Influence of biodegradation on crude oil acidity and carboxylic acid composition

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Abstract

Quantitative analysis of separated carboxylic acid fractions of 33 crude oils from the UK, Italy and California, showed that the carboxylic acid fraction is a major factor responsible for the acidity in these oils. It was apparent that biodegradation is the main process that produces high concentrations of carboxylic acids in these crude oils with the extent of biodegradation, as measured from their hydrocarbon compositions, being clearly correlated with their total acid number (TAN). Although probably not important in influencing oil TAN, the distribution of C30–C32 hopanoic acids was also seen to be controlled by biodegradation, increasing in concentration for all but the most biodegraded oils. Hopanoic acids with the 17β(H),21β(H) stereochemistry were found in many of the biodegraded oils, and were thought to be mainly derived from the bacteria that were responsible for the biodegradation of the oil. This may have implications for the timing and mechanisms of the biodegradation involved. The role of C6–C3 alkylphenols in determining oil acidity was investigated and shown not to be a significant factor in the sample set studied. However, a number of undegraded oils, with low carboxylic acid contents were seen to have relatively high acidities, showing that factors other than biodegradation, possibly related to high sulphur content can control oil acidity in certain oil types.

Keywords: Oil acidity; Total acid number; TAN; Carboxylic acids; Naphthenic acids; Hopanoic acids; Alkylphenol; Crude oil biodegradation

1. Introduction

The acidity of a crude oil is measured by its total acid number (TAN), which is the number of milligrams of KOH required to neutralise the acidity in one gram of oil (Derungs, 1956). High TAN oils (>0.5 mg KOH/g) are less desirable than low TAN oils because of the corrosion and refinery problems they cause, and they therefore attract a much lower price (Babaian-Kibala et al., 1993; Turnbull et al., 1998). Petroleum acids have been termed naphthenic acids since the earliest identified acids were saturated cyclic carboxylic acids, although there are potentially many different classes of carboxylic acids present in crude oils (Derungs, 1956). The problem of naphthenic acid corrosion was first identified in the 1920’s during the refining of oils from Romania, California and Russia (Derungs, 1956), with the first major study on acid structures published by Lochte and Littman (1955). The occurrence of high TAN oils is widespread, e.g. Venezuela, Nigeria, Texas, North Sea (Jayaraman et al., 1986), and their exploitation is becoming more important with time (Babaian-Kibala et al., 1998). Oils can vary in TAN from <0.1, to as high as 8 mg KOH/g (Babaian-Kibala et al., 1998).

There have been reports of the detailed analysis of the carboxylic acid fraction of crude oils, with compounds identified including linear fatty acids, isoprenoid acids,
monocyclic, polycyclic and aromatic acids (e.g. Lochte and Littman, 1955; Carson and Graham, 1965; Seifurt and Teeter, 1970; Seifert, 1975). The cyclic acids have been classified according to their ring structure (e.g. Fan, 1991).

There appears to have been relatively little detailed work published on identifying the compound groups responsible for high TAN values in oils, although clearly the carboxylic, (including naphthenic) acids, which are known to be produced during the in-reservoir biodegradation of petroleum hydrocarbons, could be one such group (Mackenzie et al., 1983). The role of saturated $n$-acids in the control of oil TAN has been investigated, with both Jaffé and Gallardo (1993), and Barth et al. (1998) showing that these compounds exert very limited influence. It has been suggested that biodegradation is one of the processes which may lead to oils having high TAN values, though the relationship is not a simple one (e.g. Behar and Albrecht, 1984; Jaffé and Gallardo, 1993; Olsen, 1998). It has been suggested that the increase in concentration of acidic compounds observed in many biodegraded oils could be the result of either the neoformation of acids during biodegradation, or the preferential removal of non-acidic compounds, leading to a relative increase in the concentration of the acidic components (Behar and Albrecht, 1984). Organic acids will also be incorporated into the oil during source rock maturation (e.g. Mackenzie et al., 1983), although acetic acid is the major product of organic acid generation, with the concentration of longer chain acids produced rapidly decreasing with chain length (Barth and Bjørlykke, 1993). These short chained acids are probably not a major contributory factor to oil TAN, since they are very water soluble and will rapidly partition into the aqueous phase (e.g. Reinsel et al., 1994). The relatively high water solubility of lower molecular weight carboxylic acids mean that extensive water washing, often associated with biodegradation, will deplete these components, thus resulting in acid concentrations and distributions which may reflect the extent and timings of these two processes (Mackenzie et al., 1983). Thermal degradation may also control the fate of the acids produced during biodegradation, with the linear acids, and especially any unsaturated acids being removed preferentially to the other oil constituents (Mackenzie et al., 1983).

