads in the detector react preferentially with non-aromatic hydrocarbons. In addition, the non-polar solvent that is used to extract the sea water will not extract more polar compounds, such as waxes or fatty acid esters, as well as the non-polar hydrocarbons. However, in order to be certain that the non-hydrocarbon compounds, which are far more abundant in nature, are also not measured, the column is filled with silica gel (0.063–0.200 mm grain size) that has been activated overnight at 110° and then partially deactivated with 10% (w/w) of distilled water. The area of the curve that is measured is indicated in Fig. 4 with hatched lines, and the response per unit weight relative to *n*-nonadecane of various compounds is shown in Table I. *n*-Nonadecane was chosen as the standard because it has an "intermediate" size among the hydrocarbons that are most likely to be found in a natural environment. It can be seen from Table I that the silica gel prevents both fatty acid esters and waxes from having an influence on the hydrocarbon measurements. The saturated hydrocarbons have responses per unit weight that range from 76 to 110% of that of *n*-nonadecane. In general, there is a tendency for the response to decrease with increasing chain length, but branching, judging from squalane and pristane, has no effect. Unsaturation, however, does decrease the response per unit weight. This decrease is not too pronounced with the mono-unsaturated compounds, heptadecene, nonadecene and docosene, but squalene, with six double bonds, has a response that is only 32% that of *n*-nonadecane, and carotene shows

no response at all. This means that this method will tend to underestimate the hydrocarbon content when there is a large proportion of unsaturated compounds present. If one assumes that 50% of the hydrocarbons present are unsaturated and that the unsaturated hydrocarbons have, on average, a response per unit weight that is 50% of that of *n*-nonadecane, the value determined will be 25% too low. This is naturally only a very rough estimate, but even under these extreme conditions, one can see that the underestimate is still at a tolerable level, when one considers the complexity of the problem of measuring hydrocarbons in the aquatic environment.

PROCEDURE

The sample consists of 1 litre of water, which must be taken with a sampling device that is scrupulously free from hydrocarbon material. When it is possible to take larger samples, it is better to do so. The sample is shaken for 5 min with 10 ml of Uvasol-grade TCF (E. Merck, Darmstadt, G.F.R.) and then allowed to stand for 10 min so as to permit the solvent to settle. It is then removed from the bottom of the shaking container with a pipette and transferred to a small test-tube, which is placed under vacuum in a desiccator. The desiccator is partially filled with water in order to take advantage of the relatively high specific heat of the liquid and by this means to prevent the temperature in the desiccator from becoming too low, which would retard the concentrating rate too much. This concentration step is the most time-consuming, but the extracts from a large number of samples can be placed in the desiccator at the same time, thus making this step considerably more efficient when several samples are being processed at the same time. When the extract in the test-tube has been concentrated to about 1 ml, the extract is transferred by means of an Eppendorfer pipette into a concentration tube, which is simply a small test-tube that has part of a sealed pipette with 10-μl gradiations fused on it so that the amount of extract that remains after concentration can be read with an accuracy of 5 μl. The extract is concentrated to 100 μl by means of a stream of nitrogen gas. The nitrogen used was the purest commercially available and was further purified by the use of a 1-nm molecular sieve. All glassware was pre-cleaned with dichromate–sulphuric acid in the laboratory and then wrapped in solvent-cleaned aluminium foil until required for use. The Eppendorfer pipette tips were pre-cleaned with solvent in the laboratory and disposed of after use.

The flow-rate of the solvent in the HPLC system is 5.2 ml/h and 50 μl of the concentrated extract is injected. As the standard, about 25 μg of *n*-nonadecane in a 50-μl volume are injected before the first sample and after every fifth sample. The peak on the recorder is complete after about 3 min, and 4 min after each injection, the next injection can be made. Every time the standard is injected, a blank consisting of 50 μl of solvent is also injected. Sometimes a peak results from the blank, presumably due to a pressure front, and the area of this peak is then subtracted from the peak areas of the standard and of the samples. The peak area of the sample is then divided by the peak area of the standard and multiplied by the weight of *n*-nonadecane that was injected in order to give the amount of hydrocarbons that were present in 500 ml of sample. The smallest amount of *n*-nonadecane that could readily be detected in a 50-μl injection was 2.0 μg. Several sub-samples from the

same water sample were extracted, in order to determine the standard deviation of the method, which was found to be ± 1.7 μg. Because the method, as used here, gives only the value for 500 ml, this value must be multiplied by 2, which means that the smallest amount of hydrocarbons that can be detected in 1 litre is 4.0 ± 3.4 μg. When larger amounts of sea water are extracted or when a larger percentage of the concentrated sample extract is injected into the system, these values can be considerably improved.

It is desirable to know to what extent the TCF extracts the hydrocarbons from the water, but there is no simple way of spiking a sample and maintaining natural conditions at the same time. In another study[5], it was determined that $87 \pm 8\%$ of the phenanthrene introduced into a sea water sample was recovered. Presumably the recovery of the more hydrophobic paraffins should be even better.

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