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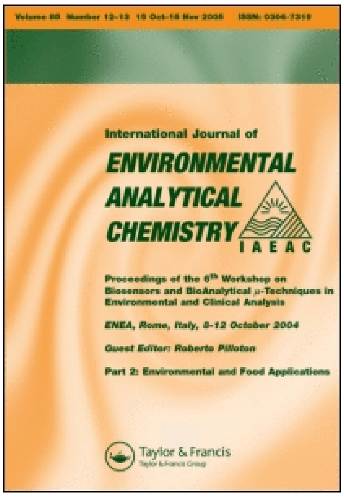

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Analysis of Water-Soluble Acidic Compounds Derived from Spilled Oil in a Controlled Marine Enclosure

F. Berthou[a]; J. Ducreux[b]; G. Bodennec[c]
[a] Faculté de Médecine, service de Biochimie, Brest-Cédex, France [b] Institut Français du Pétrole, Rueil-Malmaison, France [c] IFREMER, Brest-Cédex, France

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Analysis of Water-Soluble Acidic Compounds Derived from Spilled Oil in a Controlled Marine Enclosure

F. BERTHOU
Faculté de Médecine, service de Biochimie, BP-815 29279-Brest-Cédex, France

J. DUCREUX
Institut Français du Pétrole, BP-311 92506-Rueil-Malmaison, France

and

G. BODENNEC
IFREMER, Centre de Brest, BP-337 29273-Brest-Cédex, France

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The fate of Arabian light oil (1 litre), spilled in a controlled sea-water enclosure (320 litres), was studied. In addition to the usual monitoring of aliphatic and aromatic hydrocarbons contained in the crude oil, the formation of acidic compounds through the natural photochemical oxidation has been investigated while the biodegradation was shown to be insignificant.

A liquid–liquid extraction procedure was developed in order to obtain acidic and basic-neutral compounds. Water-soluble acidic fraction was analysed after methylation by gas-chromatography on capillary columns coated with either SE-52 or PEG-20 M. On the other hand, the level of hydrosoluble acidic compounds in six samples (initial, 19 h 30 min, 8, 20, 45 and 70 days) was evaluated by fluorimetry and by reversed-phase HPLC combined with fluorimetric detection.

Preliminary results indicated the formation of numerous oxygenated products derived from the spilled oil. The photo-oxidative process led to the formation of fatty acids and oxygenated two-ring aromatic compounds. The results showed that the concentration of the fluorescent acidic products attained after 70 days of experience a level significantly higher (6 mg/l) than that of parent PAH (0.4 mg/l). This result suggests that the photochemical oxidation could be accountable for the degradation of ca 0.3% of the initial crude oil.

The analytical approach can be considered as a first step towards the development of routine assay procedures for analyzing such acidic compounds. These "new" molecules may contribute to the global toxicity of oil towards marine biota.

KEY WORDS: Natural photochemical oxidation, hydrosoluble acidic compounds, spilled oil, HPLC-fluorescence analysis.

INTRODUCTION

Although petroleum is a complex mixture of compounds such as aliphatic, alicyclic and aromatic hydrocarbons in addition to a variety of polar aromatics and high molecular-weight polymers, the monitoring of oil marine pollution consists usually in the analysis of only the aliphatic and aromatic hydrocarbons.[1] This approach may not be realistic because it is unaware of compounds undergoing chemical structural alterations in aquatic environment. Indeed as soon as petroleum is spilled in the sea, it is subjected to a series of processes that cause distribution of oil in the environment and, at the same time, produces weathering,[2,3] thus changing its physico-chemical properties. So it is well known that a variety of oxygenated and long-chain polymers are formed through photochemical reactions.[4] These transformations yield pollutants of higher water solubility and maybe potential toxicity.

Responding to the need of a detailed monitoring of petroleum pollution, the formation of hydrosoluble acidic compounds was studied in a sea-water enclosure of 320 litres where 1 litre of Arabian light crude oil was spilled. The thin film of oil was subjected to natural irradiation during 70 days. The acidic compounds solubilized in the sea-water column were analyzed by high performance chromatographic methods. Such analysis are considered as a continuing challenge to the ingenuity of the analytical chemists.[5] This study demonstrates that numerous acidic compounds are present in the sea-water polluted by a crude oil.

