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APPLICATION OF STEAM DISTILLATION IN THE DETERMINATION OF PETROLEUM HYDROCARBONS IN WATER AND MUSSELS (*Mytilus edulis*) FROM DOSING EXPERIMENTS WITH CRUDE OIL

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SUMMARY

Steam distillation is shown to provide recoveries in excess of 80% for petroleum hydrocarbons in the volatility range encompassed by toluene and pyrene from water and mussel tissues. These recoveries were achieved with an apparatus based on Dean and Stark water estimators which are commercially available at low cost. Saponification is shown to aid hydrocarbon recovery from mussel tissue. The steam distillates derived from tissues were analysed by u.v. spectrophotometry after clean-up on alumina, or directly by gas-liquid chromatography or normal- and reverse-phase high-performance liquid chromatography (h.p.l.c.). Steam distillates of water did not require prior clean-up. Normal-phase h.p.l.c. of steam distillates on an amino-cyano phase provided a particularly convenient method for petroleum-derived aromatic hydrocarbons. These techniques were examined extensively in laboratory experiments with crude oil, but preliminary results suggest that they may be used also in environmental monitoring of hydrocarbons.

The objective of these investigations was to develop a procedure for determining some of the major components of crude oil known to be important in oil spills and chronic oil pollution, with emphasis on the particularly toxic aromatic compounds. The extraction procedure should permit rapid screening of numerous samples without excluding the possibility of subsequent detailed examination of the extracts. The techniques most commonly used for the extraction of hydrocarbons tend to be complex, give poor recoveries of alkylbenzenes and some naphthalenes, and perform badly in interlaboratory comparisons [1—3].

Steam distillation has been used successfully to extract hydrocarbons from water, sediment and biota [4—13] and forms the basis of the proposed method.

EXPERIMENTAL

Apparatus

The apparatus comprised a 2-l round-bottom flask fitted with a 12.5-ml capacity Dean and Stark water estimator (for heavy entrainers) the riser part

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of which was wrapped in aluminium foil for insulation, and a Liebig condenser of 50-cm overall length; all items were Quickfit products (Fig. 1). The extraction systems were heated on a six-place electric mantle. Cooling water at 16 ± 2°C was passed through the six condensers linked in series at a flow rate of 1.7 l min^{-1}.

Extraction of hydrocarbons from water

The water was placed in the round-bottom flask followed by extracting solvent (4—10 ml) and boiling chips and then the apparatus was assembled as shown in Fig. 1. Suitable distillation rates were within the range 0.9—1.4 ml min^{-1} for the water condensing, but for each batch of extractions the distillation rates in each of the six apparatus were adjusted to within 0.08 ml min^{-1} of each other. At the end of the distillation period, the recovered solvent was removed from the water estimator which was then rinsed with <1 ml of solvent to recover residual traces of hydrocarbon. Extracts were stored at −17°C.

The efficiency of extraction of crude oil components from water by steam distillation was compared with that of direct extraction by extracting 20-ml samples of the water-accommodated fraction (w.a.f.) of North Sea crude oil [14], or w.a.f. dilutions, with three successive 3.3-ml aliquots of 2,2,4-trimethylpentane (TMP), or by extracting 1-l samples of oil-contaminated aquarium water with four successive 5-ml aliquots of TMP (see Table 2).

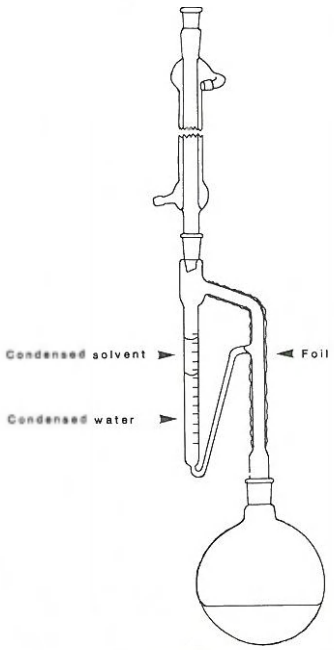

Fig. 1. Steam-distillation apparatus.

