An Ultraviolet Spectrophotometric Method for the Determination of Naphthalene and Alkylnaphthalenes in the Tissues of Oil-Contaminated Marine Animals

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Introduction

Petroleum is an extremely complex mixture of thousands of different hydrocarbons and related compounds. It is generally agreed that the most toxic components of oil to marine organisms are the mono- and bicyclic aromatic hydrocarbons (BLUMER et al., 1970; BOYLAN and TRIPP, 1971; MOORE et al., 1973). ANDERSON et al. (1974a) found a relationship between the concentration of naphthalene and alkylnaphthalenes in water-soluble fractions prepared from four oils and their relative toxicities to six species of estuarine animals.

When exposed to oil-contaminated sea water, marine animals rapidly accumulate in their tissues a wide spectrum of petroleum hydrocarbons. Detailed gas chromatographic-mass spectrometric analyses of the tissues of oil-exposed animals revealed that the naphthalenes were accumulated to a greater extent than were other aromatic hydrocarbons and n-paraffins (ANDERSON et al., 1974b). When returned to oil-free sea water, the marine animals rapidly released the accumulated hydrocarbons, the naphthalenes being the last of the petroleum hydrocarbons analyzed to reach undetectable levels in the tissues.

Because gas chromatographic techniques for the determination of naphthalene and alkylnaphthalenes in tissues are both time-consuming and expensive, a simpler, more rapid technique for determining these compounds in tissues and water was sought. Direct ultraviolet spectrophotometry has been used to identify petroleum products in sea water (LEVY, 1972) and marine animals (WOODIN et al., 1972) and for the quantitative determination of benzene, alkyl benzenes and kerosene in blood (GUERTIN and GERARDE, 1959). This paper describes a direct UV spectrophotometric method for the semiquantitative determination of naphthalene, methylnaphthalenes and dimethylnaphthalenes in sea water and the tissues of marine animals.

Experimental

Oysters Crassostrea virginica and clams Rangia cuneata were collected from the Galveston Bay system, Texas. They were maintained in the laboratory in large aquaria containing Instant Ocean artificial sea water (Aquarium Systems, Inc.) at 15 or 20 o/oo salinity and 20 $\pm$ 2°C.

The three oils used in this investigation, South Louisiana crude oil (API reference oil II), a No. 2 fuel oil (38% aromatic, API reference oil III), and a Venezuelan bunker C residual oil (API reference oil IV) were supplied by the American Petroleum Institute. Oil-in-water disperisons (OWD) and water-soluble fractions of oil (WSF) were prepared in 15 or 20 o/oo salinity sea water by the methods described in detail previously (ANDERSON et al., 1974a). Fifty ml aliquots of the aqueous phase of the oil-water mixtures were siphoned from below the surface oil layer, care being taken to avoid contamination of the sample by the surface oil. The water samples were acidified with 1 ml of concentrated HCl and then extracted with 20 ml of spectrophotometric grade n-hexane (Matheson, Coleman & Bell or Burdick & Jackson). The extracts, after suitable dilution with n-hexane to reduce the absorbance maxima to below 1.5, were placed in 1 cm path length Suprasil cuvets and their ultraviolet spectra were scanned between the wavelengths of 210 and 240 nm with a Pye-Unicam SP-1800 dual beam recording spectrophotometer with the temperature regulated cuvet chamber maintained at 20 $\pm$ 0.1°C. The concentrations in the sample of naphthalene, methylnaphthalenes and dimethylnaphthalenes were computed by means of simultaneous equations constructed from the absorbance spectra of authentic standards in hexane (FRIEDEL and ORCHIN, 1951). The standards, naphthalene, 2-methylnaphthalene and 2,6-dimethylnaphthalene, were purchased from Chemical Samples Co. and were 98-99% pure.