The carboxylic acid fraction of crude oils contains biomarkers which could potentially yield important information on the geological history of petroleum, although these compounds are not routinely analysed in petroleum exploration. However, the concentration and distribution of $C_{30}$–$C_{32}$ hopanoic acids have been used as indicators of oil maturation (Jaffé and Gardinali, 1990), migration (Jaffé et al., 1988a,b), and biodegradation (Behar and Albrecht, 1984; Nascimento et al., 1999). Recently, hopanoic acids have been shown to be formed by the oxidation of the corresponding hopanes during the laboratory biodegradation of crude oils (Watson et al., 1999).

Other groups of compounds which could influence the TAN of an oil include low molecular weight alkylphenols ($C_0$–$C_3$ alkylphenols), which occur widely in crude oils (Ioppolo et al., 1992). For example, Samedova and Guseinova (1993) noted that in a study of low wax, high TAN crude oils from Azerbaijan, the phenol contents were between two and seven times higher than those of the carboxylic acids.

In this study we investigate the influence of the carboxylic acid fraction on oil acidity, by comparing the TAN values of 14 undegraded, 13 biodegraded and 6 mixed crude oils from various geological settings and geographical locations world-wide, with the quantitative analysis of their separated carboxylic acid fractions. The relationship between oil TAN, carboxylic acid fraction, sulphur and $C_0$–$C_3$ alkylphenol content with the extent of biodegradation is also investigated in these oils.

2. Materials and methods

2.1. Samples

The sample set comprised 33 oils, 24 from offshore UK (from 13 fields), 4 from Italy (4 fields), and 5 from a single Californian field.

2.2. TAN and sulphur analysis

The TAN value, and total sulphur content of 19 of the oil samples were obtained by standard methods ASTM D 664 and ASTM D 2622 respectively. The TAN value for each of these oil samples was measured in duplicate. Details of the methodology of the TAN analysis, together with accuracy and precision data are given in the ASTM D 664 method description. Although this is a standard method for the measurement of oil acidity there are a number of potential causes of inaccuracy which are described by Piehl (1988). The data for the remaining 14 oils was obtained from oil company assay reports, with analysis by the same standard methods.

This sample set contains a number of oils from single fields, e.g. 5 oils from field M and 5 oils from field J. In both cases measured TAN and sulphur data was only measured for one of the oils from each field, with data for the other oils coming from oil company assay reports. Individual assays were not available for all the oils, and so in some instances a single assay was used for oils from adjacent or nearby wells. However, since many of other parameters measured from these oils were virtually identical it is assumed that the TAN values would also be similar.
2.3. Hydrocarbon analysis

The saturated and aromatic hydrocarbon fractions were isolated from each oil by thin layer chromatography using 0.5 mm thickness Merck Kieselgel 60G plates, and light petroleum (b.p. 40–60°C) as developer, in a similar way to that described in Farrimond et al. (1994). The fractions were then analysed by gas chromatography (GC), and gas chromatography–mass spectrometry (GC–MS), in order to assess the degree of biodegradation, maturity and source characteristics of the oils. Two samples were analysed in triplicate to assess the repeatability of the technique, with procedural blanks analysed to assess contamination.

Gas chromatographic analyses were carried out on a Hewlett Packard 5890A-II instrument fitted with a Hewlett Packard 7672 autosampler. A Hewlett Packard HP-5 phenylmethylsilicone coated (0.25 μm film thickness) fused silica column (25 m × 0.25 mm i.d.) was employed, using hydrogen as carrier gas, and a flame ionisation detector. Splitless injection was used, with an oven programme of 50°C (2 min) to 300°C at 4°C min⁻¹, where it was held for 20 mins. Data were acquired and processed using a PC based, LabSystems XChrom data system.

