CONCENTRATION, SEPARATION AND DETERMINATION OF HYDRO-CARBONS IN SEA WATER

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SUMMARY

    A method for determining the hydrocarbons dissolved in sea water, including their concentration and separation into homogeneous classes, is described. Extraction with organic solvents, determination of the optimum volumes for *n*-hexane and carbon tetrachloride and concentration procedures for the extracts were investigated. Separation of hydrocarbons from polar compounds and their fractionation into five classes (aliphatic, monoaromatic and polynuclear with 2, 3–4 and 5–6 rings) were achieved using adsorption chromatography on a two-step microcolumn of silica gel and aluminium oxide. The overall recovery efficiency of the procedures and the detection limits of several hydrocarbons in 1 l of sea water are reported.

INTRODUCTION

    A concentration procedure must be used to determine hydrocarbons dissolved in sea water and the method must fulfill the conditions that the identities of the hydrocarbons should not be changed and contaminants in the extracting solvents or in the sorbents must not interfere.
    Two methods are generally used for concentrating the hydrocarbons from sea water: extraction with organic solvents or sorption on columns of macroreticular resins, activated charcoal and other materials. The disadvantages of the second method are different depending on the sorbent used; on activated charcoal the recovery is affected by incomplete elution of the adsorbed substances and by possible chemical changes[1-3]; on macroreticular resins, which are the most widely used, the recovery is affected by contaminants and/or by resin decomposition products, notwithstanding the numerous and tedious purification processes applied to the resins before their use[4-6]. The disadvantages associated with the use of macroreticular resins become negligible only if large volumes of water (50 l) are treated or if specific detection systems are available, as with halogeno derivatives.
    We therefore used a liquid–liquid extraction method. The aims of this work were (a) to obtain the maximum recovery of hydrocarbons from 1 l of sea water with the minimum volume of organic solvent, (b) to achieve the best separation of the hydrocarbons into homogeneous classes using microcolumns and eluent volumes not

exceeding 5–6 ml and (c) to employ a simple and inexpensive apparatus that could also be suitable for *in situ* determinations.

Most papers in the pertinent literature report only procedures for concentrating organic compounds from water[7–10]. The results achieved in separating hydrocarbons from other organic materials and from each other do not seem satisfactory, as the separations reported are incomplete or the procedures are too complex[11–15].

EXPERIMENTAL

Reagents and materials

The solvents used were *n*-hexane (pesticide grade) (Merck), carbon tetrachloride (analytical-reagent grade) (Merck), *n*-pentane (for spectroscopy) (Merck), dichloromethane (for HPLC) (Riedel de Haen) and methanol (for spectroscopy) (Merck). Carbon tetrachloride and *n*-hexane were further purified by passing them through a column of aluminium oxide activated at 700°C for 3 h.

As supporting materials silica gel 60 (70–230 mesh) and basic aluminium oxide E type (activity 1), both purchased from Merck, were used. Before use they were maintained at 130°C for 12 h.

Anhydrous sodium sulphate was treated at 400°C for 2 h to remove any organic matter.

The aqueous test solutions were prepared by addition of 5 μl of a standard hydrocarbon solution (1 mg/ml of each hydrocarbon in 1:1 dichloromethane–methanol) to 1 l of sea water. The sea water was previously freed from hydrocarbons and suspended materials by filtration on Millipore filters (0.45 nm) and by passing it through a C_{18} silanized silica gel (40–60 (μm) column.

Apparatus

The extractions were effected in glass separating funnels with PTFE caps and plugs. When using a solvent with a specific gravity lower than that of water, a separating funnel similar to that described by Grob *et al.*[16] was used. The extracts were concentrated with Kuderna–Danish microevaporators or under a controlled flow of nitrogen. The separation of hydrocarbons was carried out on a two-step microcolumn of silica gel and aluminium oxide.

