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Note

Sephadex LH-20 chromatography of extracts of marine sediment and biological samples for the isolation of polynuclear aromatic hydrocarbons

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Determination of hydrocarbons in environmental samples customarily requires at least three basic steps: (a) extraction of the components from the sample matrix, (b) isolation of compounds of interest from naturally occurring interfering compounds by chromatography of the extract, and (c) analysis of the isolated extract to detect and measure the components[1–4]. To permit trace-level detection of hydrocarbons in sample extracts by current highly sensitive gas chromatographic (GC) techniques, extremely efficient isolation procedures are needed[5,6].

Several procedures for separating hydrocarbons from interfering compounds have been reported. Gel permeation–adsorption chromatography, using Sephadex LH-20 with a low-boiling alcohol or solvent, has been utilized to separate polynuclear aromatic hydrocarbons (PAHs) from lipids and pigments[7,8]. Silica gel chromatography[9] has been used to isolate aromatic hydrocarbons in marine substrates, but this procedure alone provided inadequate sample clean-up for analysis by current GC techniques. Giger and Blumer[10] reported a two-step procedure, using silica gel–alumina chromatography followed by Sephadex LH-20 chromatography (using benzene–methanol, 1:1), to improve the isolation of PAHs from soil and sediment extracts. However, recent data concerning the carcinogenicity of benzene[11,12] make substitution of a safer solvent system desirable.

We report a two-step procedure that combines silica gel chromatography and Sephadex LH-20 chromatography using safer, contaminant-free solvent systems to isolate PAHs from extracts of sediments and tissues from the marine environment.

EXPERIMENTAL**

Preparation of solvents

Commercial, distilled-in-glass solvents (Burdick & Jackson Labs., Muskegon, MI. U.S.A.; J. T. Baker, Phillipsburg, NJ, U.S.A.) were purified prior to column

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chromatography by redistillation in the azeotropic ratios in which they were to be used: cyclohexane–methanol (3:2) and cyclohexane–isopropanol (2:1). The distillation of azeotropic mixtures resulted in a higher degree of solvent purity than was possible by distillation of the individual solvents. Cyclohexane and methanol are not miscible at room temperature, therefore a small portion of dichloromethane was added to dissolve both solvents into a single phase, resulting in a mixture of cyclohexane–methanol–dichloromethane (6:4:3). In addition, the dichloromethane increases the solubility of sample extract components in the solvent mixture.

Extraction and hydrocarbon fractionation

Sediment samples were extracted with methanol, then with dichloromethane–methanol[7,13]. Biological samples were digested with caustic solution (4 N NaOH), then extracted with diethyl ether[+]. The extracts were filtered through a short column packed with granular copper and silica gel to remove elemental sulfur and many interfering polar compounds, including some lipids. The filtrate was concentrated and hexane was added; then the filtrate was concentrated again and transferred to another silica gel column for separation into saturated hydrocarbon fractions[7,13].

Rechromatography of unsaturated fraction

Sephadex LH-20 gel (25–100 μm/size; Sigma, St. Louis, MO, U.S.A.) was prepared for column chromatography by swelling ca. 20 g overnight in the elution solvent: I, cyclohexane–methanol–dichloromethane (6:4:3), or II, cyclohexane–isopropanol (2:1). The gel was then poured into 300 × 19 mm I.D. columns. These columns were calibrated by eluting a mixture of azulene and perylene of sufficient concentration to be visible under UV light. If there was a distinct separation between azulene and perylene, the columns were further characterized by eluting a sample extract spiked with PAH standard. PAHs eluted from the gel in the 40–100-ml fraction using solvent I and in the 40–140-ml fraction with solvent II. The unsaturated fraction from the silica gel separation of the sample extracts was then rechromatographed on each of the Sephadex LH-20 columns to isolate the PAHs. Eluates were concentrated to ca. 1 ml and the solvents exchanged with hexane.

Gas chromatography and mass spectrometry

Portions (2 μl) of the unsaturated hydrocarbon and PAH fractions were each injected, splitless, into a Hewlett-Parkard 5840A gas chromatograph equipped with a flame-ionization detector and fused-silica capillary column coated with SE-54 (30 m × 0.25 mm; J & W Scientific, Orangevale, CA, U.S.A.). Helium carrier was adjusted to a linear velocity of ca. 28 cm/sec at 150°C; split ratio was 20:1. Injections were made at 50°C, and column temperature was programmed from 50–280°C at 4°/min. GC–mass spectrometry (MS) was performed using an identical GC system interfaced with a Finnigan 3200 mass spectrometer used with an Incos 2300 data system.