Extraction of hydrocarbons from tissues

Homogenised wet tissue, usually not more than 20 g, was added to the round-bottomed flask which already contained boiling chips, 100 ml of distilled water and organic solvent (4—10 ml). Then 10 ml of sodium hydroxide solution (4 M) was added, followed by water to a total volume of 400 ml. The apparatus was then heated sufficiently to maintain the temperature of the aqueous homogenate at 80 ± 5°C for 2 h, after which time 50 ml of 0.68 M hydrochloric acid was added by pouring down the condenser, followed by a rinse of 50 ml of distilled water. The acid addition should reduce the pH of the homogenate to between 7.5 and 11; acidification increased the recovery of non-petroleum u.v.-absorbing material.

Distillation was then continued and the distillate recovered as described above. For many aromatic hydrocarbons, distillation rates of 0.9—1.4 ml min^{-1} for water condensing proved satisfactory, but the recovery of chrysene and involatile alkanes, the latter prior to gas-liquid chromatography (g.l.c.), was improved by higher rates.

Spiking experiments. Standard compounds were dissolved in acetone and spike volumes of 100 or 200 µl of solution were added directly to water in the distillation flask before solvent addition, or to the tissue homogenate before transfer to the flask.

Treatment of distillates

Gas-liquid chromatography. Distillates, concentrated by evaporation if required, could be injected directly. G.l.c. was conducted as described elsewhere [14].

For routine g.l.c. of petroleum-contaminated mussel tissue, the homogenate, plus internal standards [14], was saponified and distilled along with 4 ml of hexane at a distillation rate of approximately 1 ml min^{-1} for water condensing. The distillation was stopped after 2 h, the hexane removed, 5 ml of hexane/cyclohexane (4:1, v/v) added to the cooled distillation flask, and the distillation was then continued for a further 14 h at a rate of 1.7—2.0 ml min^{-1} for water condensing to recover involatile alkanes. Benzenes with three or fewer alkyl carbons were measured directly in the first distillate, and then the distillates were fractionated on 2.5-g, 4% water-deactivated silica columns [15]. Alkanes were recovered in 7 ml of pentane and aromatics in 10 ml of 10% (v/v) diethyl ether in pentane.

U.v. spectrophotometry. Distillations were done with either hexane or TMP (h.p.l.c. grade). Distillates of aquarium water were measured directly. The u.v. spectrum was recorded in the range 200—350 nm.

For u.v. spectrophotometric determination of oil in mussels, a maximum wet weight of 6 g of tissue was used, and the maximum distillation time, after a preliminary 2-h saponification, was 2 h. Biogenic interferences were removed by chromatography on 2-g alumina columns [16]. Distillate (10 ml) previously dried over anhydrous sodium sulphate was loaded onto each column and the column was eluted with the same solvent as used for the

distillation until 15 ml was recovered. Alternatively, 5 ml of distillate was run onto the column, and elution was continued until 10 ml was collected.

The u.v. spectrum of the product, diluted if necessary to give an absorbance of less than 1 in the 220—225 nm region, was then recorded against an appropriate solvent blank.

High-performance liquid chromatography (h.p.l.c.). Both normal-phase and reverse-phase systems were used. Tissue weights distilled ranged from 1 to 6 g; extracting solvent was generally hexane (5 ml).

The normal-phase system comprised a Shandon column (250 mm long, 5 mm bore) and precolumn, packed with Partisil-10 PAC (polar amino-cyano; Whatman). The mobile phase was hexane at a flow rate of 2 ml min^{-1}. Eluting aromatic hydrocarbons were detected by means of a Perkin-Elmer LC-75 u.v. spectrophotometric detector with wavelength settings of 225 nm or 254 nm and sensitivity set at 0.01 to 0.04 absorbance for f.s.d. At least 200 injections of distillate could be made into the PAC system without noticeable deterioration of column performance. Procedures for regenerating contaminated columns have been devised [17—19].