GC–MS analyses were carried out on a Hewlett Packard 5890A-II-5972 MSD quadrupole instrument fitted with a similar HP-5 column, with hydrogen as the carrier gas. Automated splitless (1 min) injection was used and, for the saturated hydrocarbons, the GC oven temperature programme was 40–175°C at 10°C min⁻¹, 175°C (1 min) to 225°C at 6°C min⁻¹ and 225°C (1 min) to 300°C at 4°C min⁻¹, held for 18 min. Aromatic hydrocarbon fractions were analysed with an oven programme of 40°C (3 min) to 300°C (12 min) at 4°C min⁻¹. Quantification was by comparison with the peak area of hydrocarbon analysis. Quantification was by comparison of analyte peak areas with that of the internal standard. The response factors of the analyte compounds were assumed to be unity, whilst the response factors of the surrogate standards were calculated and corrected for. Recovery of the surrogate standards throughout the procedure was found to be approximately 90%. Oils spiked with a suite of n-alkanoic acid standards in the range n-C₁₀:0 to n-C₃₀:0 were analysed, and recoveries of approximately 61% for n-C₁₀:0, 90% for n-C₁₄:0, and 45% for n-C₃₀:0 were obtained. The recoveries of aromatic acids were much lower, e.g. benzoic acid 16% and 2-naphthoic acid 30% (Jones et al., unpublished results). It was thought that the poor recovery of the aromatic acids may be due to the inefficient methylation of these acids by BF₃-methanol (e.g. Behar and Albrecht, 1984). The re-analysis of acid fractions from seven of these oils using diazomethane as a methylaing agent showed no significant difference in appearance in the gas chromatograms of the extracted acids (as methyl esters), and the total concentrations of the extracted acid fractions were within ±10% of the results obtained using BF₃-methanol. Therefore it was assumed that the aromatic acids were not a major component of the total carboxylic acid fraction of these oils. Five samples were analysed in triplicate to assess repeatability of the method used, with procedural blanks analysed to check for contamination. It was found that n-C₁₆:0 and n-C₁₈:0 fatty acid contaminants were introduced during the ion-exchange separations; these were quantified and corrected for during final quantitation.

The concentrations and distributions of the hopanoic acids were investigated by GC–MS, under the same conditions as the saturated hydrocarbon analysis, with the data acquired in both the selective ion monitoring (SIM), and scan modes. The concentrations of the C₃₀−C₃₂ hopanoic acids were measured by comparison of their peak areas in their respective m/z 235, 249, 263 mass chromatograms, with that of the added standard 5β-cholanic acid in the m/z 217 mass chromatogram. Response factors of unity were used, so the hopanoic acid data should be considered as semi-quantitative.

2.4. Carboxylic acid extraction

The carboxylic acid fraction was extracted from each oil sample by a novel non-aqueous ion exchange method (unpublished results). This method is, briefly, as follows. Aliquots (0.4 g) of each oil were loaded onto a 4 g SAX quaternary amine solid phase extraction (SPE) column, with the non-acid compounds removed by successive elutions with n-hexane, and dichloromethane (DCM). The crude carboxylic acid isolate was then eluted with diethyl ether containing 2% (v/v) formic acid. This fraction was methylated with boron trifluoride-methanol (BF₃-methanol) (60°C for 1 h), and cleaned up by eluting through a silica SPE column with 60% / 40% (v/v) n-hexane/DCM. 1-adamantane carboxylic acid (Fluka, U.K.) and 5-cholanic acid (Sigma, UK) were used as surrogate (extraction efficiency) standards, and the methyl ester of 1-phenyl-1-cyclohexane carboxylic acid as an internal standard for quantification purposes. Analysis was by gas chromatography as for the saturated hydrocarbon analysis. Quantification was by comparison of analyte peak areas with that of the internal standard. The response factors of the analyte compounds were assumed to be unity, whilst the response factors of the surrogate standards were calculated and corrected for. Recovery of the surrogate standards throughout the procedure was found to be approximately 90%. Oils spiked with a suite of n-alkanoic acid standards in the range n-C₁₀:0 to n-C₃₀:0 were analysed, and recoveries of approximately 61% for n-C₁₀:0, 90% for n-C₁₄:0, and 45% for n-C₃₀:0 were obtained. The recoveries of aromatic acids were much lower, e.g. benzoic acid 16% and 2-naphthoic acid 30% (Jones et al., unpublished results). It was thought that the poor recovery of the aromatic acids may be due to the inefficient methylation of these acids by BF₃-methanol (e.g. Behar and Albrecht, 1984). The re-analysis of acid fractions from seven of these oils using diazomethane as a methylaing agent showed no significant difference in appearance in the gas chromatograms of the extracted acids (as methyl esters), and the total concentrations of the extracted acid fractions were within ±10% of the results obtained using BF₃-methanol. Therefore it was assumed that the aromatic acids were not a major component of the total carboxylic acid fraction of these oils. Five samples were analysed in triplicate to assess repeatability of the method used, with procedural blanks analysed to check for contamination. It was found that n-C₁₆:0 and n-C₁₈:0 fatty acid contaminants were introduced during the ion-exchange separations; these were quantified and corrected for during final quantitation.

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2.5. Alkylphenol analysis

The alkylphenols were extracted from each oil sample by the C18 silica solid phase extraction method, described by Bennett et al. (1996). After extraction, the alkylphenol fractions were derivatised with N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) (60°C for 1 h) and analysed by GC–MS, as for the hydrocarbon analysis except for the following oven program: 35°C (10 min) to 150°C at 2°C min⁻¹ and from 150°C to 300°C at 8°C min⁻¹, held for 20 min. Data were acquired in the SIM mode. Quantification was by comparison with the peak area of
the internal standard, 2-naphthol, with correction for its response factor. Two samples were analysed in triplicate to assess repeatability, with procedural blanks analysed to check for contamination.