MATERIAL AND METHODS

Experimental

A stainless steel enclosure (ca. 1.6 m diameter and 1.6 m depth) was filled with 320 litres of sea-water from the Rade de Brest. One litre of Arabian light crude oil (Institut Français du Pêtrole) was spilled at the surface and was spread as a thin film of 0.45 mm thickness. During the experience the sea-water was unrenewed. Six water samples were collected at different times (initial, 19 h 30 min, 8, 20, 45 and 70 days) by means of a sampling valve. One litre water was extracted by a liquid/liquid procedure in order to fractionate the sample into acidic and basic-neutral compounds.[6]

Fluorescence analysis

For UV-fluorescence the sea-water extracts were dissolved into acetonitrile (HPLC quality, SDS, France) and analyzed into a 1 cm cell. Wavelengths were set for excitation at 355 mm and emission at 400 nm. Results are reported as Arabian light equivalents using this crude oil to calibrate the fluorimeter.

Synchronous excitation-emission fluorescence scans (SEES) were obtained on the same extracts using a 25 nm offset over the range 240–500 nm on a Perkin–Elmer (Norwalk, U.S.A.) apparatus model 300.

Gas chromatography

Analyses were performed using a Carlo–Erba GC model 4160 (Milano, Italy) equipped with a cold on-column injector and flame ionization detector (FID) or flame photometric detector (FPD). Acidic fractions were analyzed with a 50 m × 0.3 mm OV-1 glass capillary column home-made according to the Grob's recommendations[7] or with a 25 m × 0.3 mm CP-Wax-57-CB (Chrompack, The Netherlands). After an initial temperature of 66°C for 2 minutes, the oven was ballistically heated at 100°C, then temperature programmed at 4°C/min up to 280°C for OV-1 column and 230°C for CP-Wax-57-CB column.

Peaks were quantified on the basis of internal standard—

perdeuterated tetracosane $C_{24}D_{50}$—with a response coefficient of the unity for all peaks.

Before GC analysis all the acidic extracts were derivatized with diazomethane generated from N-methyl-N′-nitroso-N-nitrosoguanidine (Aldrich, U.S.A) according to the Aldrich procedure.

When FPD was used, GC analysis was carried-out on a methyphenyl-silicone (RSL-500) gummified *in-situ* by heat-curing technique.[8,9]

High performance liquid chromatography

Separation of the acidic fractions was carried-out with a reversed-phase octadecylsilane 5 μm type Vydac 201-TP (Cunow, France) protected with a guard column. The column was 20 cm × 4.6 mm I.D. The detector was a dual fluoromonitor from LDC (Riviera Beach, U.S.A.) with excitation at 254 nm and observation beyond 360 nm. Gradient elution was performed with 1.47% (v/v) acetic acid in water (solvent A) and acetonitrile (solvent B) in a 25 min linear gradient from 30 to 90% solvent B with a flow-rate of 1 ml/min. Injections were made via a 50 μl sample loop in acetonitrile.

RESULTS

Fluorescence

Quantitative evaluation of fixed-wavelength fluorescence of extracts of water samples indicated that fluorescent compounds were dissolved in the water column (Figure 1). Although data were reported in arbitrary units of Arabian light, it is obvious that aromatic hydrocarbons were oxidized during the weathering process and then dissolved in water. After 70 days of exposure to the sunlight, the concentration of fluorescent acidic compounds in the sea-water was ca. 6 mg/l, i.e. a total amount of 1,920 mg in the 320 l tank. Accordingly the photochemical oxidation could be accountable for the degradation of ca. 0.3% of the initial crude oil.

Identification and quantitation of these "new" molecules because of their low volatility and their high polarity is considered as a difficult task.[10] So direct fluorescence provides only a limited per-