We also employed a Carlo Erba Fractovap 4160 gas chromatograph equipped with a linear temperature programmer with flame-ionization detection (FID), coupled with a Spectra-Physics 4100 computing integrator. The glass capillary column used was coated with SE-52 (length 25 m, phase thickness 0.15 μm). The carrier gas was hydrogen at a linear velocity of 50 cm/sec. The temperatures were injection 50°C (on-column injection technique) and detector 320°C. The temperature programme was 50°C for 1 min, increased from 50 to 300°C at 8°C/min, 300°C for 10 min. Finally, we also used a Perkin-Elmer 3B liquid chromatograph equipped with an LC 75 spectrophotometric detector. The column used was a Perkin-Elmer C_{18} silanized silica column (25 × 0.46 cm I.D., particle size 10 μm). The operating conditions were as follows: flow-rate, 2 ml/min; room temperature; mobile phase, acetonitrile–water. Solvent program: First, 40% acetonitrile for 15 min, in the next 40 min the acetonitrile content is raised to 80%, and is kept there for another 10 min.

The hydrocarbons were determined by comparison of the peak areas on the chromatograms of the extracts with those on standard chromatograms.

RESULTS AND DISCUSSION

Fig. 1 shows the entire analytical scheme for the determination of the hydro-carbons dissolved in sea water. The scheme consists of three steps: (a) extraction with an organic solvent (*n*-hexane or carbon tetrachloride) and separation of the hydro-carbons from the ionizable substances; (b) concentration of the extracts; and (c) separation of the hydrocarbons from polar compounds and their fractionation into homogeneous classes.

Extraction with organic solvents
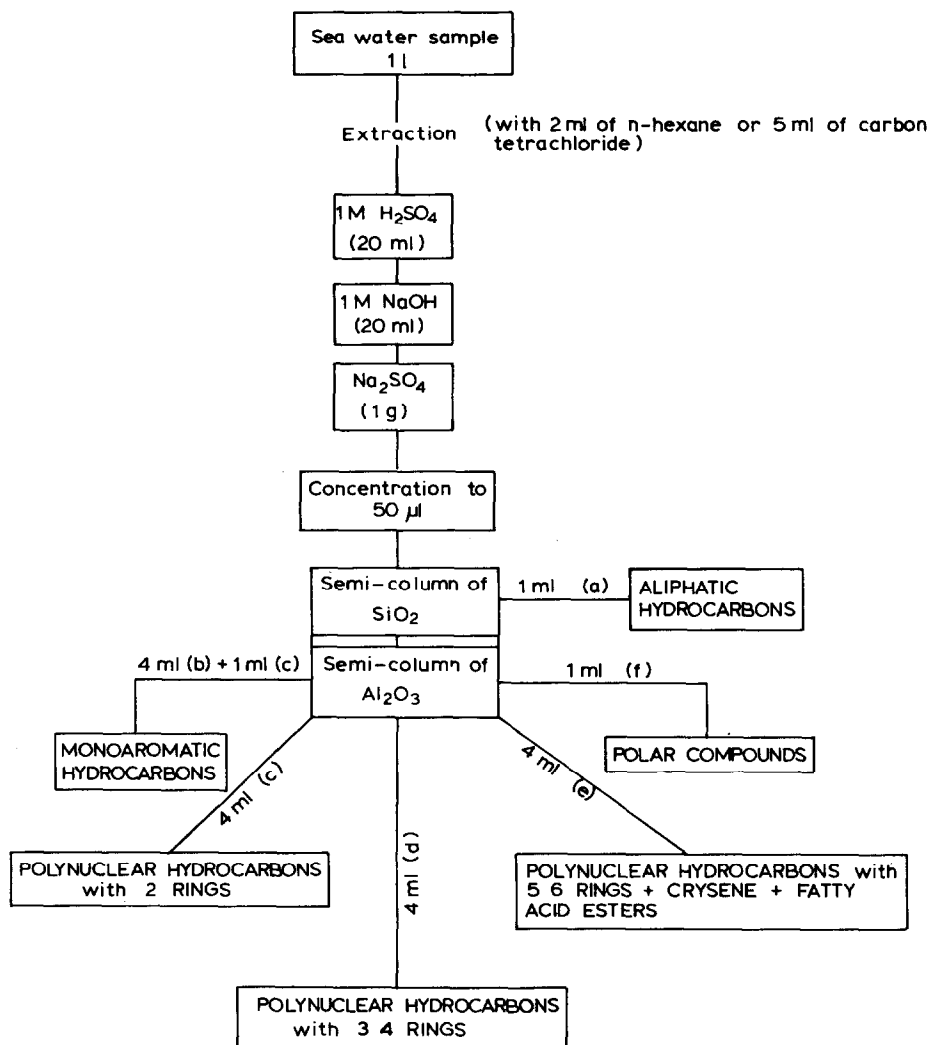   Of the organic solvents used in the extraction of the hydrocarbons, *n*-hexane