For reverse-phase h.p.l.c., a column of the same dimensions as that described above was packed with 5-μm ODS-Hypersil (Shandon) and eluted with 20% (w/v) water in methanol; u.v. detection was used as above. Prior to injection into this system, methanol was added to the distillates, and the hexane was evaporated off.

Samples were injected with a syringe via a Rheodyne valve fitted with loops of 100- or 200-μl capacity. The larger loop was used with the normal-phase system only.

RESULTS AND DISCUSSION

Recovery of hydrocarbons by steam distillation

Recovery of specific hydrocarbons from water. The results in Table 1 show that good recoveries can be obtained for hydrocarbons which are major components of aqueous dispersions of crude and fuel oils [20]. A distillation time of 1 h was adequate to recover hydrocarbons encompassing a wide range of volatility.

Recovery of u.v.-absorbing components of North Sea (Auk Field) crude oil from water. The u.v. spectrum of Auk crude oil in hydrocarbon solvents (Fig. 2) has a broad peak with a maximum at approximately 225 nm, largely from alkylnaphthalenes, and a less strongly absorbing shoulder, partly from phenanthrenes and benzenes, at approximately 257 nm. The region of high absorbance below 220 nm which is predominant in w.a.f. of the oil is due mainly to the high concentration of benzenes [14].

Typically, more than 70% of the components absorbing at 220—225 nm could be recovered within 1 h (Table 2). Recovery at 255 nm was less than at 220—225 nm.

TABLE 1

Recovery of hydrocarbons from water by steam distillation

Hydrocarbon	Hydrocarbon concentration in water[a] ($\mu g\ l^{-1}$)	Distillation time[b] (h)	Hydrocarbon recovery[c] (%)
Aromatics			
Toluene	50	2	90.3 ± 3.5 (3)
	130	0.5, 1 and 2	100.9 ± 2.8 (6)
	870	2	98.5 ± 3.4 (4)
	4,320	3	84.6
o-Xylene	50	2	92.5 ± 3.7 (3)
	130	0.5, 1 and 2	110.0 ± 4.9 (6)
	4,380	1 and 3	98.9 ± 4.2 (2)
n-Propylbenzene	230	3	93.1
Naphthalene	1	1	99.5 ± 1.7 (3)[d]
	6	1	95.1 ± 1.7 (3)[e]
	2,510	1	95.7 ± 7.2 (3)
2-Methylnaphthalene	2,510	1 and 3	93.1 ± 2.2 (4)
1,5-Dimethylnaphthalene	530	2	90.2 ± 9.2 (4)
	2,640	1 and 3	87.7 ± 5.2 (4)
Biphenyl	250	3	92.3
	2,420	1 and 3	93.2 ± 1.4 (4)
Phenanthrene	1	1	89.0 ± 8.3 (3)[d]
	650	2	89.7 ± 8.7 (4)
	2,700	1 and 3	97.9 ± 3.5 (4)
Pyrene	1	1	88.4 ± 6.2 (3)[d]
	4	1	102.0[f]
	10	1	97.2 ± 8.1 (2)[e]
Chrysene	1	2	87.7 ± 1.4 (3)[d]
Alkanes			
Undecane (n-C_{11})	3,690	1	95.4 ± 2.2 (2)
Eicosane (n-C_{20})	130	3	80.3
	220	2	86.9 ± 2.9 (4)

[a]The hydrocarbons were spiked into 0.4 l or 1 l of filtered sea water and steam-distilled with either 4 ml or 10 ml of hexane or TMP or 10 ml of pentane. Pentane was not used for the extraction of toluene, xylene or undecane. [b]Where more than one distillation time is indicated, recoveries obtained with each individual distillation are averaged; no account is taken of distillation time. [c]Mean ± range where $n = 2$ or ± standard deviation where $n > 2$; n, the number of determinations is shown in parentheses. Distillates were analysed by g.l.c. except where denoted otherwise. [d]H.p.l.c. [e]U.v. spectrophotometry. [f]U.v. fluorescence.