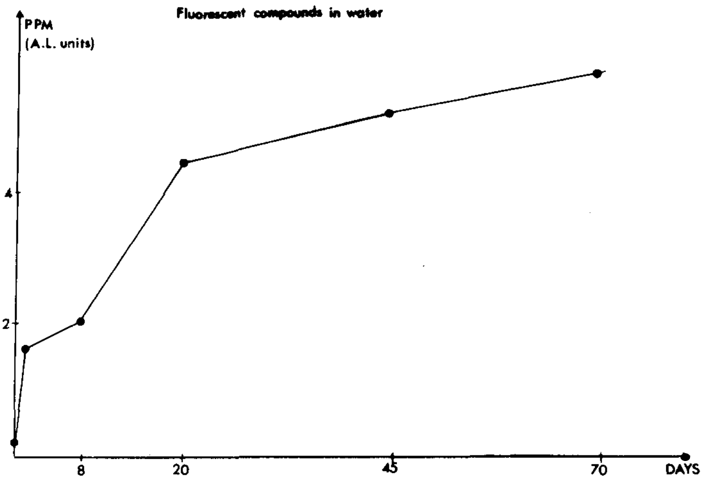

FIGURE 1 Acidic fluorescent compounds concentration in sea-water. Measurement at 355 nm excitation and 400 nm emission; calibration by the Arabian light (AL) reference oil.

spective of these compounds. A more detailed perspective is obtained by SEES fluorescence and by high resolution chromatographic techniques.

Synchronous excitation-emission scan fluorescence

SEES scans representing the acidic fractions extracted from the sea-water at various times are shown in Figure 2. For comparison is included the SEES from the Arabian light reference oil. Aromatic ring number designations were made according to Wakeham[11] and Ostwald.[12] All the samples, except the 70-days sample, showed a major peak at ca. 285 nm and a minor peak at 300 nm representing two-ring aromatic compounds. In contrast the 70-days extract differed markedly in that the major peak was observed at 330 nm representing three- and four-ring aromatic compounds. The 45- and 70-days samples showed a minor shoulder in the above 370 nm region, suggesting the presence of five-ring aromatic molecules.

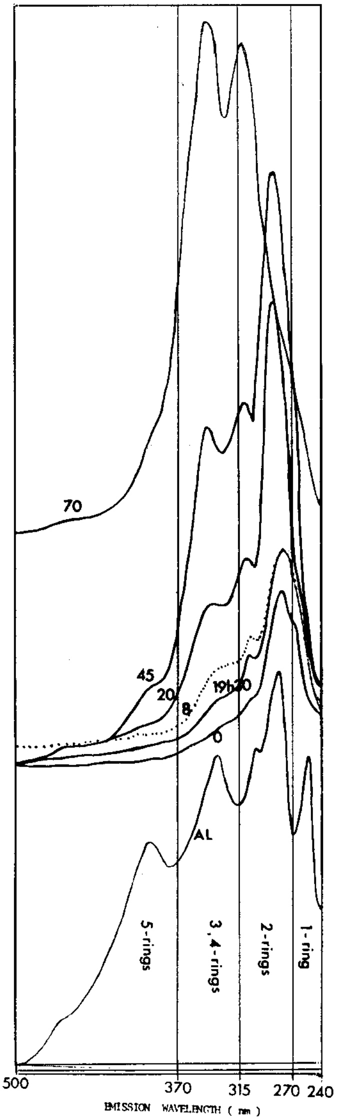

FIGURE 2 SEES fluorescence profiles of the acidic extracts from sea-water at different times. Also indicated are the respective fluorescence regions for different size aromatic compounds. SEES profile for Arabian light (AL) is included for comparison.

Gas chromatography

Gas chromatographic profiles of the methylated acidic extracts from the sea-water are given in Figure 3. Qualitatively these profiles showed notable changes during the time of experience. The control sea-water contained numerous peaks corresponding to biogenic fatty acids in addition to phtalate esters. After 20 and 70 days of experience, the major fatty acids were palmitic acid (peak *a*) and stearic acid (peak *b*). The concentration of palmitic acid showed a maximum at 20 days (Figure 4). Other identified normal fatty acids were C-12, C-14 and C-20. The 20- and 70-days samples contained other oxidized compounds quantified either as GC peaks or as unresolved complex mixture (Figure 4). The most notable change in the chromatographic profile between the methylated and unmethylated (not shown) was in the sharp, more symmetrical peaks in the methylated sample. This indicated that polar compounds bearing phenolic and carboxylic functions have been derivatized to substances more amenable to gas chromatographic analysis.

If the UCM increased dramatically from 100 to 1000 ppb (ng/l) between the 45 and 70 days, the individual resolved GC peaks showed a maximum at 20 days.

