Analysis of Water Extracts of Crude Petroleum by Gel Permeation Chromatography

Richard A. Larson and Joan C. Weston
Stroud Water Research Center
of the Academy of Natural Sciences of Philadelphia
Avondale, Pa. 19311

The successful use of recent techniques for removal of floating petroleum from water has led to increased emphasis on the ecological effects of its water-soluble fraction. Water extracts of petroleum products are complex and often greatly enriched in benzenoid aromatics, naphthalenes, phenols, carboxylic acid salts and carbonyl compounds relative to starting material (BOYLAN and TRIPP, 1971; ANDERSON, et al., 1974; LEE, et al., 1974). Many of these materials could be toxic to aquatic organisms, or exert sublethal (behavior modification, e.g.) effects. There is accordingly a need for rapid characterization of petroleum extracts and for monitoring their environmental alterations.

We report a novel procedure using Sephadex LH-20 gel for analysis of water extracts of crude oils. The method exploits the differential interactions of this gel with aliphatic, aromatic and phenolic substances as well as its property of fractionating molecules by size and shape (HSIEH, et al., 1969).

Materials and Methods

Samples of four crude oils were obtained from the Sun Oil Company (Marcus Hook, Pennsylvania), designated by the Company as Nigerian, Iranian, Mirando (Venezuelan) and Solvent Mix (Texas) crudes.

Water extracts were prepared by slow magnetic stirring (BOYLAN and TRIPP, 1971) of the oil with 500 ml of water from White Clay Creek (Chester County, Pennsylvania). After 48 hours, the stirrer was shut off and the extract was allowed to stand for one hour before 450 ml was siphoned into a 1-l separatory funnel with a Teflon stopcock. This portion of the water extract was back-extracted with three 25 ml portions of chloroform and the combined chloroform extracts were concentrated under vacuum to 2.0 ml. The Sephadex LH-20 column

Bulletin of Environmental Contamination & Toxicology,
Vol. 16, No. 1 © 1976 by Springer-Verlag New York Inc.

(volume 110 ml, 26.5 x 2.3 cm) was prepared by swelling 30 g of resin in 105 ml of redistilled reagent grade tetrahydrofuran.

The chloroform concentrate was added to the column; development was carried out with redistilled tetrahydrofuran. The first 20 ml of the eluate was discarded, and 3.0-ml fractions were collected thereafter.

Ultraviolet (Beckman DBGT) and fluorescence (Turner 110) spectra were measured on the eluate fractions. Fluorescence emission above 420 nm (Turner filter #2A) was measured with an excitation maximum of 360 nm (Turner filter #7-60).

Fractions were combined for gas chromatographic analysis on the basis of ultraviolet spectral characteristics. A Hewlett-Packard Model 5750 gas chromatograph with dual flame detectors and a 6 mm x 1.8 m glass column (3% OV-210, 60-80 mesh Chromosorb W) was used. Temperature was programmed from 60° to 280° at 10°/min.

A mixture of known aliphatic and aromatic hydrocarbons and phenols known to occur in crude oils was prepared, dissolved in chloroform, and passed over the column using the same conditions. Gas chromatography (using the standard program) was used to establish the elution behavior of each known substance.

Results

Elution patterns for the four crudes are shown in Figures 1-4. Quantities plotted in the figures are total fluorescence, optical density at 257 nm (OD_{257}) and the ratio OD_{275}/OD_{257}.

Benzene absorbs at 254 nm; methyl-substituted benzenes, at somewhat longer wavelengths (o-xylene 262 nm, mesitylene 266 nm). Because the aromatic fractions of the crude extracts examined had maximal absorption at 257 nm, this wavelength was chosen to monitor aromatic hydrocarbon content.

Phenolic compounds from petroleum absorb near 275 nm (phenol, 270; p-cresol, 279; 2-naphthol, 275). However, it was not possible to monitor phenolic content in the fractions using this wavelength, since many aromatic hydrocarbons, which elute earlier, also exhibit strong absorption there. Better results were obtained by use of the ratio of absorbances at 275 and 257 nm; most phenols have weak absorption at 257 nm.