Hydrocarbon recovery from mussel tissues. The results presented in Table 3 show that alkaline saponification can substantially increase the recovery of hydrocarbons from mussel tissue by steam distillation. This observation is consistent with other evidence that the presence of lipids in biota can adversely affect recovery of non-polar compounds by vapour-phase procedures [21, 22].

TABLE 3

Effect of saponification on the recovery of hydrocarbons from mussel tissues by steam distillation

Hydrocarbon	Hydrocarbon added to tissue ($\mu g\ g^{-1}$ wet wt.)	Hydrocarbon recovery[a] (%)		Relative increase in recovery[b] (%)
		+NaOH	−NaOH	
Specific hydrocarbons[c]				
Toluene	57.7	93.3 ± 5.5	85.9 ± 12.3	8.6
1,5-Dimethylnaphthalene	35.0	97.4 ± 3.2	86.1 ± 4.6	13.1
Phenanthrene	43.0	90.5 ± 6.8	73.7 ± 3.3	22.8
Chrysene	29.8	23.2 ± 2.1	16.9 ± 2.8	37.3
		Concentration in tissue ($\mu g\ g^{-1}$ wet wt.)		
		+NaOH	−NaOH	
Crude oil[d]				
Alkylnaphthalenes		1.86 ± 0.18	1.31 ± 0.14	42.0
Alkylphenanthrenes		0.99 ± 0.11	0.57 ± 0.11	73.7

[a] Recoveries and tissue concentrations are means ± standard deviation for 3 replicate determinations on the tissue homogenates. Analysis of "control" (i.e., uncontaminated) tissues was also triplicated and the results were used to correct the concentrations given for w.a.f.-exposed tissues. [b] $[R_{(+NaOH)} - R_{(-NaOH)}/R_{(-NaOH)}] \times 100$, where R denotes recovery. [c] 6 g of spiked tissue (all wet tissue bulked) was saponified for 2 h in the presence of 6 ml of hexane and then steam-distilled for 2 h; procedural details were as described under Experimental; g.l.c. was used. [d] Distillation was done as described above, using tissues from mussels which had been exposed to a diluted w.a.f. of North Sea crude oil for in excess of 1 month. Normal-phase h.p.l.c. was quantified by reference to 2,3-dimethylnaphthalene (225 nm detection) or 1-methylphenanthrene (254 nm detection).

Compound recoveries achieved from both water and tissues by the water estimator-based apparatus were comparable with those obtained with more specialised equipment [8—10, 12, 13, 23].

Measurement techniques applicable to steam distillates containing petroleum hydrocarbons

Gas-liquid chromatography. Here, g.l.c. was used to examine mussel distillates for hydrocarbons with volatilities in the range encompassed by toluene and C_1-alkylnaphthalenes. However, the chromatographic peaks of less volatile hydrocarbons are masked by biogenic compounds which increase in concentration with distillation time. These biogenics are eluted from silica clean-up columns with the aromatic hydrocarbons; removal requires gel-permeation chromatography [24] or normal-phase h.p.l.c. [22].