Figure 5 shows the chromatographic profiles of the same extracts as in Figure 3 analyzed on a polar stationary phase CP-Wax-57, the polarity of which is closely related to PEG-20 M. Thus the predominance of saturated fatty acids was confirmed.

The samples contained evidence of other oxidized hydrocarbons. Although additional analyses are needful to obtain a reasonably complete identification of these oxygenated compounds, the initial data present clear evidence for the formation of such molecules during the photochemical process. Thus in the dibenzothiophenes series, the formation of oxygenated sulfur aromatic hydrocarbons is illustrated in Figure 6. Compounds detected by flame photometric detection were not parent alkyl dibenzothiophenes present in the crude oil but more polar compounds such as sulfones, sulfoxides or alkyl dibenzothiophenic acids.[4]

The GC analysis of water column samples are in agreement with the previous results of Overton *et al.*[4] Concerning the origin of fatty acids, it is suggested that aliphatic hydrocarbons are degraded by the action of sunlight at the water surface and then dissolved in the water column. The mechanism of this reaction is yet unknown.

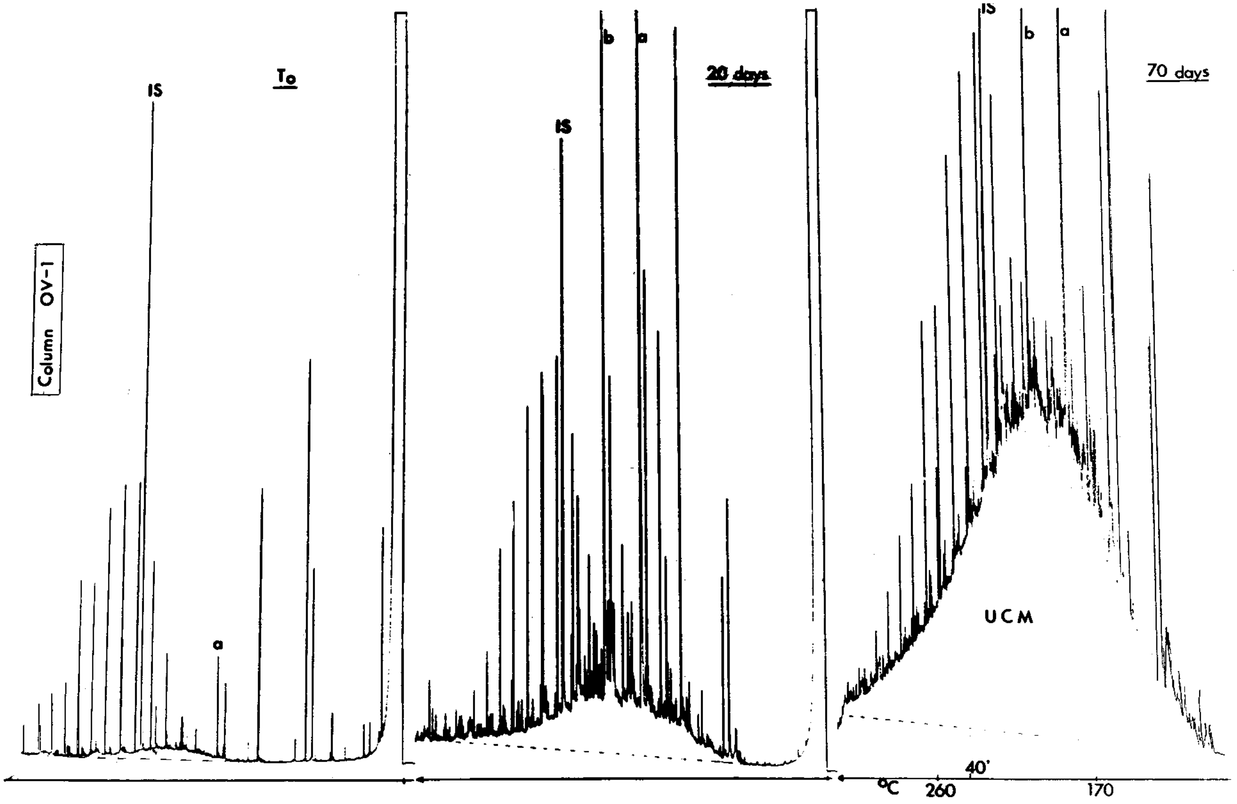

FIGURE 3 Gas chromatograms of the methylated acidic extracts of the water column at initial, 20- and 70-days times. For analytical conditions on OV 1 column, see the text. FID detection.