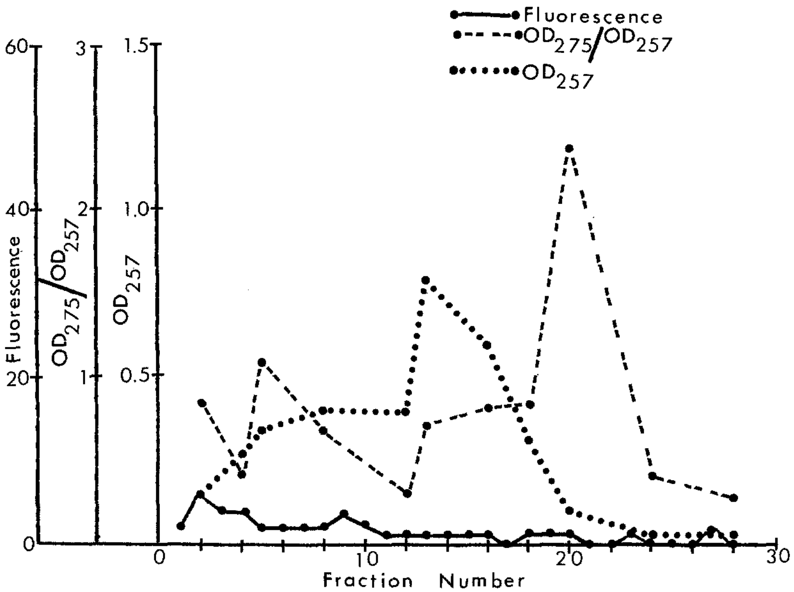

Fig. 1 - Iranian crude

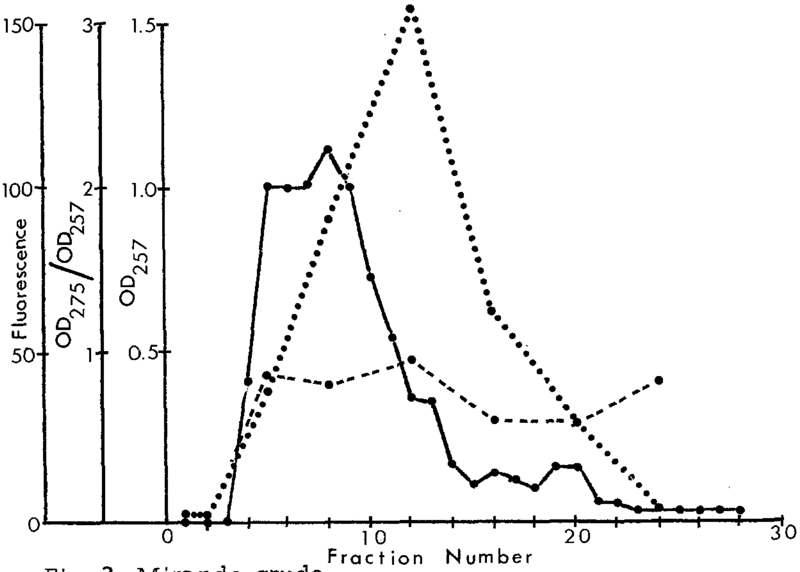

Fig. 2- Mirando crude

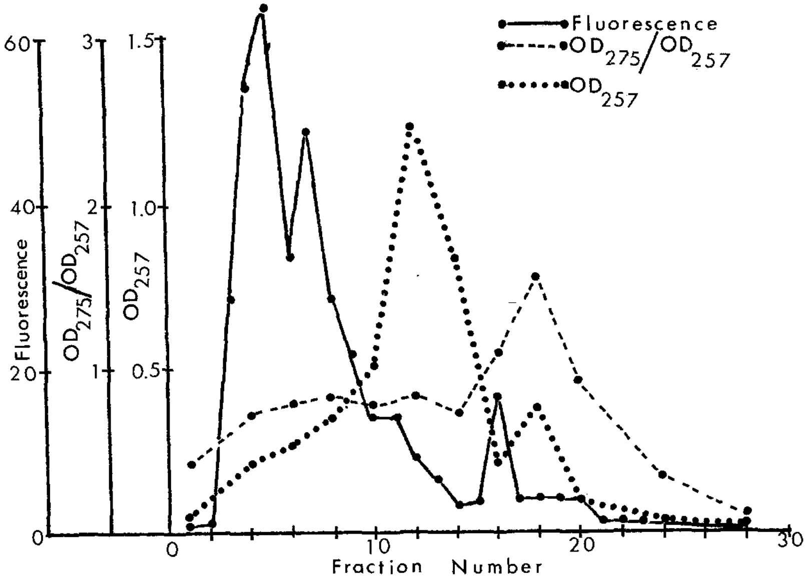

Fig. 3 - Solvent Mix

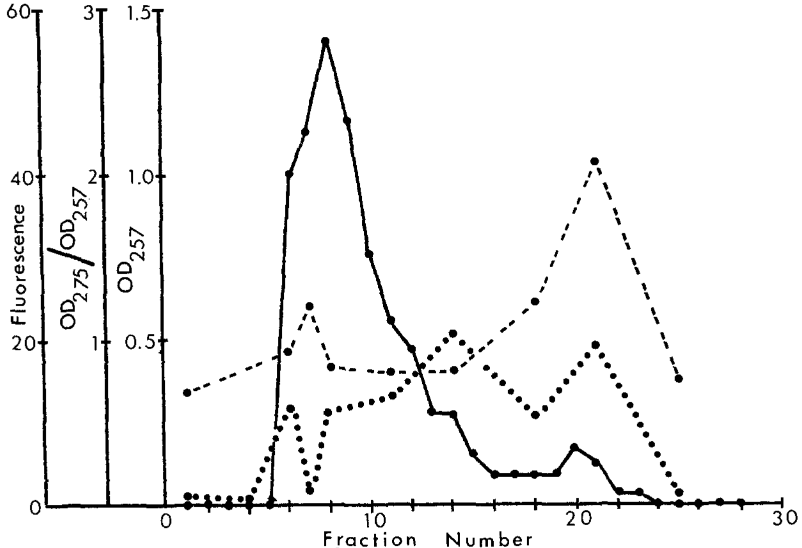

Fig. 4 - Nigerian crude

Table 1 shows the fraction number in which the largest quantity (based on GC peak height) of each compound (in the mixture of known substances from petroleum) eluted. Although most of the compounds listed were present in one or two fractions before and after the fraction listed in the table, phenolic compounds eluted more sharply.

Table 1. Elution of known petroleum substances from Sephadex LH-20.

Compound	Ret. Time (min.)	Fraction No.
_n_-octacosane	19.8	5
_n_-tetracosane	14.6	6
_n_-hexadecane	7.2	8
2-Methylnaphthalene	6.4	13
Naphthalene	4.8	14
Biphenyl	7.0	16
Anthracene	12.7	17
Phenol	1.8	19
3, 5-xylenol	4.4	19
_m_-cresol	2.9	19
2-Naphthol	9.8	20

In Table 2, absorption data is converted into approximate aromatic and phenol content for each fraction containing these substances. The conversion factors are derived from the extinction coefficients for benzene (204 l/mole cm) and phenol (1450 l/mole cm) at their maxima (SCOTT, 1964).

Discussion

The extracts of the four tested crudes show marked differences in content of fluorescent compounds, aromatic hydrocarbons and phenolic substances.

For example, the Iranian crude extract contains almost no material which fluoresces under the experimental conditions (although it may contain substances which fluoresce at shorter excitation wavelengths), whereas the other three extracts are similar in fluorescence intensity. The occurrence of the fluorescence maximum in the early fractions indicates that the compounds responsible for it must be of considerable size: the exclusion limit for LH-20 is near 5, 000 molecular weight. The substances are probably polynuclear aromatics with multiple or very long chain alkyl substituents.