If 20 g (wet weight) of mussel tissue is extracted by steam distillation and

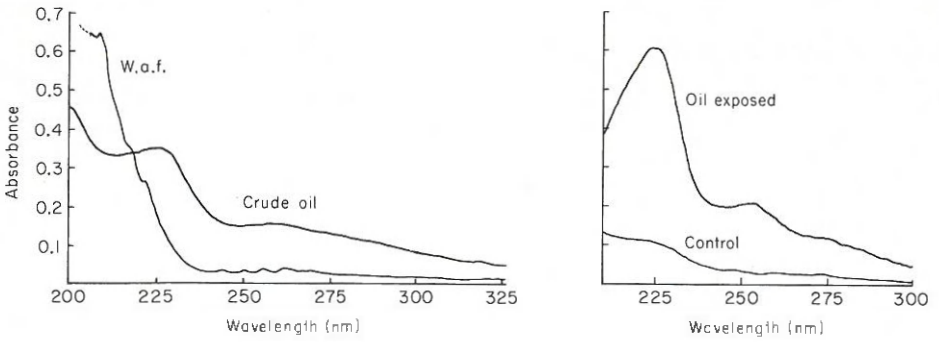

Fig. 2. U.v. absorbance spectra in 2,2,4-trimethylpentane: spectrum of North Sea (Auk Field) crude oil and spectrum of a 2,2,4-trimethylpentane extract of water-accommodated fraction (w.a.f.) of Auk Field crude oil [14].

Fig. 3. U.v. absorbance spectra of alumina cleaned-up steam distillates of mussel tissue (all remaining tissues after removal of the digestive glands); mussels exposed for 1 month to Auk Field w.a.f. with a total hydrocarbon content of 30 $\mu g\ l^{-1}$ [14] and "control" animals. Estimated aromatic hydrocarbon content of the oil contaminated tissue was 9.3 $\mu g\ g^{-1}$ wet weight. Solvent, 2,2,4-trimethylpentane.

Table 4 shows that the proposed saponification/steam distillation procedure is capable of efficient recovery of a range of hydrocarbons known to be accumulated by oil-contaminated mussels [3]. The less volatile compounds proved difficult to recover within 2 h, but recovery could be improved by extending the distillation time (see chrysene, eicosane and tetracosane) and/or increasing the distillation rate.

TABLE 2

Recovery of crude oil from water[a]

Form of oil	Conc. in water ($\mu g\ l^{-1}$)	Vol. of water (l)	Dist. time (h)	λ used (nm)	Recovery[b] (%)
Crude	1,580	0.2	0.75, 2.0	224	72.3 ± 1.8 (5)
				255	49.4 ± 4.7 (5)
W.a.f.[c]	4,200[d]	0.4	1.0	221	72.0 ± 5.0 (2)
Diluted w.a.f.	1,400[d]	1.0	1.0	221	68.2
			2.0 more	221	9.7
Aquarium[e]	25[d]	1.0	1.0	221	125.1 ± 22.6 (3)
	20[d]	1.0	1.0	221	107.0 ± 4.6 (2)

[a]Filtered sea water was extracted by steam distillation with 10 ml of 2,2,4-trimethylpentane and the distillates were examined by u.v. spectrophotometry. [b]Mean ± range where n (in parentheses) = 2 or ± standard deviation where $n > 2$. 100% recovery was considered to be that achieved by direct partition extraction into TMP (see Experimental). [c]Water-accommodated fraction of crude oil. [d]Hydrocarbon concentrations determined by g.l.c. [e]Aquarium containing mussels and dosed with w.a.f. plus algae [14].

TABLE 4

Recovery of hydrocarbons from mussel tissues by steam distillation[a]