3. Results

3.1. TAN and sulphur data

The TAN data from both the direct analysis of the samples, and from oil company assay reports are summarised in Table 1. The samples have a range of TAN from 0.1 to 2.67 mg KOH/g, with those oils having a TAN > 0.5 mg KOH/g being classified as high TAN.

The sulphur content of these samples range from 0.24 to 4.8% by weight. With the exception of one sample with a sulphur content of 3.1%, the oils from the UK are generally low sulphur oils. In contrast, the oils from Italy and California are high sulphur, with a content greater than 1% by weight.

3.2. Hydrocarbon analysis

Gas chromatograms of the saturated hydrocarbon fraction from typical examples of an undegraded oil, a moderately degraded oil, a severely degraded oil and a mixed oil are shown in Fig. 1. The chromatograms of the undegraded oils (e.g. oil no. 5 — UK) show a complete envelope of \( n \)-alkanes, which are reduced in a moderately degraded oils (e.g. oil no. 30 — California), whilst heavily degraded oils (e.g. oil no. 13 — UK) are dominated by resistant biomarker compounds. Some of the oils (e.g. oil no. 22 — UK) appeared undegraded, although they contained 25-norhopanes which was attributed to the mixing of a fresh oil with a heavily degraded component on which is superimposed a homologous series of saturated \( n \)-acids from each oil is shown in Table 1.

The ratios of a wide range of source and maturity dependent biomarkers were determined from GC–MS analysis of both the saturated and aromatic hydrocarbon fractions. These ratios could potentially be influenced by the extensive biodegradation apparent in many of the oils. All of the oils were thought to be Type II (except the Californian oils which were Type III) marine sourced oils. The maturity of the oils was variable with the data of one of the maturity parameters, the relative concentration of the \( C_{29} \) hopanoic acid fraction, the relative concentration of the saturated \( n \)-acids and the concentration of the aromatic steroid fraction. Together these ratios give an indication of the maturity of the oils.

The C-Ratios, C-Hopanoic acids and C-Aromatic steroids are shown in Table 1. Gas chromatograms of the aromatic steroid fraction from an undegraded oil, a moderately degraded oil, a highly degraded oil and a mixed oil are shown in Fig. 1. The gas chromatogram of a typical undergraded oil (e.g. oil no. 5) is dominated by a homologous series of saturated \( n \)-acids (\( C_{29} \)) with a low total concentration of \(< 300 \mu g/g\). The \( n \)-acids are also apparent in the gas chromatogram of a moderately degraded oil (e.g. oil no. 30), together with a large number of unidentified peaks and an unresolved complex mixture (UCM) of medium molecular weight branched and cyclic carboxylic acids. The \( n \)-acids were not prominent in the gas chromatograms of a typical highly degraded oil (e.g. oil no. 13), which is instead dominated by a higher molecular weight UCM, with a much higher total concentration of acids (up to 9000 \( \mu g/g\)).

3.3. Carboxylic acid analysis

The concentration of the total carboxylic acid fraction, and the total concentration of \( n \)-acids from each oils are shown in Table 1. Gas chromatograms of the carboxylic acid fraction from an undegraded oil, a moderately degraded oil, a highly degraded oil and a mixed oil are shown in Fig. 1. The gas chromatogram of a typical undergraded oil (e.g. oil no. 5) is dominated by a homologous series of saturated \( n \)-acids (\( C_{29} \)) with a low total concentration of \(< 300 \mu g/g\). The \( n \)-acids are also apparent in the gas chromatogram of a moderately degraded oil (e.g. oil no. 30), together with a large number of unidentified peaks and an unresolved complex mixture (UCM) of medium molecular weight branched and cyclic carboxylic acids. The \( n \)-acids were not prominent in the gas chromatograms of a typical highly degraded oil (e.g. oil no. 13), which is instead dominated by a higher molecular weight UCM, with a much higher total concentration of acids (up to 9000 \( \mu g/g\)).