Clams and oysters were shucked. The soft tissues were blotted, weighed and then homogenized for 1-2 minutes over ice in spec-grade n-hexane using a Sorval Omni-mixer. Generally 10 ml of hexane was used for tissue samples smaller than 1 g and 20 ml for tissue samples of 1 - 10 g. The homogenate was centrifuged for 5 minutes in a clinical centrifuge. The hexane supernate was poured into a 2 dram glass vial and approximately 2 g of activated Florisil was added. The Florisil was activated by heating it to 200°C for 24 hours. The vial was capped with an aluminum foil-lined cap and shaken several times over 12-24 hours. The extracts were then filtered through glass wool and after suitable dilution with n-hexane, their UV spectra were determined as described above. In some cases, a second treatment with Florisil was required to bring the background absorbance down to an acceptable level.

Results and Discussion

Naphthalene, 2-methylnaphthalene and 2,6-dimethylnaphthalene in hexane have strong sharp absorbance maxima in the far UV region at approximately 221, 224 and 228 nm respectively. At their absorbance maxima, all three compounds have molar extinction coefficients in the range of log ε = 5 (FREIDEL and ORCHIN, 1951). These were the absorbance peaks used for quantitative analyses.

In order to determine the efficiency of the extraction procedure for recovering naphthalenes from sea water, quantities of naphthalene or 2-methylnaphthalene equivalent to from 0.1 to 10.0 ppm were dissolved in synthetic sea water. The sea water samples

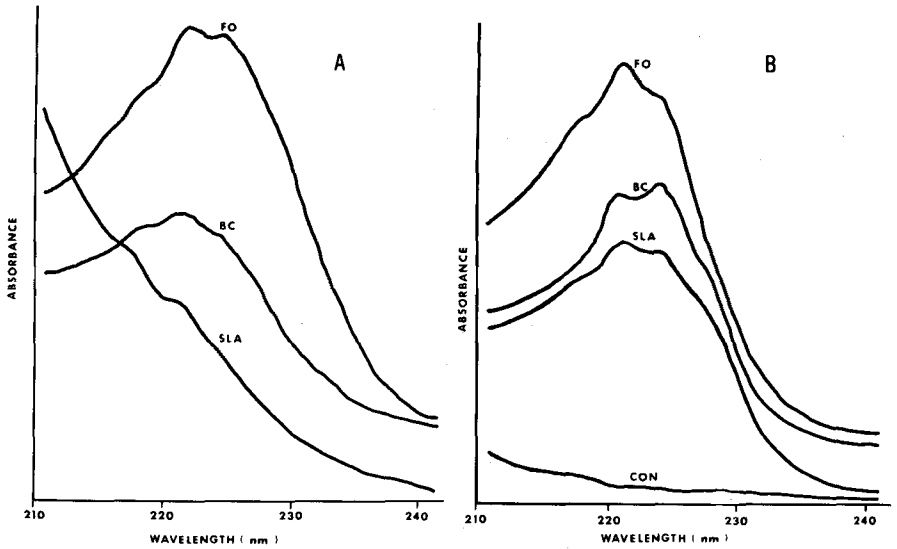

Fig. 1 A. UV spectra of hexane extracts of water-soluble fractions of No. 2 fuel oil (FO), South Louisiana crude oil (SLA) and Venezuelan bunker C residual oil (BC).

B. UV spectra of Florisil-treated hexane extracts of the soft tissues of clams Rangia cuneata following 24-hour exposure to dilute oil-in-water dispersions in comparison to the extract of an unexposed control clam (CON).

were extracted and analyzed as described above, giving average recoveries of 104 $\pm$ 9.5 and 98.8 $\pm$ 1.8% for naphthalene and 2-methylnaphthalene respectively.

Typical UV spectra of the hexane extracts of water-soluble fractions prepared from the three test oils are shown in Figure 1A. The spectra of the #2 Fuel oil and bunker C residual oil extracts resemble each other qualitatively. Both have a sharp absorbance maximum at 221 nm with a prominant shoulder at 224 nm. On the other hand, the hexane extract of the WSF of South Louisiana crude oil has a strong absorbance below 211 nm with only a shoulder evident at 221 nm. The concentrations in the three WSFs of naphthalene, methylnaphthalenes and dimethylnaphthalenes as determined by gas chromatography (WARNER, 1974), and UV spectrophotometry are listed in Table 1. The two analytical techniques gave roughly comparable results for the WSFs of #2 Fuel oil and bunker C residual oil, the UV technique giving slightly higher values in both cases. However, concentrations of naphthalene, methylnaphthalenes and dimethylnaphthalenes in the WSF of South Louisiana crude oil as determined by the UV technique were about 2 times higher than values obtained by gas chromatography.

