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Use of oxidative degradation followed by capillary gas chromatography–mass spectrometry and multi-dimensional scaling analysis to fingerprint unresolved complex mixtures of hydrocarbons

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ABSTRACT

Unresolved complex mixtures (UCMs) of hydrocarbons are a common feature of the gas chromatograms of bacterially degraded crude oils, some refined oils (*e.g.*, lubc oils) and of oil-polluted sediment extracts. It is often difficult to identify the original source oil in polluted sediments by existing methods. A study was made of the use of chemical oxidation of ten UCMs to yield gas chromatographically resolvable compounds and analysis of the oxidation products by quantitative gas chromatography–mass spectrometry. The data were then used to calculate inter-sample Euclidean distances. These values were input into a multi-dimensional scaling program which allows similar samples to be clustered. This technique allows UCMs in a number of different sediments to be compared with their likely source oils.

INTRODUCTION

When crude oil is altered either by weathering processes in the environment, in oil reservoirs by bacteria or refining by man, the resulting residues often consist of chromatographically unresolved complex mixtures (UCMs) of hydrocarbons. The characterization of these UCMs is an important goal in oil pollution studies [1] and in oil–source rock and oil–oil correlation exercises in oil exploration geochemistry [2]. The distributions of the small amounts of resolved alkanes which survive weathering and bacterial degradation (usually called biomarkers [3–8]) are often used for these correlations, but these may not reflect accurately the identity of the original oils. Biomarkers rarely constitute more than 1% of the total hydrocarbons [9] and if mixtures of oils with different biomarker concentrations are present, their profiles may be misleading. Recent work has shown that oxidative degradation of UCMs yields some gas chromatographically resolvable products [10–12]. It has been suggested

that these products (e.g., acids, lactones and ketones) may be useful for "fingerprinting" UCMs [10]. Indeed, the oxidation products of UCMs isolated from sediments known to be contaminated with fresh Nigerian crude [13] and a tank oil [14] were successfully used to identify the source oils.

This paper describes modifications to the existing method for application to smaller amounts of UCMs (2 mg compared with 50 mg in the original study [10]), duplicate oxidations to test the reproducibility and quantification of the individual resolved oxidation products. The resulting data are analysed by cluster analysis and multi-dimensional scaling (MDS) in an attempt to improve upon the subjective comparisons made in the initial study. Application of the modified technique to a number of case studies is described.

EXPERIMENTAL

Samples

Samples (Table I) of Sullom Voe sediment (Garths Voe), Ninian crude oil, Mersey sediment (Dungeon's Lane) and Tia Juana Pesado crude oil were supplied by the Field Studies Council Research Centre (FSCRC), and a sample of the Esso *Bernicia* fuel oil was supplied by the Sullom Voe Oil Terminal.

Isolation and oxidation of UCMs

Oils and sediments were extracted and hydrocarbon fractions isolated by published methods [15]. Each hydrocarbon fraction was further separated by silver ion thin-layer chromatography to yield the "saturated aliphatic fraction", then desulphurized [16], and any resolved components were removed by urea clathration (and in some instances thiourea clathration [17]) to yield the UCMs.

Oxidation of the UCMs (*ca.* 2 or 50 mg) was carried out with chromium(VI) oxide–acetic acid [11] for 1 h at 70 $\pm$ 2°C (molar ratio of oxidant to substrate = 10:1). Oxidation of 2 mg samples was performed using microscale apparatus (Wheaton) with all reagents, where practical, scaled down in proportion to the original method. This was not possible with solvent volumes, and therefore all 2-mg oxidations were carried out in 1 cm^3 (*cf.*, 10 cm^3) of solvent.

Gas Chromatography (GC)

GC was carried out on a Carlo-Erba Mega series gas chromatograph fitted with a fused-silica capillary WCOT column (30 m $\times$ 0.32 mm I.D.) coated with DB-5 (0.1 μm film thickness) (J&W Scientific). On-column injection with temperature programming from 40 to 300°C at 5°C/min, followed by an isothermal period of 10 min, and hydrogen as the carrier gas at a flow-rate of 2 cm^3/min was employed.

Gas chromatography–mass spectrometry (GC–MS)

The GC conditions were as above except that helium was used as the carrier gas. A Kratos MS25 double-focusing mass spectrometer operating in the electron impact mode with source temperature 250°C, ionizing voltage 40 eV and filament emission current 400 μA was used.

Two internal standards (naphthalene-d$_8$ and perylene) were added to each sample at 5 μg mg^{-1} of oxidation products. Major oxidation products, *i.e.*, *n*-carboxylic acids, γ-methyl-γ-lactones and alkyl ketones, were identified by GC–MS as reported previously [10].