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Table 1
Location of and geochemical data from the 33 oils in the sample set

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Field Location</th>
<th>Degree of biodegradation</th>
<th>TAN (mg KOH/g)</th>
<th>Total acids (µg/g)</th>
<th>Calculated TAN (mg KOH/g)</th>
<th>% Of measured TAN</th>
<th>Total n-acids (µg/g)</th>
<th>Total ββ hop acids (ng/g)</th>
<th>Total βα hop acids (ng/g)</th>
<th>Total ββ hop acids (ng/g)</th>
<th>Sulfur wt.%</th>
<th>Total C0–C3 alkylphenols (µg/g)</th>
<th>Pristane / nC17 C29 sterane (zβ/zβ + zββ) ratio</th>
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<tbody>
<tr>
<td>1</td>
<td>A UK 1</td>
<td>0.05a</td>
<td>178 0.03</td>
<td>61</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.38a</td>
<td>35</td>
<td>0.60</td>
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<tr>
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a Data from assay reports.

b Scale of Peters and Moldowan (1993).

c See text for details.
d n.d., not detected.
Fig. 1. Gas chromatograms of saturated hydrocarbon fractions and corresponding carboxylic acid fractions of: A — undegraded oil (no. 5); B — moderately degraded oil (no. 30); C — extensively degraded oil (no. 13) and D — mixed oil (no. 22). Peak numbers denote carbon number of homologues, IS and SS denote internal and surrogate standards, respectively.

### Diagram Description

- **Saturated Hydrocarbon Fraction**
  - A: Undegraded oil (no. 5)
  - B: Moderately degraded oil (no. 30)
  - C: Extensively degraded oil (no. 13)
  - D: Mixed oil (no. 22)

- **Carboxylic Acid Fraction**
  - A: Internal and surrogate standards (IS and SS)
  - B: Increased degree of biodegradation
  - C: Further increased degree of biodegradation
  - D: Highest degree of biodegradation

### Retention Time (mins)

Retention time ranges from 0 to 80 minutes, showing a progression from left to right in the diagram.
3.4. Alkylphenol analysis

The concentration of the C₀–C₃ alkylphenols were measured from the relevant mass chromatograms (m/z 151 for phenol, m/z 165 for the cresols, m/z 179 for the dimethylphenols and m/z 193 for the trimethylphenols), as reported by Bennett et al. (1996). As shown in Table 1 the total concentration of C₀–C₃ alkylphenols in these samples varies between 1 and 116 μg/g.

4. Discussion

4.1. Contribution of isolated carboxylic acids to oil TAN

The strong correlation ($r^2 = 0.91$) between the concentration of the carboxylic acid fraction and TAN (shown in Fig. 3), suggests that this fraction is a major contributor to the TAN of these oils. This correlation can be seen to be valid for oils from a variety of different areas and different geological settings (UK, Italy, California), with different source environments and maturation histories.

Below TAN values of 0.5 mg KOH/g the correlation between TAN and total acid concentration is not as clear as that for the higher TAN oils. This indicates that compounds other than carboxylic acids contribute significantly to the TAN in these oils. The poor correlation for the low TAN oils may also be in part due to the analytical errors. Variation in duplicate TAN analysis was around ±0.1 mg KOH/g, which is of greater significance in low TAN compared to high TAN oils. In addition relative errors in the acid fraction quantitation are greater in low TAN oils, for example, in replicate analyses of oils with acid concentrations below 200 μg/g the relative standard deviation can be up to 17% (at higher acid concentrations of 4000 μg/g this error decreased to around 7%).

However, some individual oils lie away from the trend line and may have unique features which require further investigation that could provide key insights into what other geochemical characteristics control oil TAN. For example, sample nos. 7, 26 and 31 (from the UK, Italy...
and California, respectively), all of which have high sulphur contents, can be seen to have high acidities, (0.6, 0.72 and 1.0 mg KOH/g), despite having low carboxylic acid contents (245, 210 and 534 μg/g). The recovery of the surrogate standards for these oils were comparable to that of the remainder of the sample set, and so the low concentration of carboxylic acids isolated from these samples is not an analytical artefact.

If one assumes that the “average” acid is a C22 compound, with a saturated tricyclic structure, an alkyl side chain and one carboxylic acid group (e.g. Olsen, 1998; Tomczyk et al., 1998), it would have an average molecular weight of 334. This is consistent with the retention position of the UCM maxima in the chromatograms of our isolated acid fractions. It is therefore possible to calculate the expected TAN of each oil, if it was controlled by its carboxylic acid content alone. The results of these concentrations are shown in Table 1. Excluding the low TAN oils (<0.5 mg KOH/g), the calculated TANs are between 26 and 65% of the measured values. The exceptions are three high sulphur oils (sample nos. 7, 26 and 31) whose carboxylic acid content appears to contribute less than 10% to the total oil TAN, indicating that some other group of compounds is controlling the TAN of these oils. The apparently low contribution to the total TAN of the isolated carboxylic acid fraction may be because either the average structure assumed is incorrect (in molecular weight, or with dicarboxylic acids being a major component), or that non-GC amenable, higher molecular weight acids are also contributing to the TAN of the oils. However, it is still apparent that in the majority of these oils, the carboxylic acid fraction is a major contributor to oil TAN.