RESULTS

Following calibration of the Sephadex LH-20 columns using solvent I or II, recoveries of the added standards were determined (Table I). In addition, the extract from a highly contaminated environmental sample was analyzed by GC–MS before

TABLE I

RECOVERY OF PAHs FROM COLUMN CHROMATOGRAPHY ON SEPHADEX LH-20

PAH	Amount added (µg)	Solvent I		Solvent II	
		Amount recovered (µg)	Recovery (%)	Amount recovered (µg)	Recovery (%)
Naphthalene	5.45	5.07	93	5.12	94
2-Methylnaphthalene	5.00	4.60	92	4.59	92
Biphenyl	5.75	5.23	91	5.25	91
Phenanthrene	6.10	5.98	98	5.67	93
1-Methylphenanthrene	5.20	5.01	97	5.04	97
Fluoranthene	5.85	5.56	95	5.55	95
Chrysene	7.50	7.48	100	7.41	99
Benzo[e]pyrene	5.40	5.24	97	4.72	87
			$\bar{x} = 95$		$\bar{x} = 93.5$

and after Sephadex LH-20 column chromatography (solvent I). PAH percent recoveries for the environmental sample, determined by comparison of concentrations before and after Sephadex, were similar to those for the added standards. Elution volumes of several PAHs on a Sephadex LH-20 column using solvent I are given in Fig. 1.
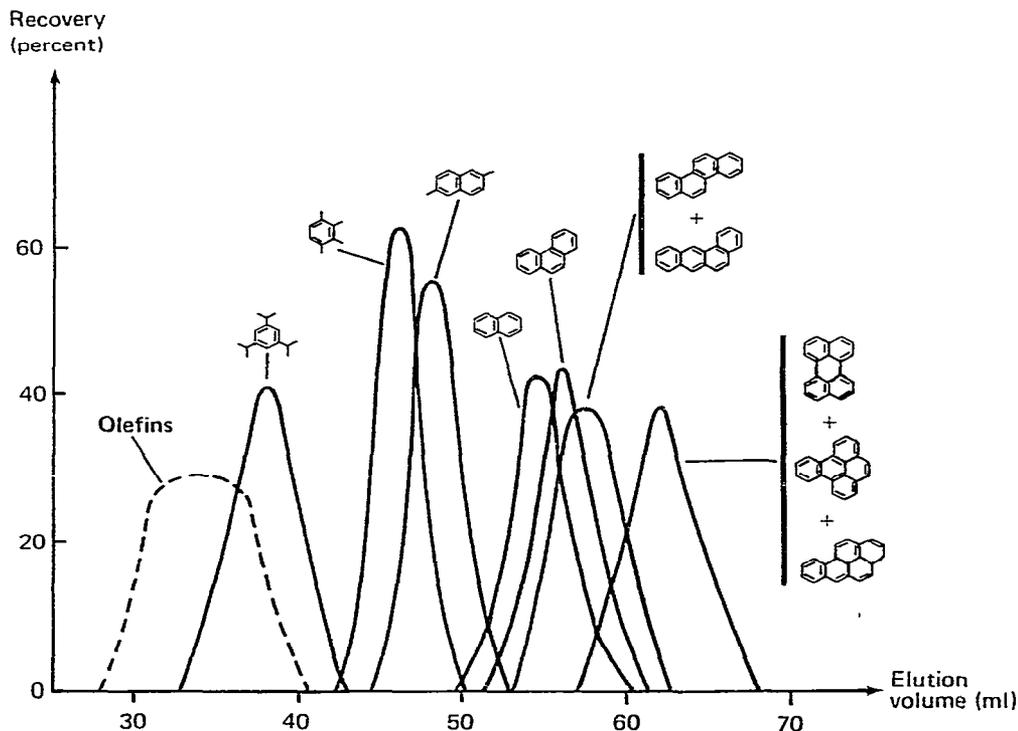

Fig. 1. Elution pattern of selected aromatic hydrocarbons on Sephadex LH-20 using cyclohexane–methanol–dichloromethane (6:4:3).