Fig. 1. Analytical scheme. For eluents (a)–(f), see Fig. 3.

and carbon tetrachloride exhibit the highest recovery efficiency[17,18]. Table I lists the recovery of a series of hydrocarbons (*n*-alkanes, monoaromatic, polynuclear) from 1 l of sea water with different volumes of *n*-hexane and carbon tetrachloride. The volumes are those obtained after one extraction with *n*-hexane and two successive extractions with carbon tetrachloride. Smaller *n*-hexane volumes (200 μl)[19] cannot

TABLE I

RECOVERY OF HYDROCARBONS FROM 1 l OF SEA WATER WITH *n*-HEXANE AND CARBON TETRACHLORIDE

The recoveries are average values from five determinations.

Hydrocarbon	Efficiency (recovery, %)				
	C_6H_{14}			CCl_4	
	1 ml	2 ml	5 ml	2 ml	5 ml
n-C_8	75	81	84	71	80
n-C_{10}	79	83	87	71	81
n-C_{12}	80	87	91	72	83
n-C_{14}	81	90	94	72	89
n-C_{16}	83	91	95	73	90
n-C_{18}	85	91	96	74	90
n-C_{20}	85	92	96	74	90
n-C_{22}	86	93	97	75	91
n-C_{24}	86	94	97	75	92
n-C_{28}	87	94	97	75	92
n-C_{32}	87	95	97	75	93
m-/*p*-Xylene	65	81	88	74	89
o-Xylene	63	79	88	73	89
Cumene	77	87	91	74	90
Propylbenzene	77	88	91	74	90
Mesitylene	79	89	92	75	90
p-Cymene	81	91	92	75	91
Butylbenzene	83	91	92	76	90
Hexylbenzene	83	92	92	75	88
Decylbenzene	83	94	94	73	88
Naphthalene	70	84	90	77	89
1-Methylnaphthalene	80	91	92	77	91
2-Methylnaphthalene	81	92	93	77	91
Biphenyl	79	91	93	75	90
Acenaphthene	77	88	92	71	88
Acenaphthylene	82	86	89	67	88
Fluorene	82	91	94	74	90
Phenanthrene	84	93	93	73	90
Anthracene	83	91	94	73	90
Fluoranthene	86	93	94	74	92
Pyrene	85	93	94	70	88
1-Methylpyrene	87	92	94	70	85
Benzo[*a*]pyrene	84	91	92	68	88
1,3,5-Triphenylbenzene	93	93	95	77	91
Dibenz[*a,h*]anthracene	86	93	94	73	85
Benzo[*ghi*]perylene	87	93	95	73	90

be used as they are not well separated from the sea water and therefore no reproducible results are obtained.

The recoveries in Table I are average values for five determinations. The standard deviations are between 2% and 5%. As regards the optimum solvent volume for the extraction of the hydrocarbons, the data in Table I suggest 2 or 5 ml of *n*-hexane and 5 ml of carbon tetrachloride.

Concentration of the extracts

In the case of hydrocarbons, the FID sensitivity is below the ng/μl level, so the concentration of the extracts must allow for the following: (1) the presence of contaminants in *n*-hexane and carbon tetrachloride must not produce a significant gas chromatographic response if the solvents are concentrated about 100-fold; (2) the loss of hydrocarbons during the evaporation step increases dramatically when the solvent volume is reduced below 50 μl in the case of *n*-alkanes, monoaromatic and polynuclear hydrocarbons with two rings; polynuclear hydrocarbons with more than two rings, in contrast, can be recovered in even smaller solvent volumes (10 μl). On this basis, 50 μl is regarded as the optimum final volume.