The Mirando and Solvent Mix extracts are relatively high in aromatics, and the Nigerian and Solvent Mix extracts high in phenols.

Table 2. Aromatic and phenol content of extract fractions.

Oil	Fraction No.	OD_{257}	OD_{275}	Aromatic content mg	Phenolic content mg
Iranian	12	0.38		0.44	
	13	0.78		0.90	
	16	0.58		0.67	
	18		0.24		0.05
	20		0.19		0.04
	24		0.06		0.01
Mirando	8	0.91		1.05	
	12	1.55		1.78	
	16	0.62		0.71	
	20		0.18		0.03
	24		0.05		0.01
Nigerian	8	0.32		0.37	
	12	0.52		0.60	
	16	0.26	0.32	0.30	0.06
	20		0.98		0.19
	24		0.03		0.01
Solvent Mix	10	0.50		0.58	
	12	1.19		1.37	
	14	0.82		0.94	
	16		0.29		0.06
	18		0.56		0.11
	20		0.09		0.02

The early fractions (numbers 1-9) contain (in addition to fluorescent material) straight-chain aliphatic hydrocarbons, as predicted from the properties of LH-20 (HSIEH, et al., 1969). A characteristic gas chromatographic pattern (cf. Figure 5) of repeating n-alkane isomers ranging up to C_{40} is observed. All the extracts showed this general pattern, with the exception of the Mirando extract, which contained only two strong peaks at retention times characteristic of C_{12} and C_{14} n-alkanes.

The alkane peaks were at low intensity, but probably do not represent direct water solubilization. BOEHM and QUINN (1974) have described several mechanisms by which hydrocarbons can be accommodated in water, including formation of particulate aggregates and colloidal micelles with natural organic macromolecules.

Gas chromatographic patterns of samples from the aromatic (Figure 6) and phenolic (Figure 7) regions were much more complex. Pronounced high-boiling "unresolved envelopes" were evident and few peaks could be tentatively assigned to known compounds. The Nigerian extract had fairly strong peaks at retention times characteristic of tetrahydronaphthalene, naphthalene, a monomethyl naphthalene, phenol and m-or p-cresol.