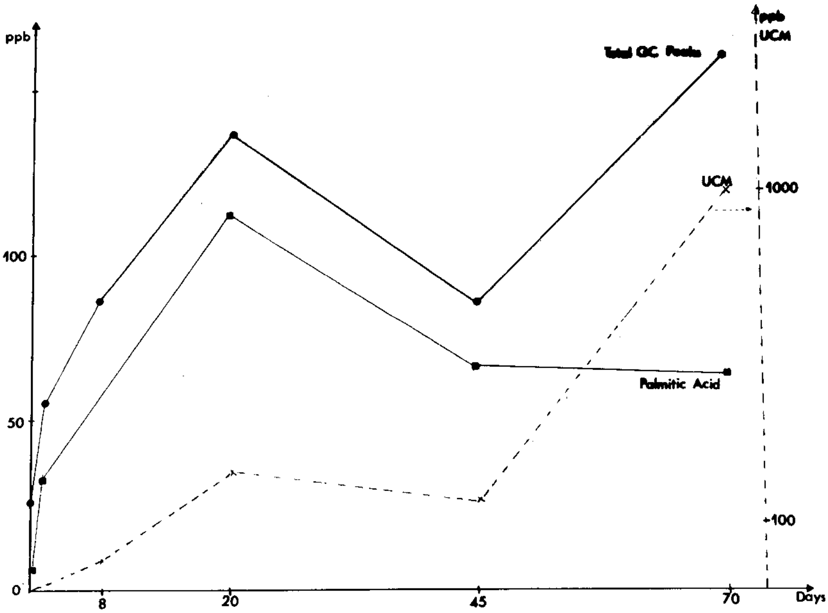

FIGURE 4 GC peaks quantitation in the methylated acidic fractions from seawater. UCM = unresolved complex mixture (OV-1 column).

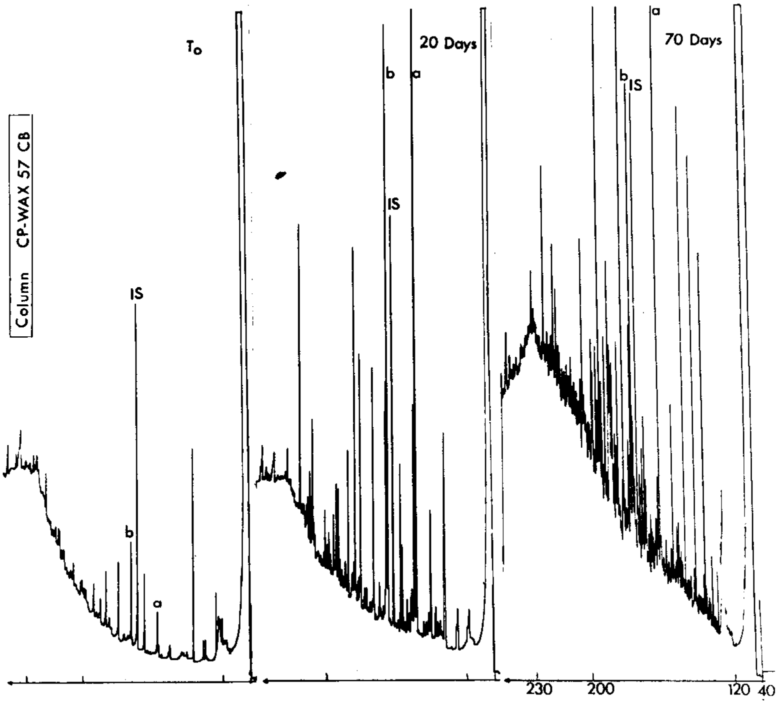

FIGURE 5 Gas chromatograms of the methylated acidic extracts of the water column at initial, 20- and 70-days times. For analytical conditions on CP-Wax-57-CB column, see the text. FID detection.