In order to reduce the volume of the extracts we used: (1) Kuderna–Danish (K–D) evaporators; (2) evaporation at 25°C under a stream of nitrogen (0.1 l/min). The size and the shape of the apparatus are important when using the second method. We tested experimentally that a conical shape allows a larger recovery than a cylindrical shape. Moreover, by using different conical vessels, we found that the loss during the evaporation was largely influenced by the size of the tube. According to our experiments the best dimensions of the evaporation vessel are height, 9 cm; higher inner diameter, 1.6 cm; and inner diameter corresponding to a volume of 0.05 ml, 0.3 cm. The optimum evaporation rate at 25°C varies from 0.1 to 0.2 ml/min, depending on the solvents.

The K–D evaporators are very suitable with volatile solvents such as diethyl ether and *n*-pentane, but exhibit some disadvantages if used with carbon tetrachloride (b.p. = 76.5°C). In fact, in this instance a very long time (about 4–5 h) is required for the concentration of the extracts and the hydrocarbons may decompose during the evaporation step. Further, the dead volume of the K–D evaporators is about 0.3 ml[4] and the extracts must be subsequently concentrated under a stream of nitrogen to the final solution volume (50 μl).

Table II lists the recovery efficiencies of a series of hydrocarbons after concentration of *n*-hexane extracts from starting volumes of 2 and 5 ml to the final volume of 50 μl with K–D evaporators and with the above-described apparatus. The standard deviations of the values in Table II are usually between 1.5 and 3.5% for both concentration systems. Exceptions are the aliphatic and monoaromatic hydrocarbons with the lowest molecular weight, for which standard deviations of about 5% are obtained with K–D evaporators.

The data show that the two systems give rise to similar results and indicate that the optimum solvent volume for the extraction of the hydrocarbons from 1 l of sea water is 2 ml of *n*-hexane, as the lower recovery compared with a volume of 5 ml is compensated for by a cleaner blank. With carbon tetrachloride, for which values similar to *n*-hexane are obtained by evaporation at 25°C under a stream of nitrogen, the optimum solvent volume is 5 ml.

TABLE II

RECOVERY OF HYDROCARBONS IN 2 OR 5 ml OF *n*-HEXANE AFTER CONCENTRATION TO 0.05 ml

The recoveries are average values from five determinations.