Many microorganisms capable of degrading aliphatic hydrocarbons have been described (AHEARN and MEYERS, 1973). The environmental fates of other classes of petroleum-derived substances are not as well known. It should be practical to use the gel chromatographic method for rapid assessment of changes in petroleum slicks or their water extracts during environmental exposure. Experiments of this nature are now being undertaken in our laboratory.

Acknowledgement

We thank the National Science Foundation-RANN program for financial support (Project #G 42282).

References

AHEARN, D. G. and S. P. MEYERS, eds.: The microbial degradation of oil pollutants.Center for Wetlands Resources, Louisiana State Univ., 1973.

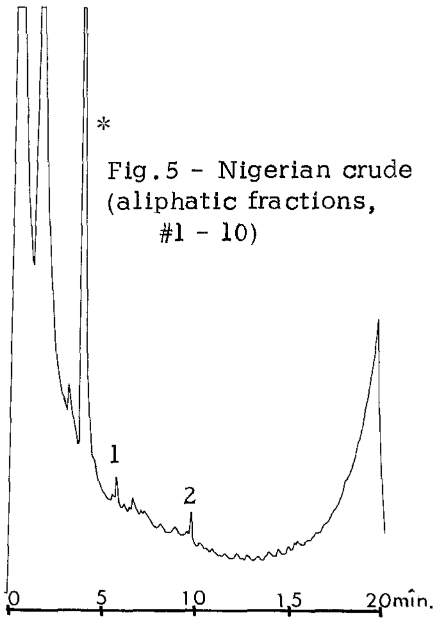

Fig. 5 - Nigerian crude (aliphatic fractions, #1 - 10)

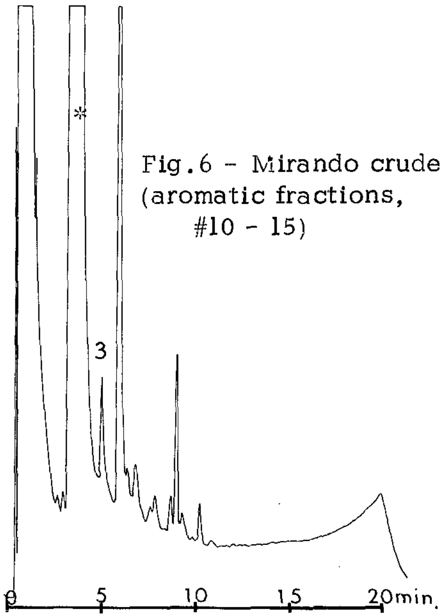

Fig. 6 - Mirando crude (aromatic fractions, #10 - 15)

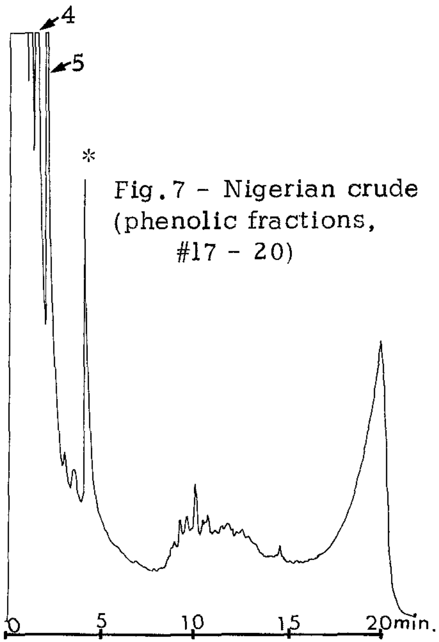

Fig. 7 - Nigerian crude (phenolic fractions, #17 - 20)

Gas chromatographic conditions, see text. * = BHT (Impurity in tetrahydrofuran).
Tentative identifications: 1, n-$C_{16}H_{34}$; 2, n-$C_{22}H_{46}$; 3, monomethylnaphthalene(s); 4, phenol; 5, m- or p-cresol.

ANDERSON, J. W., J. W. NEFF, B. A. COX, H. E. TATEM and G. M. HIGHTOWER: Marine Biol. 27:75 (1974).

BOEHM, P. D. and J. G. QUINN: Marine Poll. Bull. 5:101 (1974).

BOYLAN, D. B. and B. W. TRIPP: Nature 230:44 (1971).

HSIEH, B. C. B., R. E. WOOD, L. L. ANDERSON and G. R. HILL: Anal. Chem. 41:1066 (1969).

LEE, C. C., W. K. CRAIG and P. J. SMITH: Bull. Environ. Contam. Toxicol. 12:212 (1974).

SCOTT, A. I.: Interpretation of the ultraviolet spectra of natural products. Pergamon Press, Oxford, 1964.