TABLE

Table 1. Concentrations in ppm of naphthalene (N), methylnaphthalenes (MN) and dimethylnaphthalenes (DMN) in water soluble fractions of 3 oils as determined by gas chromatography (GC) and ultraviolet spectrophotometry (UV).

Analytical Method	South Louisiana Crude			Bunker C Residual Oil			No. 2 Fuel Oil		
	N	MN	DMN	N	MN	DMN	N	MN	DMN
GC	0.12	0.11	0.06	0.21	0.39	0.20	0.84	0.82	0.24
UV	0.26	0.22	0.19	0.30	0.48	0.31	0.92	0.94	0.52

There are relatively few hexane-soluble compounds likely to be found in oil-contaminated sea water that have a sufficiently strong absorbance in the 220-230 nm region to cause interference in the determination of naphthalenes by the UV technique. Among the petroleum hydrocarbons, the alkylbenzenes are the most likely to cause interference. Because these aromatic hydrocarbons have molar extinction coefficients one to three orders of magnitude lower than those of the naphthalenes in the 220-230 nm region, they will not be a problem unless they are present at concentrations several times higher than those of the naphthalenes. The strong UV absorbance of the South Louisiana crude oil WSF extract below 220 nm is indicative of the presence of relatively high concentrations of benzene and alkylbenzenes. We have shown (ANDERSON et al., 1974a) that the WSF of this oil contains 13.6 ppm benzene and alkylbenzenes. This nearly 50 fold excess of benzenes over naphthalenes undoubtedly is the cause of the high values obtained for naphthalenes by the UV technique. Polycyclic aromatic hydrocarbons larger than the alkylnaphthalenes usually have strong absorbance maxima at wavelengths longer than 230 nm. Their presence in hexane extracts can be ascertained by scanning the 230-270 nm region.

Untreated hexane extracts of the tissues of marine organisms usually have UV spectra characterized by a strong absorbance maximum in the 198-205 nm region. In many cases a shoulder of this maximum extends to about 225 nm resulting in interference in the spectral region of interest. These UV-absorbing biogenic substances are probably primarily polyunsaturated lipids and steroids. In an effort to remove this background absorbance without removing the naphthalenes, the hexane extracts were treated with 3 chromatographic adsorbants, alumina, silica gel and Florisil. Activated alumina was relatively inefficient in removing the background. Activated silica gel and activated Florisil were quite effective. However, silica gel also removed some of the naphthalenes. Therefore, Florisil was chosen as the adsorbent for subsequent work.

BRIDIE et al. (1973) used Florisil to selectively remove non-hydrocarbons from pentane extracts of oil-in-water mixtures. They found that Florisil removed nearly all the non-hydrocarbons but also removed up to 20% of the naphthalenes from the pentane extracts when these were present at high concentrations (20-200 mg/l).

The efficiency of the extraction technique and the effect of Florisil treatment on the recovery of naphthalene from tissues was therefore evaluated. Five to 15µg of either naphthalene or 2,6-dimethylnaphthalene in acetone was injected into the visceral mass of Oysters Crassostrea virginica. The soft tissues were then processed and analyzed as described above. The amounts injected resulted in calculated tissue concentrations of 0.5 to 4.8 ppm depending on the tissue weight of the oysters. Average recoveries were 92 ± 13.5 and 95 ± 22.5% for naphthalene and 2,6-dimethylnaphthalene respectively. When hexane extracts of uncontaminated oysters were spiked with 2,6-dimethylnaphthalene at levels between 0.1 and 10 ppm and then treated with Florisil, more than 90% of the added hydrocarbon was recovered in all cases. The better recoveries of naphthalenes in the present investigation as compared to those of BRIDIE et al. (1973) may be due to the solvents used (hexane vs. pentane) or to the lower concentrations of the solutes used in the present investigation.