| Hydrocarbon	Efficiency (recovery, %)			
	2 ml		5 ml	
	K–D	N_2	K–D	N_2
n-C_8	62	62	57	55
n-C_{10}	78	82	76	76
n-C_{12}	83	87	83	85
n-C_{14}	89	88	89	87
n-C_{16}	97	90	95	88
n-C_{18}	99	97	99	96
n-C_{20}	100	100	100	99
n-C_{22}	100	100	100	99
n-C_{24}	100	100	100	100
n-C_{28}	100	100	100	100
n-C_{32}	100	100	100	100
m-/p-Xylene	62	62	55	54
o-Xylene	64	64	57	57
Cumene	69	71	63	64
Propylbenzene	72	74	66	67
Mesitylene	75	77	69	71
p-Cymene	77	81	72	74
Butylbenzene	79	83	75	77
Hexylbenzene	84	87	83	86
Decylbenzene	95	94	95	94
Naphthalene	81	84	80	82
1-Methylnaphthalene	84	86	83	85
2-Methylnaphthalene	84	87	85	86
Biphenyl	87	88	86	87
Acenaphthene	88	88	88	88
Acenaphthylene	89	89	89	89
Fluorene	92	91	91	91
Phenanthrene	97	96	97	96
Anthracene	98	99	96	97
Fluoranthene	100	100	100	99
Pyrene	100	99	100	100
1-Methylpyrene	100	100	100	100
Chrysene	100	100	100	100
Benzo[*a*]pyrene	100	100	100	100
1,3,5-Triphenylbenzene	100	100	100	100
Dibenz[*a,h*]anthracene	100	100	100	100
Benzo[*ghi*]perylene	100	100	100	100
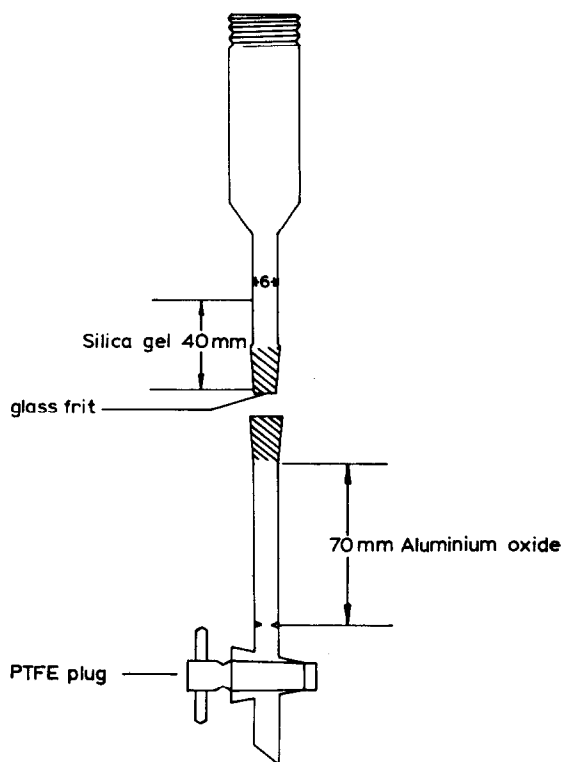
Fig. 2. Silica gel–aluminium oxide two-step microcolumn.

The results indicate that the procedure is reliable and the method can be extended to the analysis of unknown hydrocarbons dissolved in sea water.

Separation of the hydrocarbons into homogeneous classes

The hydrocarbons were fractionated into five classes (aliphatic, monoaromatic and polynuclear with 2, 3–4 and 5–6 rings) and separated from the polar compounds using sorption chromatography on a two-step microcolumn of silica gel and aluminium oxide. The microcolumn (see Fig. 2) is formed by a first column of silica gel and by a second of aluminium oxide, separated by a glass frit. A standard mixture of hydrocarbons, fatty acid esters, phthalic acid diesters with 1–12 carbon atoms and other polar compounds (alcohols, ketones, aldehydes, phenols and chlorinated phenols) is eluted on the silica gel column with 1 ml of *n*-pentane; in this way the aliphatic hydrocarbons (including *n*-alkanes with more than 30 carbon atoms) are separated from all the other compounds (see the histogram for alkanes in Fig. 3). It should be noted that the highest molecular weight alkanes cannot be eluted from a mixed column of silica gel and aluminium oxide using *n*-pentane, as such compounds are strongly adsorbed on aluminium oxide[20].

By combining the two columns and eluting with solvent mixtures of increasing polarity, fractionation of the aromatic hydrocarbons into four classes and their sep-
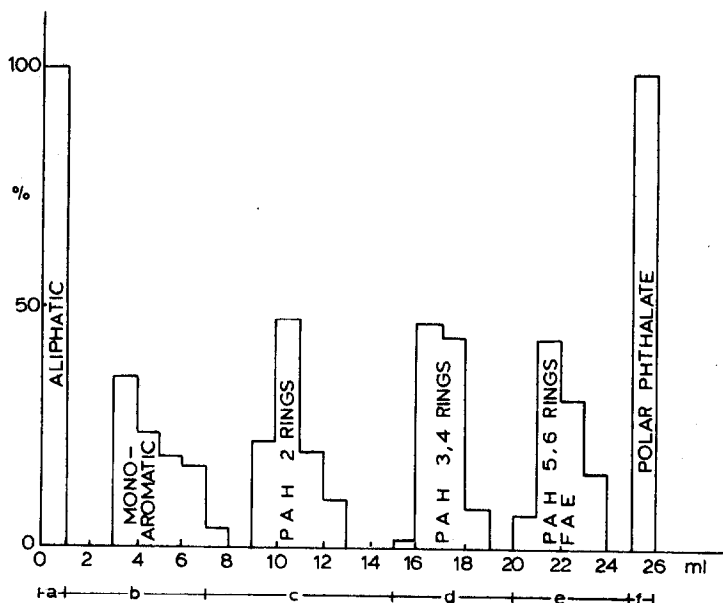
Fig. 3. Histograms relative to the two-step microcolumn elution with eluents of increasing polarity: (a) *n*-pentane; (b) *n*-pentane–carbon tetrachloride (8:2); (c) *n*-pentane–dichloromethane (9:1); (d) *n*-pentane–dichloromethane (7:3); (e) *n*-pentane–dichloromethane (2:8); (f) methanol. FAE = fatty acids esters.