GC analysis of the unsaturated hydrocarbon fraction of both a sediment extract (Fig. 2a) and a crab hepatopancreas extract (Fig. 3a) following silica gel chromatography revealed a large number of compounds that coelute with PAHs and interfere with their determination by GC. These compounds were shown by GC–MS analysis to be large polyunsaturated aliphatic hydrocarbons. The interfering compounds were removed by chromatography of this fraction on a Sephadex LH-20 column, as shown in Figs. 2b and 3b.
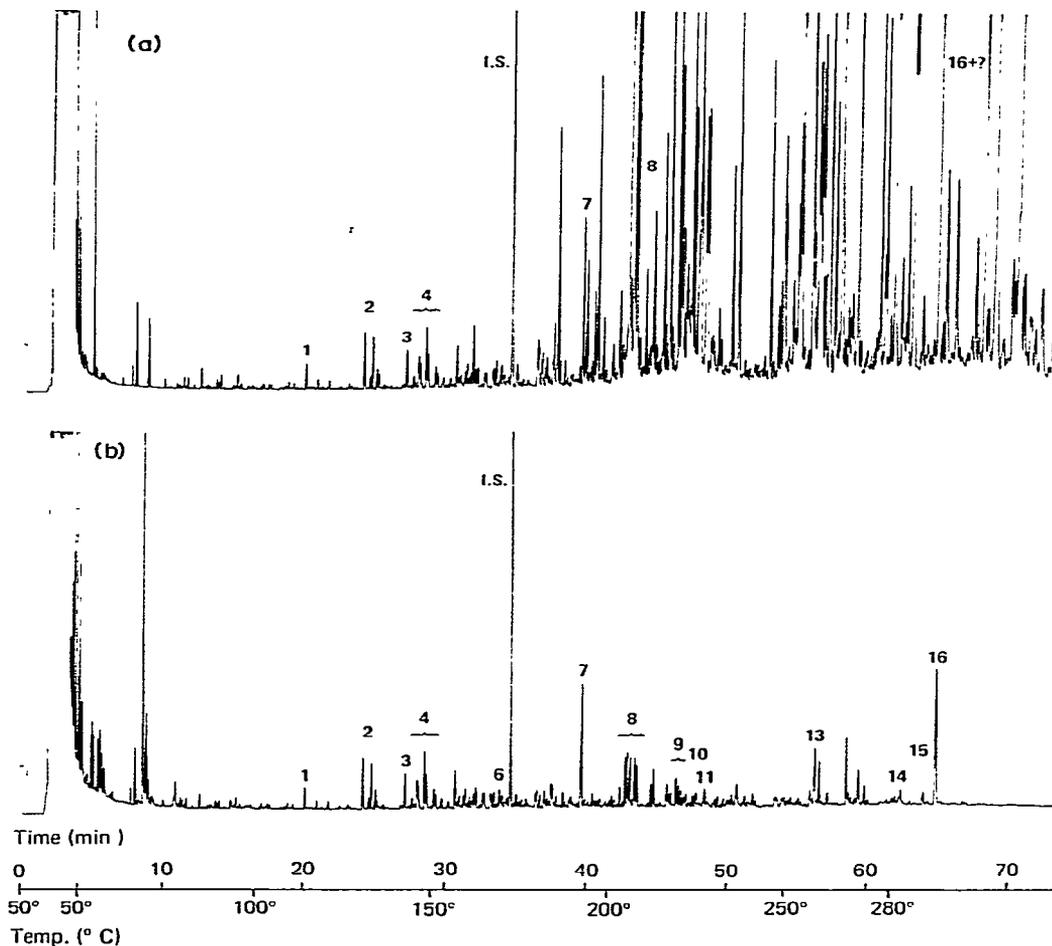