Hydrocarbon	Hydrocarbon concentration in tissue ($\mu g\ g^{-1}$ wet wt.)	Mass of tissue (g wet wt.)	Time[b] (h)	Hydrocarbon recovery[c] (%)
Aromatics				
Toluene	14	6	1 (0.5)	104.3 ± 4.4 (3)
	30	6		85.8 ± 4.3 (2)
o-Xylene	15	6	1 (0.5)	109.3 ± 4.4 (3)
	90	20		83.3
Naphthalene	1	6		88.8 ± 4.2 (3)[d]
	50	20		85.1
2-Methylnaphthalene	50	20		92.3
1,5-Dimethylnaphthalene	20	6		94.5 ± 2.5 (2)
	50	20		94.3
Biphenyl	5	20	1 (1)	100.6
	40	15		103.5 ± 4.7 (6)
	40	2		95.5
Phenanthrene	1	6		85.6 ± 9.5 (3)[d]
	20	6		97.0 ± 2.0 (2)
	50	20		102.0
Pyrene	1	6		84.0 ± 9.4 (5)[d]
Chrysene	1	6	2 (2)	29.4 ± 5.0 (3)[d]
			14 more	47.6 ± 5.7 (3)[d]
Alkanes				
Undecane (n-C_{11})	70	20		86.7
Tetradecane (n-C_{14})	2	15	14 (2)	89.1 ± 1.2 (2)[e]
	30	6		105.2 ± 1.9 (2)
Hexadecane (n-C_{16})	30	6		91.3 ± 5.9 (5)
Eicosane (n-C_{20})	3	15	14 (2)	77.5 ± 6.5 (3)[e]
	7	6		31.9 ± 5.7 (3)
Tetracosane (n-C_{24})	15	15	2 (2)	5.3 ± 0.9 (6)
			14 more	43.6 ± 0.1 (2)

[a]Either all tissues were combined for analysis or digestive glands were removed and the remaining tissues were combined. Extraction was with 4 ml of either hexane or hexane/cyclohexane (2:1, v/v) mixture; the latter was used for 14-h distillations. [b]Saponification time was 2 h and distillation time (in parentheses) was 2 h unless otherwise tabulated. [c]See Table 1. [d]H.p.l.c. [e]Recovery after steam distillation and silica-gel column chromatography.

column fractions are reduced to 1 ml, individual hydrocarbons can be measured down to a concentration of 0.1 $\mu g\ g^{-1}$ (wet weight) with a precision generally within ±10% when quantification is by reference to internal standards.

Steam-distillation extraction of 1.5 l of water followed by concentration of the distillate to 1 ml enables individual hydrocarbons to be determined with acceptable precision by g.l.c. at concentrations down to 1 $\mu g\ l^{-1}$.

U.v. spectrophotometry. Aromatic hydrocarbons can be detected selectively in a matrix of biogenic compounds by measuring their characteristic u.v. absorbance/fluorescence; this enables clean-up procedures to be simplified. Englehardt et al. [11] quantified petroleum hydrocarbons in distillates of seal tissue by u.v. fluorescence, but did not provide recovery data for the fluorescent components. Present observations suggest that recovery by steam distillation of hydrocarbons fluorescing at long wavelengths is incomplete. By combining steam-distillation extraction with u.v. spectrophotometry, it proved possible to measure the naphthalenes, a petroleum component efficiently recovered from mussel tissue, with better precision than could be achieved with an alternative spectrophotometric procedure [25].

Aromatic hydrocarbons ranging in molecular weight from benzene to pyrene were recovered with an efficiency of more than 90% from the alumina clean-up column. Recovery of u.v.-absorbing components of crude oil from this column was 76.7 ± 1.3% at 225 nm and 68.5 ± 0.0% at 256 nm (mean ± s.d., $n = 3$ in each case).

The u.v. spectrum of a typical cleaned-up distillate of mussels experimentally exposed to Auk field crude oil is shown in Fig. 3, along with a distillate from control animals. Comparison of these spectra with that derived from a w.a.f. extract (Fig. 2), demonstrates more efficient accumulation of diaromatics by the mussel than monoaromatics.

The spectrophotometric results were quantified by calibrating against naphthalene, readings from both sample and standard spectra being taken at the peak wavelength for naphthalene (221 nm). The measurements were then related to aromatic hydrocarbon concentrations determined simultaneously by g.l.c. on a limited number of samples [14, 25]. Because of selectivity of hydrocarbon accumulation [3, 14], reference to a di- or tri-alkyl substituted naphthalene at 225—230 nm is more appropriate for tissue analysis.