aration from the polar compounds and from the phthalic acid diesters are obtained (see Fig. 3). The exception is chrysene, which is eluted with the polynuclear hydrocarbons with 5–6 rings. These last compounds cannot be separated from the fatty acid esters. However, the polynuclear hydrocarbons with 5–6 rings can be determined using high-performance liquid chromatography (HPLC), as the fatty acid esters do not adsorb in the same UV region. The fatty acid esters can be removed from the eluate by saponification with alcoholic potassium hydroxide[21].

Final recovery of the hydrocarbons

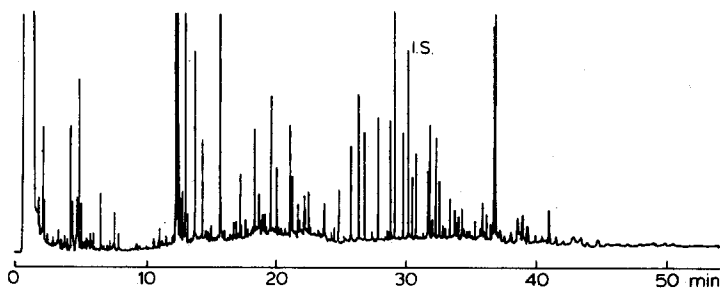   In addition to the extraction of the hydrocarbons from sea water, also the

Fig. 4. Gas chromatography of 2 ml of *n*-hexane extract of 1 l of S. Rossore water concentrated to 50 μl. I.S. = internal standard (0.5 μg of 1,3,5-triphenylbenzene) added to the water sample.

TABLE III

FINAL RECOVERY OF HYDROCARBONS FROM SEA WATER AFTER THEIR FRACTIONATION INTO HOMOGENEOUS CLASSES

The recoveries are the average values from five determinations.

Hydrocarbon	Efficiency (recovery, %)	Detection limit (ng/l)
n-C$_8$	28	36
n-C$_{10}$	46	20
n-C$_{12}$	57	18
n-C$_{14}$	70	14
n-C$_{16}$	80	12
n-C$_{18}$	84	12
n-C$_{20}$	83	12
n-C$_{22}$	85	12
n-C$_{24}$	85	12
n-C$_{28}$	85	12
n-C$_{32}$	86	12
m-/p-Xylene	26	40
o-Xylene	28	36
Cumene	38	26
Propylbenzene	41	24
Mesitylene	45	22
p-Cymene	45	22
Butylbenzene	47	22
Hexylbenzene	50	20
Decylbenzene	61	16
Naphthalene	47	40
1-Methylnaphthalene	50	38
2-Methylnaphthalene	50	38
Biphenyl	52	38
Acenaphthene	55	36
Acenaphthylene	58	34
Fluorene	76	26
Phenanthrene	84	24
Anthracene	76	26
Fluoranthene	83	24
Pyrene	84	24
1-Methylpyrene	82	24
1,3,5-Triphenylbenzene	85	24
Benzo[a]pyrene	44	45
Dibenz[a,h]anthracene	77	26
Benzo[ghi]perylene	77	26