Fig. 2. Gas chromatogram of an intertidal sediment extract following chromatographic clean-up with (a) silica gel, and (b) silica gel, followed by Sephadex LH-20 using cyclohexane–methanol–dichloromethane (6:4:3). See text for GC parameters. Peak identities were verified by GC–MS. Labelled peaks are: I.S. = internal standard; 1 = naphthalene; 2 = methylnaphthalenes; 3 = biphenyl; 4 = dimethylnaphthalenes; 6 = fluorene; 7 = phenanthrene; 8 = methylphenanthrenes; 9 = dimethylphenanthrenes; 10 = fluoranthene; 11 = pyrene; 13 = chrysene; 14 = benzo[e]pyrene; 15 = benzo[a]pyrene; 16 = perylene.
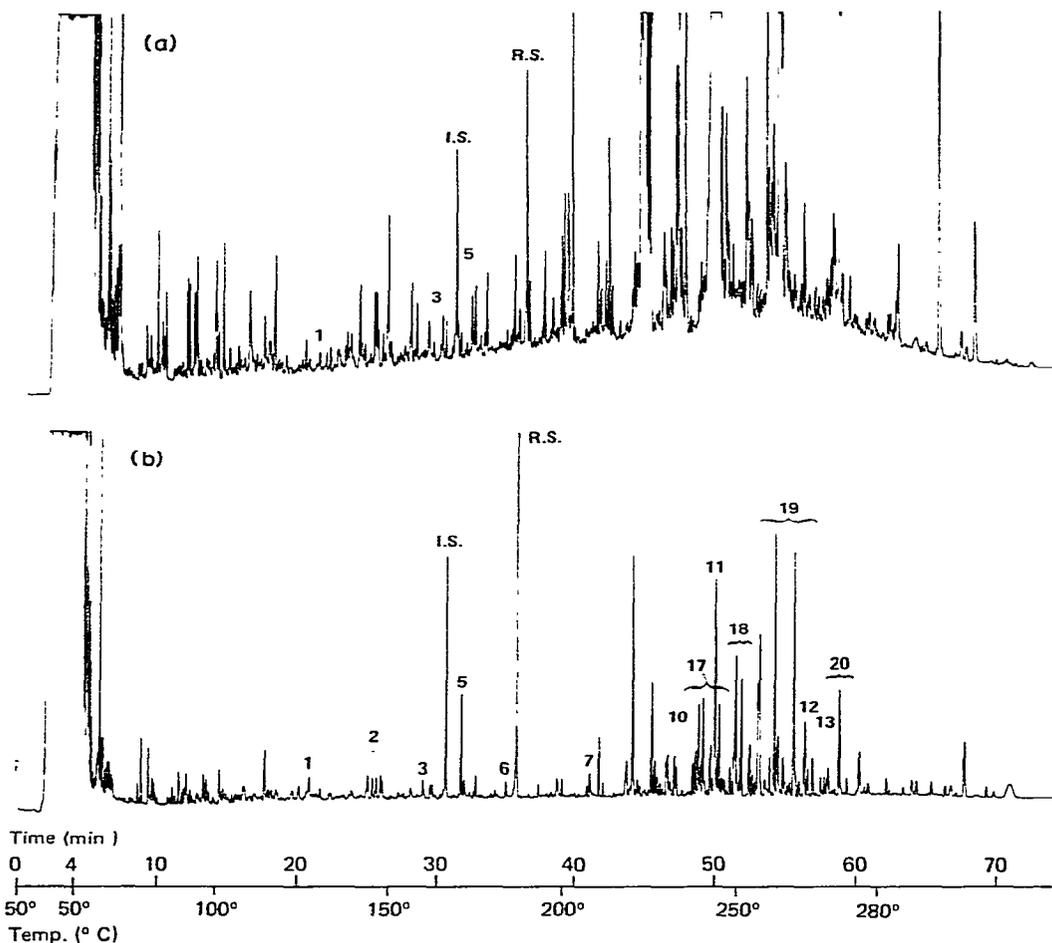
Fig. 3. Gas chromatogram of a crab (*Cancer gracilis*) hepatopancreas extract. See Fig. 2 for analytical conditions. Additional labelled peaks are: R.S. = recovery standard; 5 = acenaphthene; 12 = benz[*a*]anthracene; 17 = Cl$_4$-polychlorinated biphenyls (PCBs); 18 = Cl$_5$-PCBs; 19 = Cl$_6$- and Cl$_7$-PCBs; 20 = Cl$_8$-PCB.

DISCUSSION

Previous isolation methods have either failed to separate adequately the PAH fraction from interfering substances or have used benzene which is both carcinogenic and difficult to purify. Our two-step procedure replaced benzene with contaminant-free azeotropic solvent mixtures while achieving the desired isolation of PAHs. A comparison of the chromatograms of a sediment extract (Fig. 2a) and a crab hepato-pancreas extract (Fig. 3a) taken before Sephadex LH-20 chromatography with those taken after clean-up (Figs. 2b and 3b) shows the virtually complete removal of inter-fering compounds from the aromatic hydrocarbon fraction. In addition, this method gave high PAH recoveries (Table I) and eliminated the need for MS techniques to

separate target compounds from coeluting substances before quantitations could be made.

The cut-off point between the olefinic fraction and the PAH fraction was fairly critical with solvent I, as shown in Fig. 1. Although certain alkylated benzenes (*e.g.*, triisopropylbenzene) may not totally separate from the olefins, most PAHs were, however, isolated from interfering compounds. Solvent II separated PAHs from interfering compounds better than solvent I (*e.g.*, no elution overlap between olefins and triisopropylbenzene), so that the fraction end-point was not as critical. However, solvent II eluted PAHs only half as fast as solvent I under the same pressure, and 50 % more solvent was required. Also, the boiling point for solvent II is 15°C higher than that for solvent I, increasing the possibility of evaporative losses or oxidative changes of components during concentration steps. Solvent I was preferred for routine analyses because of its speed of elution and low boiling point.

In summary, our two-step isolation procedure separated PAHs in marine samples from interfering compounds with good recoveries of target compounds. The relatively simple gas chromatograms of PAH fractions isolated by this procedure allowed direct quantitation. Finally, to improve the safety of the procedure, the carcinogenic solvent, benzene, was replaced with safer, contaminant-free solvent systems.

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