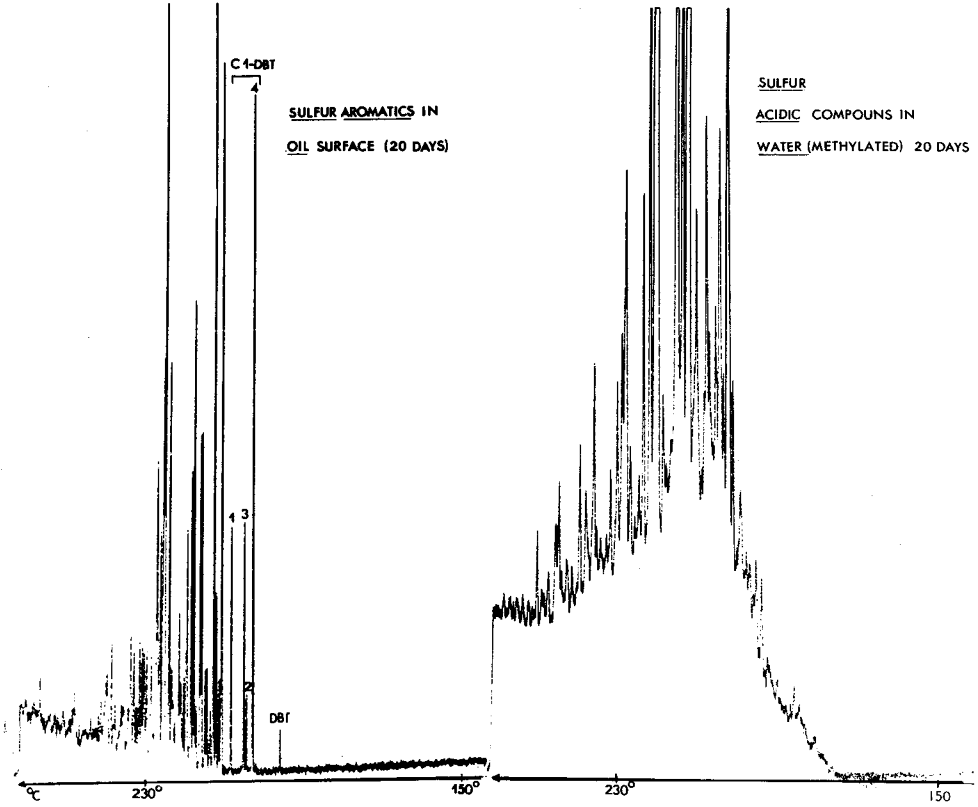

FIGURE 6 FPD-gas chromatograms of the 20-days sample from sea-water and sulfur aromatics in oil surface. Methylphenylsilicone column as in ref. 9.

HPLC

The tendency of aromatic hydrocarbons to oxidize and form polar molecules in marine environment is well known.[4,13,14] However, because of the limitations of conventional analytical methods, especially GC, in analyzing most of these products, reversed-phase HPLC was explored. In a first approach the UV detection at 254 nm was shown to be inadequate. In a second approach RP-HPLC in combination with UV-fluorescence detection at wavelengths characteristic of some representative petroleum derived compounds made the partial analysis of oxygenated aromatic hydrocarbons possible. The detected products bear certainly acidic functional groups because the addition of acetic acid as mobile phase modifier was needful to suppress the ionization of molecules and thus to involve their elution[15] by solvophobic chromatography.

The analysis of the acidic fractions is presented in Figure 7. Differences in HPLC-UV fluorescence profiles were found in function of progress of experience. These differences correlated relatively well with GC data. So the peaks areas, expressed in arbitrary units, presented a dramatic increase after the 45th day (Figure 8). This phenomenon was already observed when studying the UCM in GC (Figure 4). This result suggests that the compounds interferent in the UCM were also detected by UV-fluorescence. Detection was carried-out using for emission selection the broad-band spectral bandpass of a cut-off filter at 360 nm. Such detection conditions provided more sensitive detection of three-, four- and five-ring aromatic molecules. High selectivity can be enhanced by selecting the fluorescence excitation and emission detection wavelengths via monochromators during the chromatographic separation.

The retention times of the majority of HPLC detected compounds were shorter than those of parent PAH present in the initial crude oil. This observation corroborates that HPLC peaks were oxygenated polar products.