successive steps (treatment of the organic extract with sulphuric acid, sodium hydroxide and sodium sulphate, evaporation to 50 μl, separation on column of silica gel and aluminium oxide and evaporation of the eluates to 50 μl) involve some losses. In order to evaluate such losses, we dissolved a standard solution of hydrocarbons in 1 l of sea water and applied the analytical procedure in Fig. 1 using n-hexane (2
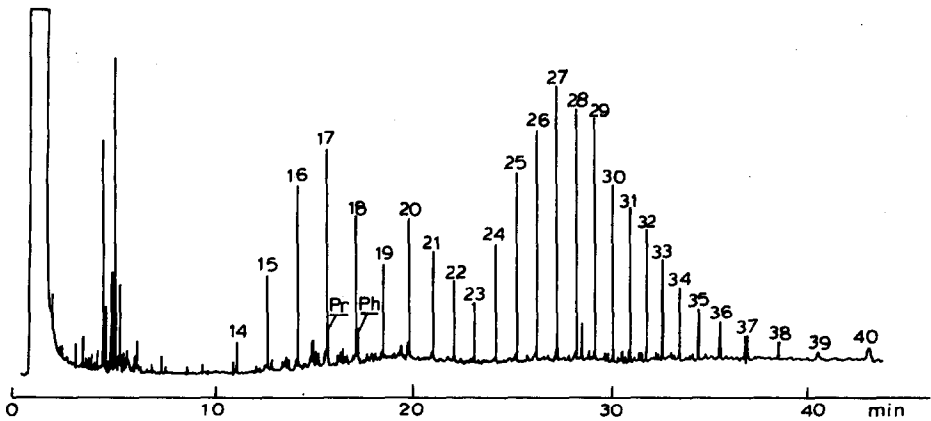
Fig. 5. Gas chromatogram of the aliphatic fraction. Numbers indicate the number of carbon atoms of *n*-alkanes. Pr = pristane; Ph = phytane.

ml) as the extracting solvent and evaporating the extracts under a stream of nitrogen. The final recovery of hydrocarbons is reported in Table III.

The greatest efficiency is observed with the highest homologues of the alkanes and with polynuclear hydrocarbons with more than two rings. Table III also gives the detection limits of the different hydrocarbons in 1 liter of sea water, calculated on the basis of the gas chromatograph sensitivity and of the percentage recovery.
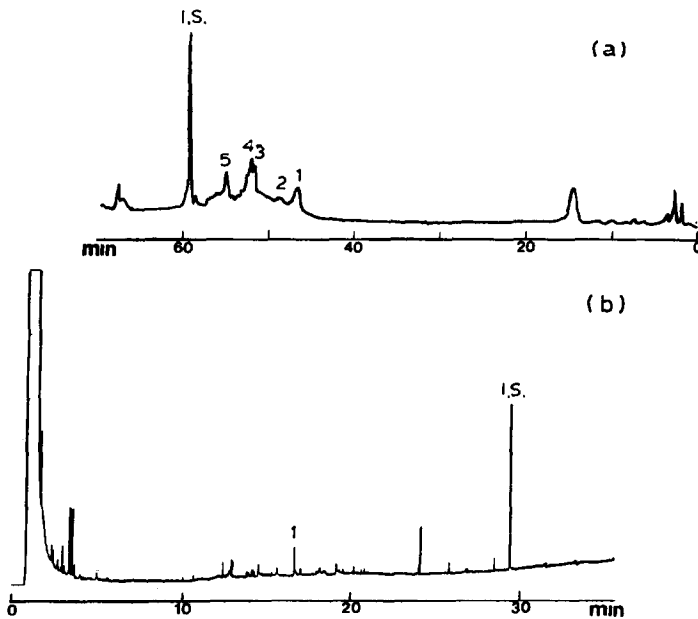