The Florisil cleanup technique has been successfully applied to hexane extracts of a large number of other vertebrate and invertebrate tissues. However, this treatment was not completely successful in removing biogenic UV-absorbing substances from the hexane extracts of fish (Fundulus similus) gall bladder and crab (Callinectec sapidus) hepatopancreas probably due to their high steroid content.

These recovery experiments give no indication of the efficiency of the extraction technique in solubilizing tissue-bound naphthalenes. Therefore, groups of 7 clams Rangia cuneata were exposed to sea water solutions of either naphthalene or 2-methylnaphthalene for 6 and 24 hours respectively. The clams were then shucked and the soft tissues weighed and frozen. Three clams from each exposure group were analyzed by GC techniques by Dr. J.S. Warner, Battelle Columbus Laboratories (WARNER, 1974) and 4 from each group were analyzed by the UV technique (Table 2).

The concentration of naphthalene and 2-methylnaphthalene in the clam tissues, as determined by the two methods, are all in the same range. The results suggest that both methods yield comparable results. Much of the variability observed is undoubtedly due to individual variations in the rates of uptake of these aromatic hydrocarbons by the clams.

Groups of clams Rangia cuneata were exposed to dilute oil-in-water dispersions of the 3 test oils for 24 hours. Typical UV spectra of the Florisil-treated hexane extracts of these clams are shown in Figure 1B. For comparison, a typical UV spectrum of a Florisil-treated hexane extract of an unexposed control clam is also given. The control extract shows practically no absorbance in the 220-240 nm region. The spectra of the hexane extracts of the #2 Fuel oil and South Louisiana crude oil-exposed clams are qualitatively

TABLE

Table 2. Comparison of the gas chromatographic and the UV spectrophotometric method for determination of naphthalene and 2-methylnaphthalene in tissues. Seven clams, Rangia cuneata were exposed to each aromatic hydrocarbon.

Compound	Clam #	Concentration in tissue ppm	
		Gas Chromatography	UV spectrophotometry
	1		62
Naphthalene	2		67
Exposure con-	3		57
centration	4		71
9 ppm	5	33	
	6	86	
	7	30	
Ave.± S.D.		50±32	64±6.3
	1		32
2-methyl	2		88
naphthalene	3		101
Exposure con-	4		58
centration	5	65	
11 ppm	6	82	
	7	103	
Ave.± S.D.		83±19	70±30

similar. Both have absorbance maxima at 221 nm and a prominant shoulder in the 224 nm region. The spectrum of the hexane extract of the bunker C-exposed clam has an absorbance maximum at 224 nm with a slightly smaller peak at 221 nm. It should be noted that the hexane extract of the clam exposed to the OWD of South Louisiana crude oil shows no indication of interference from benzene and alkylbenzenes. Apparently these monoaromatic hydrocarbons are not accumulated or retained in the molluscan tissues to the same extent as are the naphthalenes.

Because crude and refined oils are extremely complex mixtures of hydrocarbons and various heterocompounds, no single analytical technique is completely satisfactory for the quantitative determination of petroleum contamination of biological samples. The usual approach to this problem has been to determine a narrow range of petroleum hydrocarbons by gas chromatography (BLUMER et al., 1970; FARRINGTON and QUINN, 1973; CLARK and FINLEY, 1973) or spectrofluorometry (ZITKO, 1971). The concentration of these hydrocarbons is then used as an index of total petroleum contamination of the tissue sample. A similar approach was taken in the present investigation. Naphthalene and alkylnaphthalenes were chosen because of their strong absorbance in the far UV region, their

high toxicity to marine organisms (ANDERSON et al., 1974c) and their persistance relative to other petroleum hydrocarbons in the tissues of oil contaminated marine animals (ANDERSON et al., 1974b).

The ultraviolet spectrophotometric technique described in this paper has been used in our laboratory for the past year in studies of the accumulation and retention of petroleum hydrocarbons by several species of marine invertebrates and fish. As little as 0.1 ppm of naphthalene and alkylnaphthalenes have been detected in tissues without difficulty. The detection limits in sea water are in the range of 0.01 to 0.05 ppm.

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