DISCUSSION

As described in several models,[16,17] oil spilled in sea-water can be degraded or dispersed by several processes. It was suggested that the

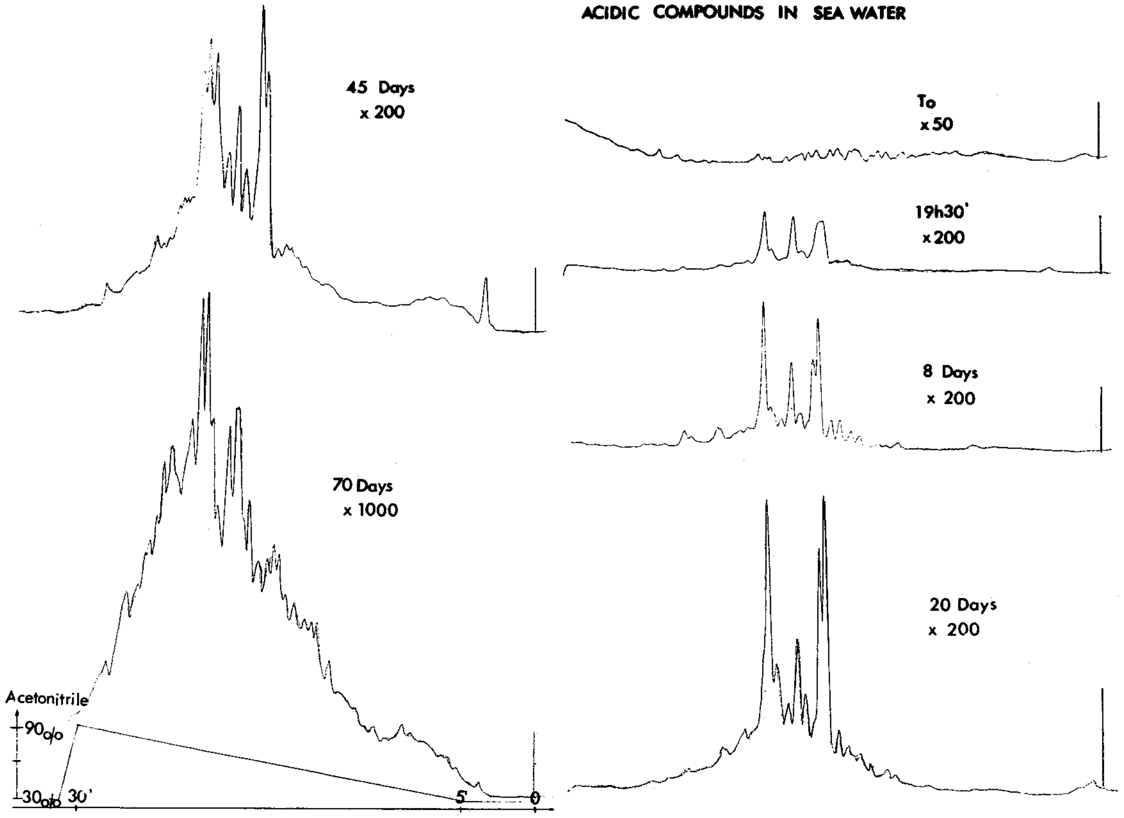

FIGURE 7 HPLC profiles of acidic extracts of sea-water. Fluorescence detection: excitation at 254 nm and emission beyond 360 nm. Gradient elution conditions: 5 min with acetonitrile/acetic acid 1.47% in water (30/70) then linear gradient in 25 min to acetonitrile 90%. Vydac-201-TP-C-18 column.

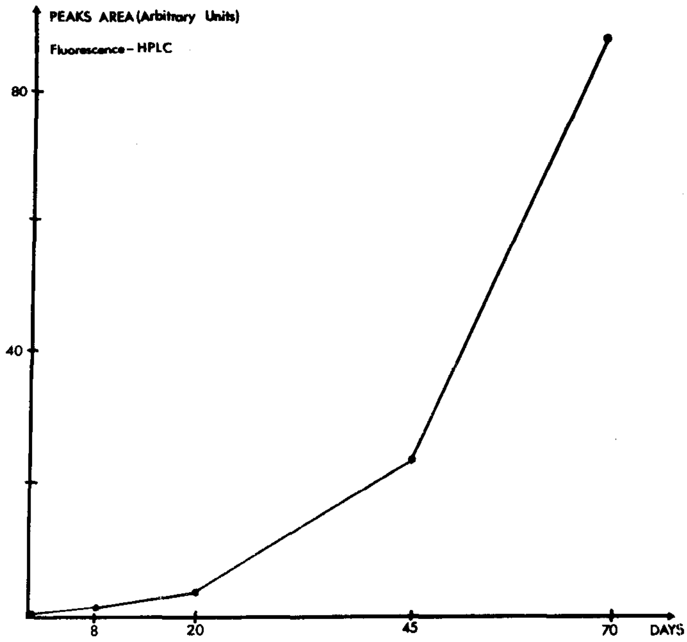

FIGURE 8 HPLC peaks area in the acidic fractions from sea-water. Fluorescence detection.