There is no clear trend in relationship between the n-acid concentration of these oils and their TAN (Fig. 4), although there is some evidence of a trend of decreasing n-acid concentration with increasing TAN. The scatter on the plot is due in part to the effect of the samples with mixed compositions, which have a n-acid distribution reflecting the undegraded component of the oil, while the acidity is due in most part to the biodegraded component. These findings support earlier work (e.g. Jaffé and Gallardo, 1993; Barth et al., 1998) which showed that n-acids do not contribute greatly to the TAN of an oil.

4.2. Role of biodegradation in controlling oil TAN

The relationship between the pristane/nC17 ratio and TAN for these oils is shown in Fig. 5. Amongst the undegraded oils from both the UK and Italy there is little variation in this ratio. This suggests that the variation in TAN apparent in these oils is due to processes other than biodegradation. Amongst the four Californian oils a trend of increasing TAN with increasing pristane/nC17 is apparent. Although these oils are thought to share a similar source, factors other than biodegradation may be influencing this ratio, which in addition to the small sample set may be reflected in the relatively poor correlation of this relationship. However, this data is sufficient to suggest that for these oils, of up to moderate degradation, the degree of biodegradation is an important controlling factor on the ultimate TAN. This conclusion is supported by the work of Olsen (1998), who demonstrated a strong correlation.
between TAN and biodegradation (assessed by the phytane / nC\textsubscript{18} ratio) for a suite of Norwegian oils.

The relationship between the degree of biodegradation (as defined by the scale of Peters and Moldowan, 1993) and TAN (Fig. 6) shows a trend of increasing TAN with an increase of biodegradation, although there is some degree of scatter ($r^2 = 0.74$). The relationship between the level of biodegradation, and the concentration of carboxylic acids in these oils (Fig. 7) shows a very similar trend to that in Fig. 6, although the correlation is stronger ($r^2 = 0.86$). There is a little scatter in the concentration of carboxylic acids in the undegraded oils, which indicates that compounds other than carboxylic acids are responsible for the high acidity of some of the undegraded oils. These data, therefore, strongly suggest that it is the degree of biodegradation of an oil which controls the concentration and distribution of carboxylic acids.

These data support the hypothesis that biodegradation is a major control of oil TAN, which has been previously alluded to (e.g. Behar and Albrecht, 1984; Jaffé and Gallardo, 1993; Olsen, 1998), but never demonstrated for a large sample set. Although some minor increase in TAN may occur by the preferential removal of other groups, the tenfold increase in the carboxylic acid concentration between the least and the most degraded oils strongly suggests that the neoformation of these acids during biodegradation is the major process leading to high TAN values.

The results of this study suggest that the primary geological and geochemical conditions that appear to give rise to most high TAN, acid crudes are those that favour biodegradation. Those conditions are generally found in reservoirs that are (or were at one time) shallow, with temperatures lower than about 100°C (e.g. Bailey et al., 1973; Connan, 1984). Traditionally, in-reservoir oil biodegradation has been associated with oxygenated meteoric waters (e.g. Palmer, 1993), and carboxylic acids are known to be intermediates during the aerobic biodegradation of petroleum hydrocarbons (for proposed pathways, see Singer and Finnerty, 1984). More recently there has been evidence that anaerobic degradation is possible, although the mechanisms have tended to be speculative (e.g. Stetter et al., 1993; Rueter et al., 1994; Wilkes et al., 1995; Heider et al., 1999). However, very recently Zengler et al. (1999) reported that long chain alkanes can be metabolised anaerobically by a consortium of bacteria resulting in the formation of methane. It is not clear whether it is aerobic or anaerobic biodegradation processes which cause the increase in acidity seen in these oils.

4.3. Distribution of hopanoic acids

The relationship between the total C\textsubscript{30}–C\textsubscript{32} hopanoic acid concentration of these oils, and both their degree of biodegradation and TAN (Fig. 8), shows that there is initially a trend of increasing hopanoic acid concentration with increasing TAN, to a maximum TAN value of approximately 2.0 mg KOH/g ($r^2 = 0.91$). Many of the undegraded oils contain no detectable hopanoic acids and in those undegraded oils which do contain them, they are present in very low concentrations (< 500 ng/g). With an increase in degree of biodegradation through the moderately degraded Californian oils, the concentration
of hopanoic acids can be seen to increase. The maximum hopanoic acids contents found were present in the extensively or severely degraded oils UK oils (~5000 ng/g). After this maximum, the concentration of hopanoic acids appears to rapidly decrease, with the most degraded sample (no. 18), containing no detectable hopanoic acids.