The detection limit for experimental hydrocarbon contamination of mussels is controlled by the variability of the background signal measured in groups of "control" animals. Twice the error (standard deviation) of measurement of an average "control" background gave a detection limit of 0.2—0.8 $\mu g\ g^{-1}$ wet weight when expressed in naphthalene equivalents or 1.3—5.2 $\mu g\ g^{-1}$ wet weight expressed as aromatic hydrocarbons, determined by g.l.c. intercalibration [14]. Precision of duplicate hydrocarbon results corrected by means of associated duplicate control results was generally better than ±10% (range) at tissue hydrocarbon concentrations greater than 2—3 $\mu g\ g^{-1}$ wet weight expressed as naphthalene. The detection limit for u.v. spectrophotometric analysis of oil-dosed aquaria based on the precision of results for uncontaminated water (500-ml samples) was approximately 0.2 $\mu g\ l^{-1}$ expressed as naphthalene of 3 $\mu g\ l^{-1}$ expressed as oil w.a.f. The relative standard deviation at ten times this detection limit was better than ±5% ($n = 3$).

Phthalates absorb strongly at 220—230 nm and were efficiently recovered by steam distillation. However, they were not eluted from the alumina clean-up columns so could only interfere with measurements of aromatic hydrocarbons where clean-up was omitted (i.e., water analysis).

High-performance liquid chromatography. By combining u.v. spectrophotometric and spectrofluorimetric detection with h.p.l.c., the procedure for aromatic hydrocarbon can be made compound-specific or compound-group specific. Reverse-phase h.p.l.c. has been applied in this way to steam distillates of industrial effluents [7]. A reverse-phase chromatogram of a steam distillate of an oil-contaminated mussel tissue and of its associated "control" is shown in Fig. 4, along with a chromatogram of the oil w.a.f. used for experimental dosing (see [14]). The detection wavelength is 222 nm, selective for naphthalenes. The increase in biological concentration factor with increasing alkyl substitution of naphthalene is clearly demonstrated [3,