The peak heights and areas of each component were measured by integration using a Kratos DS90 data system with peak baselines adjusted manually. This integration data was then downloaded to a PC for further data processing.

Chromatogram comparison

The component peak heights in each mass chromatogram for each sample were compared with the corresponding mass chromatograms of all other samples. This was achieved using a purpose-written BASIC program which adjusted peak retention times by comparison with the internal standards and then compared peaks by retention time. A "window" of $\pm$5 s was set for a positive match. Any components found to be missing in a sample were given a height value of zero. All components were measured by comparison with the peak height of the internal standard (naphthalene-d$_8$, *m/z* 136, assuming a response factor of 1). Data were then transferred to an IBM mainframe computer for statistical analysis.

Statistical analysis

Euclidean distances were calculated using a pur-

pose-written program within the SAS statistical package, according to the following:

$$D_{jk} = \sum_{i=1}^{n} (x_{ij} - x_{ik})^2$$

where D_{jk} = distance between samples j and k, X_{ij} = peak i integral for sample j and X_{ik} = peak i integral for sample k.

Thus, each sample has a distance from all others in a multi-dimensional space [18], where, if N is the number of samples, there are $N-1$ dimensions. This analysis produces a distance matrix which is then analysed by cluster analysis [19] to identify groups of samples, followed by non-parametric MDS [20], which replaces distances with ranks. This projects the information onto two dimensions while maintaining the distances as closely as possible [21]. The resulting two-dimensional plot is only important in terms of the relative closeness of samples, the axes having no scales or units.

RESULTS AND DISCUSSION

Oxidation of the isolated UCMs produced good yields (total *ca.* 90%) of resolved (typically *ca.* 15%) and unresolved (typically *ca.* 85%) products, as found oreviously [10] (Table I). This was true whether smaller (*ca.* 2 mg) or larger (*ca.* 50 mg) UCM samples were oxidized. The components produced were typical of the method [10,11] and included resolved carboxylic acids, lactones and ketones (Fig. 1). Quantification, cluster analysis and MDS (see Experimental) produced the results shown diagramatically in Fig. 2 and 3. Although pattern recognition and various statistical methods (*e.g.*, principal components analysis, discriminant analysis, cluster analysis) have been widely reported for chromatographic data [22–26], the small number of samples (twelve) and relatively large number of variables (*ca.* 100 components) in this study required a different statistical approach to those commonly used. We therefore employed techniques developed for environmental analysis [21] (*e.g.*, the analysis of volatile organic compounds in water samples on a seasonal basis), where the statistical constraints are similar. Thus, a strategy of calculating the Euclidean distance between samples and the use of this information in a multi-dimensional scaling package was adopted. The method was then tested on a series of samples involving various degrees of hydrocarbon pollution in a number of sediments.

Samples 1–3: reproducibility study

Initial work [10] showed that the oxidation method produced reproducible yields and distributions of

TABLE I

GRAVIMETRIC DATA FOR ALL SAMPLES ANALYSED

Figures in italics are taken from ref. 10.

No.	Sample	Alkanes (%)	UCM[a] (%)	Total yield (%)	Oxidized[b] yield (%)
1,2	Silkolene 150 (2 mg)	78	86	94	—
3	Silkolene 150 (50 mg)	79	86	95	*71*
4	Amoco tank oil	*16*	*68*	*87*	—
5	Newgale beach oil	*16*	*21*	*75*	—
6	*Sivand* cargo	*55*	*76*	*83*	—
7	Humber sediment	*43*	*87*	*83*	—
8	Sullom Voe sediment	66	9	95	70
9	Ninian crude oil	52	31	89	55
10	*Bernicia* fuel oil	28	62	84	69
11	Tia Juana Pesado crude oil	22	85	87	71
12	Mersey sediment	55	80	95	40