The distribution of the C_{30}-C_{32} hopanoic acids between the three stereochemical configurations are shown in Fig. 9. Both the total 17\(\alpha\),21\(\beta\) and 17\(\beta\),21\(\alpha\) acids show an initial increase in concentration with increasing TAN, although there is significant scatter in the data. This trend continues to a maximum TAN value of approximately 1.5 mg KOH/g, (and extensive levels of biodegradation) before the concentration of hopanoic acids decreased to zero in the most severely degraded oil.

The total 17\(\beta\),21\(\beta\) acids show a relatively minor increase in concentration with increasing TAN and degree of biodegradation up to moderately degraded oils, with a TAN of 0.5 mg KOH/g. The extensively degraded oils show a dramatic ten fold increase in concentration of \(\beta\) hopanoic acids to a maximum of 4500 ng/g, at a TAN of approximately 2 mg KOH/g. As with the \(\alpha\)\(\beta\) and \(\beta\)\(\beta\) acids, there was then a sharp decrease to zero in \(\beta\)\(\beta\) acid concentration in the most severely degraded oil.

The hopanoic acid concentrations in the mixed oils obscure the above trends, and so are not included on these plots. Sample no. 19 has a very high hopanoic acid content (6045 ng/g), dominated by \(\beta\)\(\beta\) acids (3964 ng/g), despite a relatively low TAN of (0.3 mg KOH/g). The other mixed oils, which are all from the same UK field (field M) show considerable variation in their total hopanoic acid content (20-2969 ng/g to 22-5099 ng/g), which are predominantly of the \(\alpha\)\(\beta\) and \(\beta\)\(\beta\) configurations. This suggests these acids may be a preserved signal of biodegradation heterogeneity within the field before the second charge of oil occurred, or that alternatively some process other than biodegradation are responsible for the distribution of hopanoic acids seen in these oils.

The observed trend of increasing hopanoic acid concentration with increasing degree of biodegradation (Fig. 8), is in contrast to a study by Behar and Albrecht (1984), which showed an apparent decrease in hopanoic acid concentration with an increasing degree of biodegradation amongst five unrelated oils. The observed initial increase in hopanoic acid content with increasing biodegradation of the oils in this present study could be due to the preferential removal of other compounds during biodegradation. However, it is more probable that this trend reflects either the incorporation of these acids during migration, or the neoformation of hopanoic acids within the biodegraded oils.

It has been suggested that the incorporation of hopanoic acids during migration can result in an isomeric distribution of these acids which can be used to describe the maturity of the rocks through which an oil had migrated (Jaffé et al., 1988a, b). The \(\beta\)\(\beta\) configuration is the least thermodynamically stable of the three isomers, and their presence in an oil has been suggested to indicate the incorporation of immature organic matter during migration (Jaffé et al., 1988a). The \(\alpha\)\(\beta\) and \(\beta\)\(\alpha\) hopanoic acids are formed from the isomerisation of \(\beta\)\(\beta\) hopanoids via diagenetic processes (e.g. Seifert, 1975). Therefore, oils containing only the \(\alpha\)\(\beta\) and \(\beta\)\(\alpha\) acids were thought to have migrated through relatively mature rocks (Jaffé and Gallardo, 1993). It is likely that many of the UK oils would have migrated through Cretaceous and Tertiary mudstones (Lovell, 1990), which due to their depth and consequent temperature would have a maturity which would preclude the presence of \(\beta\)\(\beta\) hopanoic acids. Consequently, it is unlikely that these oils would have incorporated any \(\beta\)\(\beta\) acids during their secondary migration.

An alternative mechanism for the incorporation into crude oils of \(\beta\)\(\beta\) acids at relatively high levels of maturity has been proposed by Jaffé and Gardinali (1990). Those authors indicated that between vitrinite reflectance maturity measurements of approximately 0.5–0.8% Ro, there is a secondary generation of hopanoic acids which had been tightly bound to organic geopolymers within the source rock. These acids retained their original immature configuration and could give rise to \(\beta\)\(\beta\) acids within the produced oil (Jaffé and Gardinali, 1990; Jaffé and Gallardo, 1993). However, the absolute concentrations of \(\beta\)\(\beta\) hopanoic acids produced during secondary generation have been shown to be very minor compared to the concentration of hopanoic acids produced during migration.
their primary generation at lower maturities (Jaffé and Gardinali, 1990; Bennett and Abbott, 1999). In this present study the concentration of \(\text{bb}\) hopanoic acids can be seen to significantly increase in the biodegraded oils. Therefore, the hypothesis of secondary generation of these acids is unlikely to account for the high concentrations of \(\text{bb}\) acids found in many of these highly degraded oils.