primary processes were evaporation, microbial degradation, sedimentation and photochemical oxidation.[3] Our aim was to attempt to study the relative importance of the photochemical reactions in a crude oil spilled at the water surface under natural irradiation. This phenomenon was often neglected. Thus, using the wide variety of data collected during the monitoring of the Amoco-Cadiz oil spill, Gundlach *et al.*[18] suggested that the photochemical oxidation processes were probably insignificant in the Brittany environment (high-energy tide and cold water). This assertion was supported by any quantitative result probably because of the inability to analyse the oxygenated compounds formed by such a process. Although the photo-oxidation is quantitatively minor, its importance cannot be neglected because such transformations generally yield pollutants of enhanced water solubility and higher potential toxicity.[19]

The authors did not intend to determine the relative importance of major-balance pathways. Therefore, some weathering steps were not studied (evaporation) or considered insignificant. Thus the biological degradation may be neglected in our experimental conditions where sea-water was not renewed during 70 days and not supplemented with nitrogen and phosphorus nutrients.[20] That was confirmed by the relative stability of the pristane or phytane/*n*-alkanes ratios.[6] On the other hand sedimentation depending on the turbulence and the level of suspended solids[21] is a very minor process in our conditions. It can be therefore suggested that under our model conditions the oxygenated acidic compounds present in the sea-water derived from the crude oil via photochemical reactions.

The results showed that the concentration of hydrosoluble acidic compounds increased as significantly from the 45th day to attain levels signicantly higher (ca. 6 mg/l) that those of parent PAH (ca. 0.4 mg/l) at the 70th day of weathering experience.[6] Even if concentration of dissolved hydrocarbons were minimized owing to experimental conditions, it occurs that conventional analysis of aliphatic and aromatic hydrocarbons may provide only a limited, perhaps a myopic, perspective of petroleum contamination. From the point of view of toxicity, an important question is "Are these oxygenated compounds more toxic than their parent hydrocarbons?" To answer this question, it is important that these molecules do not remain undetected and it goes without saying that these products must be identified.

At this time identification of these "new" molecules will need additional analysis by specific analytical methods such as mass spectrometry (work in progress). However it may be suggested that the degradation of crude oil induced by sunlight involves photo-oxidation and aliphatic and aromatic compounds to form fatty acids and oxygenated two- and three-ring aromatic products according to two reaction steps. So photosensitizers such as oxygenated naphthalene derivatives have been shown to accelerate the photo-oxidation of *n*-alkanes and alkyl-PAH.[22] Accordingly the first oxidative reactions could lead to the formation of reactive species of naphthalene series (see SEES results). The dramatic increase of formation of oxygenated molecules after the 45th day could be due to the highest concentration of photosensitizers in the crude oil. The second step of photo-oxidation could lead the oxidation of threeand four-ring aromatic compounds. During this step polymeric products (tar) could be formed from reactive species. The high viscosity of residual oil could restrict the diffusion of oxidation products across the oil-water interface.[6]

Because of the limitations of conventional GC in analyzing oxygenated polar compounds (phenols, acids), reversed-phase HPLC showed great promise especially in conjunction with on-line UV-fluorescence.

CONCLUSION

The data as stated above have not yet been fully interpreted because of the complexity of analysis of these "new" molecules. However the results present clear evidence of the formation of oxygenated compounds under natural irradiation during the weathering of oil spilled at the sea surface even under a relatively cold climate.

To summarize the analytical approach must be considered as a first step towards the development of routine assay procedures for analyzing the acidic molecules resulting from weathering of oil spilled in the marine environment. Current analysis of such compounds will need long series of investigations. This way will open attractive prospects in the study of the oil toxicity with regard to the marine life.

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