Fig. 6. Gas chromatogram of PAH fraction with 3–4 rings. I.S. = internal standard (1,3,5-triphenylbenzene). 1 = Phenanthrene. (b) Liquid chromatogram of PAH fraction with 3–4 rings. Analytical wavelength = 235 nm. Volume injected = 0.01 ml. 1 = Phenanthrene; 2 = anthracene; 3 = fluoranthene; 4 = pyrene; 5 = 1-methylpyrene. I.S. = internal standard (1,3,5-triphenylbenzene).
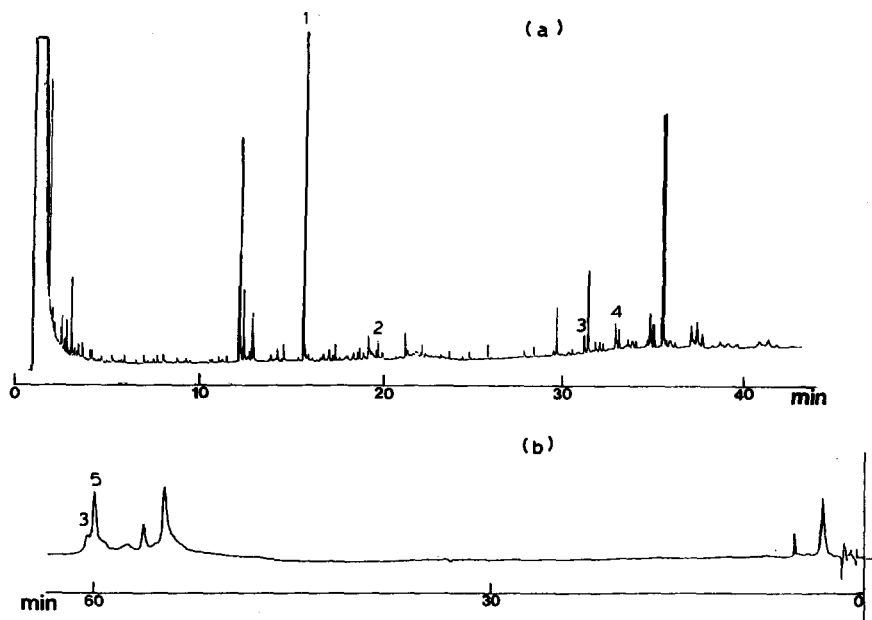
Fig. 7. (a) Gas chromatogram of PAH fraction with 5–6 rings. 1 = Methyl tetradecanoate; 2 = isopropyl palmitate; 3 = dibenz[a,h]anthracene; 4 = hexadecylpalmitate. (b) Liquid chromatogram of PAH fraction with 5–6 rings. Analytical wavelength = 295 nm. Volume injected = 0.01 ml. 3 = Dibenz[a,h]anthracene; 5 = benzo[ghi]perylene.

Analysis of sea water samples

The method has been applied to many samples from the Ligurian and High Tyrrhenian seas with satisfactory results. As an example, we report the data from sea water sampled at a depth of 0.5 m and 200 m from the coast facing S. Rossore (High Tyrrhenian Sea), which is a polluted area. The gas chromatograms in Figs. 4–7 demonstrate the applicability of the apparatus and procedure.

Fig. 5 shows a typical pattern of gas chromatograms of a mixture of n-alkanes. The n-alkanes were identified by comparing the retention times of the peaks with those of standard solutions. The important markers pristane (Pr) and phytane (Ph)[12,22,23] were also determined.

Small concentrations of monoaromatic compounds and polynuclear hydrocarbons with two rings were found in the sample from the High Thyrrenian Sea and in the other samples from the Ligurian Sea.

Fig. 6 shows (a) the gas and (b) the liquid chromatograms for the fraction of polynuclear aromatic hydrocarbons (PAHs) with 3–4 rings. The optimum volume of solution that must be injected for HPLC (0.01 ml) was obtained by evaporation of the 0.05-ml fraction of PAHs. The use of this technique allows the identification of phenanthrene, anthracene, fluoranthene, pyrene and 1-methylpyrene at levels lower than those reported in Table III. The identification of the hydrocarbons by HPLC was performed by using retention times and UV spectra.

Fig. 7 shows (a) the gas and (b) the liquid chromatograms of the PAH fraction with 5–6 rings. Gas chromatography allows the identification of methyl tetradeca-

noate, isopropyl palmitate and hexadecyl palmitate, while dibenz[*a,h*]anthracene and benzo[*ghi*]perylene were determined by HPLC.

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