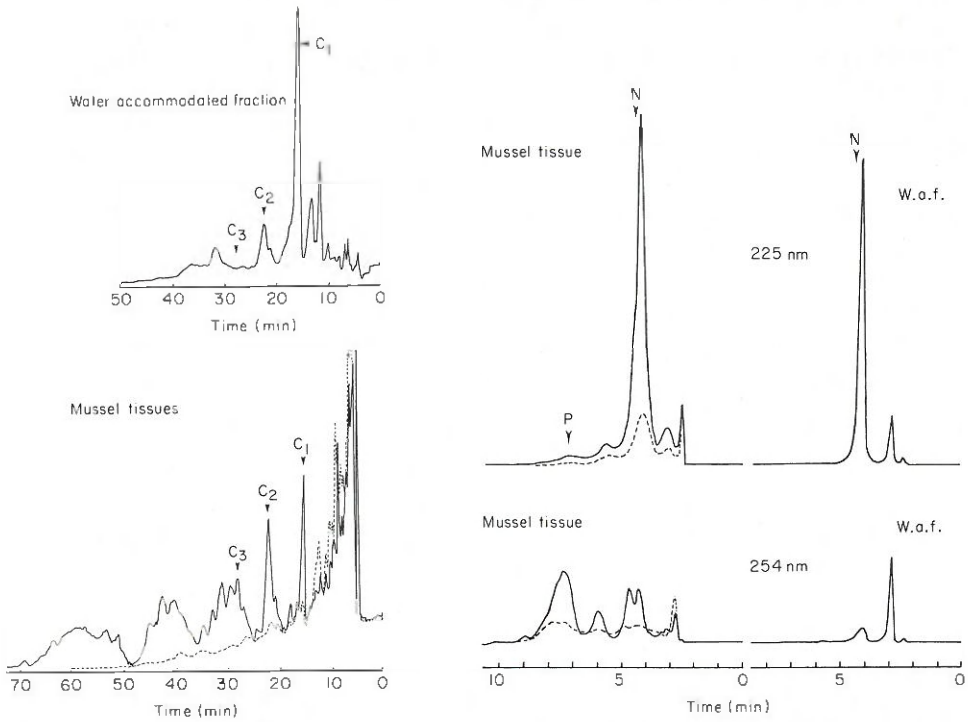

Fig. 4. Reverse-phase h.p.l.c. of a hexane extract of a w.a.f. of Auk Field crude oil and of steam distillates of mussel tissue (tissues as for Fig. 2). Mussels were exposed for 1 month to w.a.f. containing 36 $\mu g\ l^{-1}$ hydrocarbons and had a tissue aromatic hydrocarbon content of 12.6 $\mu g\ g^{-1}$ wet weight [14]. A chromatogram of a distillate derived from "control" mussels is shown by the broken line. C_1, C_2 and C_3 denote the retention times of 1-methylnaphthalene, 1,5-dimethylnaphthalene and 2,3,5-trimethylnaphthalene standards, respectively. Detection wavelength was 222 nm.

Fig. 5. Normal-phase h.p.l.c. of a hexane extract of w.a.f. of Auk Field crude oil and of a steam distillate of mussel tissue (mantle). Mussels were exposed for 20 days to w.a.f. with a total hydrocarbon content of 30 $\mu g\ l^{-1}$. A chromatogram of a distillate derived from "control" mussels is shown by the broken line. N and P denote the retention times of 2,3-dimethylnaphthalene and 1-methylphenanthrene standards, respectively. Detection wavelengths are shown.

14]. Phenanthrenes may be detected more selectively at 254 nm [26]. In this work, such chromatograms were quantified by reference to naphthalene, and representative C_1, C_2 and C_3 alkylnaphthalenes or phenanthrene and 1-methylphenanthrene, as appropriate to the monitoring wavelength. However, quantification is difficult because of the complexity of the chromatograms.

Shorter retention times and chromatograms of simpler form may be obtained by using normal-phase h.p.l.c. on amino or amino-cyano phase columns [19, 27—30]. Typical normal-phase (PAC) chromatograms derived from steam distillates of oil-contaminated mussels and an associated "control" are shown in Fig. 5, along with a chromatogram of a hexane extract of the oil w.a.f. to which the mussels were exposed [14]. The preferential accumulation of C_3-alkylnaphthalenes by the mussels is indicated by the broadening of the naphthalenes peak in comparison to that derived from the w.a.f. The quantity of u.v.-absorbing material detected by h.p.l.c. increased linearly with tissue weight extracted over a range 1—6 g wet weight for both experimentally contaminated and control animals.

The detection limit for experimental hydrocarbon contamination was determined in the same way as described for u.v. spectrophotometry. Expressed in terms of 2,3-dimethylnaphthalene and 1-methylphenanthrene equivalents, detection limits with PAC h.p.l.c. were always less than 1 $\mu g\ g^{-1}$ wet weight for naphthalenes, and less than 0.1 $\mu g\ g^{-1}$ wet weight for phenanthrenes; relative standard deviations were better than ±10% at concentrations 10 times the detection limit. Chromatograms may be quantified by reference to these standard compounds at the appropriate monitoring wavelengths (225 nm for alkylnaphthalenes, 254 nm for alkylphenanthrenes). The error introduced by using a single aromatic hydrocarbon to quantify a multicomponent peak may be estimated in selected samples by analysing appropriate h.p.l.c. fractions by g.l.c. [31].

Phthalates could not be recovered from the PAC column under the elution conditions described, so could not interfere with the analysis.

Application of steam distillation to analysis of environmental hydrocarbon residues

Although the procedures described above have been evaluated in laboratory experiments, they have also been applied successfully to a comparison of hydrocarbon contamination of mussels from a polluted dock and an estuary [32]. Examination of the steam distillates by u.v. fluorescence has enabled the distribution of aromatic hydrocarbons in an estuarine water column to be mapped [33].

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