[a] Urea non-adduct expressed as a proportion of total alkanes.
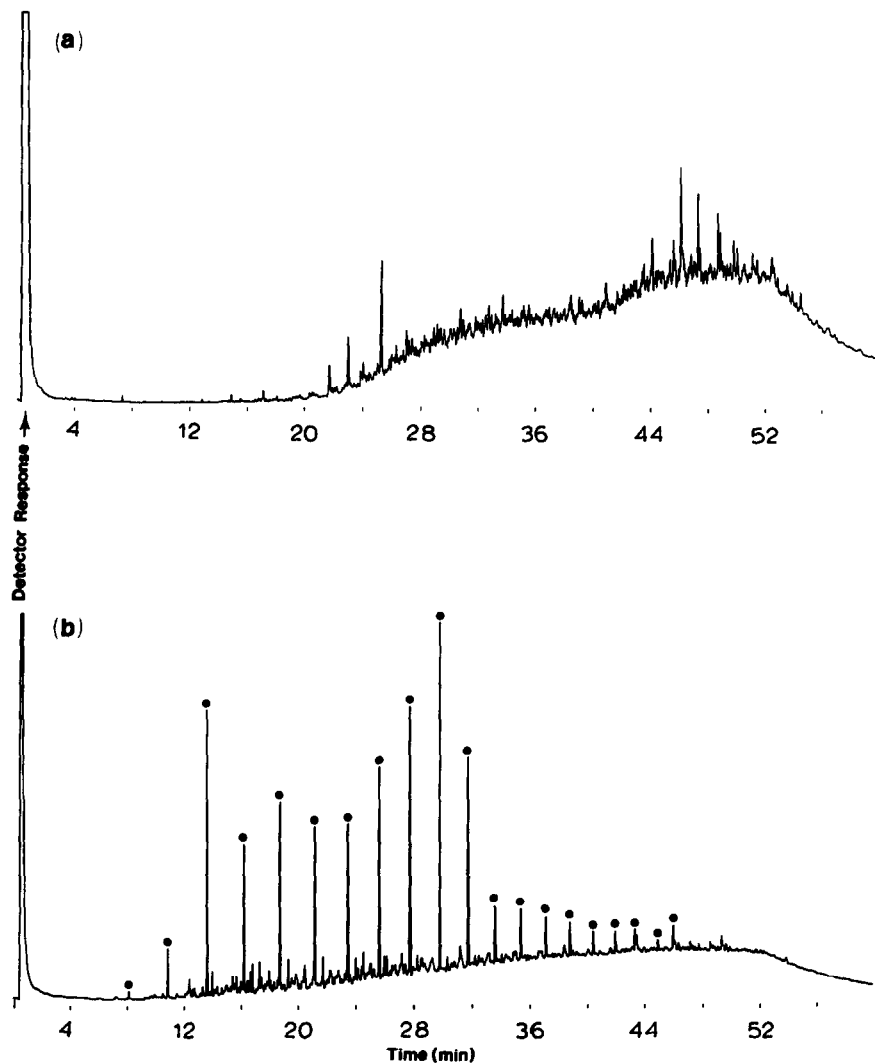[b] Oxidized material is that not eluting with hexane from a silica gel chromatographic column.

Fig. 1. Gas chromatograms of (a) fuel oil UCM and (b) total oxidation products. ● = n-Carboxylic acids.

products when 50-mg amounts of UCM isolated from grossly polluted sediments were oxidized. However, it was necessary to scale the method down for the present studies of long-term chronic hydrocarbon contamination as it was impracticable to isolate 50 mg of UCM from these sediments. About 2 mg was found to be the minimum practical working amount. To verify the oxidation method, 2- and 50-mg samples of lube oil (Silkolene 150) were oxidized (samples 1–3) and the results compared (Figs. 2 and 3). The two small-scale (2-mg) oxida-tions (samples 1 and 2) showed particularly good reproducibility and recovery (>90%), but they did not correlate well with the oxidation of the 50-mg sample (sample 3). This is not surprising. The oxidizing environments are very different owing to the different concentrations of oxidant involved (see Experimental). Initial results of the 2-mg duplicate analyses are encouraging, but further replication is obviously desirable, and will be carried out.
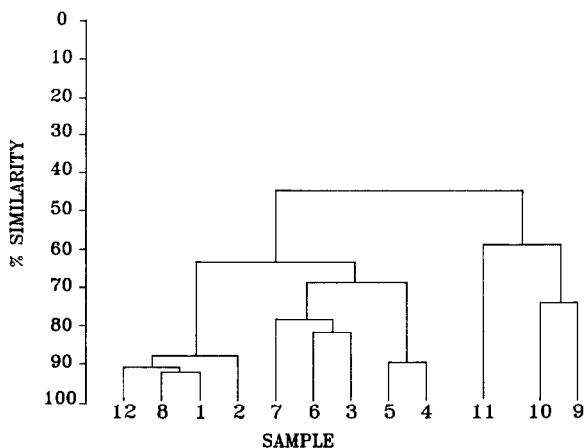
Fig. 2. Similarity dendrogram of all samples from cluster analysis. For sample identity, see Table I.

Sample 4–7: Amoco and Sivand oil spills

The initial UCM fingerprinting studies carried out by Gough and Rowland [10] involved two well documented, large oil spills. The qualitative results from these studies have been made quantitative and included in the present database for comparison. The results show a good correlation (ca. 90% similarity) between the Amoco tank oil and Milford Haven sediment as found previously [10]. The results for the Sivand oil (sample 6) and Humber sediment (sample 7) also show a good correlation (ca. 78%
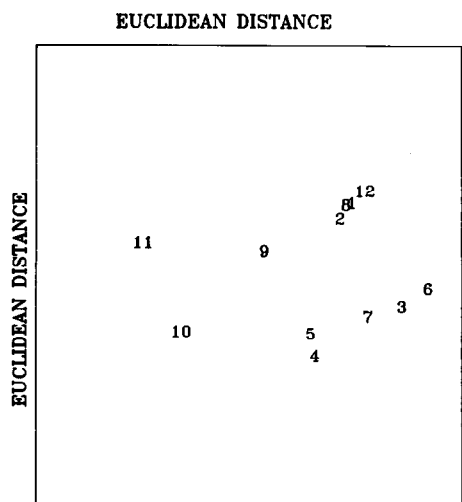

Fig. 3. All-samples plot of Euclidean distances from MDS analysis. For sample identity, see Table I.

similar), although not as good as in the previous example. The latter result is also in agreement with previous work [13], and suggests that there have been other inputs of hydrocarbons to the Humber sediment in addition to the Sivand oil.

Samples 8-10: Sullom Voe Oil Terminal

The Sullom Voe Oil Terminal (Shetland, UK) receives oil from the North Sea Ninian Field, which it then stabilizes and holds ready for transport. Despite the proximity of the oil terminal, numerous previous surveys of sublittoral sediments [27] in the area have shown that the concentrations of aliphatic hydrocarbons rarely exceed 100 μg/g dry sediment. However, in some sediments the GC profiles are mainly UCMs and interest has arisen as to their source. Several sources have previously been suggested, including a general background of chronic hydrocarbon accumulation, residues of Ninian crude oil possibly discharged with process waters, fuel oil spilled from the Esso Bernicia in 1978 [28] and a possible contribution from nearby peat deposits. Traditional biomarker techniques were not completely effective in fingerprinting these degraded oil residues, partly owing to difficulties caused by an input of biogenic triterpanes from nearby peat deposits [7,29]. The sterane distribution was more useful, but these compounds occur at lower concentrations in oils than the triterpanes and tend to be biodegraded more easily [30]. The results of UCM oxidation, GC–MS cluster and MDS analysis in this study showed that neither the Ninian (sample 9) nor the Bernicia (sample 10) oil UCMs were very similar ($\leqslant 50\%$ similarity, Fig. 3) to the UCM in the sediments. Indeed, the clustering of the sediment, close to the Silkolene lube oil samples, suggests that a chronic accumulation from sources such as road runoff may be a more likely source [31]. The peat was found to contain no UCM, so this source can also be discounted.

Samples 11 and 12: Mersey oil spill

On August 19th, 1989, there was an accidental spillage of 150 tonnes of Tia Juana Pesado (TJP; Venezuela) heavy asphaltic crude oil from the Shell refinery at Stanlow, Elsmere Port, into the River Mersey estuary [32]. Early analyses using GC–MS to monitor biomarkers found no direct evidence for the presence of TJP crude oil in the Mersey sediments

[32]. This was mainly due to the high background concentration (*ca.* 200 ppm) of biomarker hydrocarbons from other sources. As it is heavily biodegraded, gas chromatograms of TJP oil exhibit a significant UCM of hydrocarbons and it was hoped that the UCM composition of the oil could be compared with that of the sediment by the oxidation method. Results of these analyses for the sediment (sample 11) and the TJP oil (sample 12) confirmed the biomarker results (<50% similarity) that the spill of TJP has apparently had little impact on the overall sediment hydrocarbon loading (Figs. 2 and 3). TJP crude oil has a relatively low percentage of hydrocarbons (*ca.* 50%), of which 46% are aliphatic. This, combined with the already high background level of oil hydrocarbons in the sediments [32], suggests that the TJP is not a large contributor of UCM to the Mersey sediments.

CONCLUSIONS

The analysis of ten UCMs by oxidation, GC–MS and MDS has shown that the oxidation product profiles of these UCMs can be used to fingerprint different oils. The method is enhanced by the use of non-subjective multivariate statistical techniques and should prove useful in pollution studies and oil exploration as a means of characterizing and correlating degraded oils.

ACKNOWLEDGEMENTS

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