The stereochemistry of the hopanoids isolated from bacteria is always \(17\beta(H),21\beta(H)\) (Rohmer et al., 1992), and the predominance of \(\beta\beta\) acids in many of the degraded oils may suggest a biological origin. It can be seen in Fig. 2 that the distribution of hopanoic acids in the degraded oil (sample no. 13) is dominated by the \(\text{C}_{32}\) \(\beta\beta\) \([R]\) isomer. This distribution is also seen in the other severely degraded oils with high hopanoic acid contents.

The dominance of the \(\text{C}_{32}\) \(\beta\beta\) isomer over the other homologues has been previously reported in recent sediments (e.g. Buchholz et al., 1993; Ries-Kautt and Albrecht, 1989), and is thought to be due to the oxidation and side chain cleavage of the extended \(\text{C}_{35}\) tetra-functionalised biohopanoid precursors during diagenesis (Innes et al., 1997). Tetra-functionalised biohopanoids have been noted to be the most abundant type of functionalised hopanoids in recent sediments (Farrimond et al., 2000).

The presence of \(\beta\beta\) acids in relatively high proportions in some of these oils suggests that they may be derived from the biomass of the micro-organisms responsible for biodegrading the oil. Since this isomeric configuration is the least thermodynamically stable of the three isomers it also suggests that either biodegradation is on-going in these reservoirs, or that since it
ceased the oils have not been subjected to temperatures high enough to cause the isomerisation of the ββ acids to the αβ or βα forms. Another potential influence on the distribution of hopanoic acids in biodegraded oils is the neoformation of αβ and βα hopanoic acids from the microbial oxidation of hopane hydrocarbons in the original oil (Watson et al., 1999).

The observed variations in the isomeric distribution of the hopanoic acids may also give insights on the mechanisms of the biodegradation processes affecting these oils. The hopanoids which are the precursors of these acids have to date not been detected in obligate anaerobic bacteria, and are present only in aerobes or facultative anaerobes (Ourisson et al., 1987). Therefore, the presence of hopanoic acids suggests that these oils may have been biodegraded at least partly by aerobic processes. Alternatively, it may be possible that types of anaerobic bacteria as yet unidentified, which contain hopanoids were involved.

4.4. Role of sulphur content in controlling oil TAN

In the 33 oils analysed in this study there is no clear relationship between their sulphur content and TAN (Fig. 10). Generally the data is very scattered, but by dividing it into oils from geological areas it can be inferred that the extremes in the sulphur contents are probably dependent on source environment, whereas the TAN of an oil is not. When this relationship is investigated for the UK oils separately, with the exception of the one anomalously high sulphur oil, there does seem to be a slight trend of increasing sulphur content with increasing TAN (Fig. 10). This trend has been reported previously (e.g. Gransch and Posthuma, 1974), and it is probably a biodegradation effect, where the relatively more resistant sulphur containing compounds are concentrated in the oil, as the less resistant hydrocarbons are removed.

However, as noted previously sample nos. 7 and 26, are undegraded, and no. 31 is slightly degraded. These oils contain low carboxylic acid contents, but have high acidities. They also have relatively high sulphur contents, which may play a role in controlling the TAN of these oils since there have been reports that unbiodegraded high TAN oils, which have high sulphur contents occur in the Gulf of Mexico (B.A. Stankiewicz, Shell, pers. comm.).

4.5. Influence of maturity on oil TAN

An example of the relationship between the TAN and maturity (as assessed by the \( \frac{C_{29} \alpha \beta \beta}{C_{29} \alpha \alpha \alpha + \alpha \beta \beta} \) sterane ratio) of these oils is shown in Fig. 11. Within each geographical region there is little variation in maturity (with the exception of one UK outlier), but great variation in the TAN of the oils. It has been reported that thermally immature oils may have high acidities (Seifert, 1975) but this sample set does not contain sufficient low maturity oils to test this hypothesis.

4.6. Role of alkylphenols in controlling oil TAN

The relationship between the C0–C3 alkylphenol concentration and TAN is illustrated in Fig. 12, and shows a considerable scatter with no obvious trends. The absence of an observed increase in alkylphenol concentration with an increase in TAN suggests that alkylphenols do
not exert a dominant influence over the TAN in these oils, although due to their acidic nature it is envisaged that they may play a minor role.

The distribution of alkylphenols within these oils may be due to many interacting factors, including migration biodegradation and water washing (Taylor, 1994). Alkylphenols are an intermediate in a number of degradative pathways of petroleum components and it has been proposed that they may be formed during the microbial alteration of aromatic hydrocarbons in crude oil (e.g. Kaphammer et al., 1990; Gibson, 1991). The anaerobic degradation of aromatic hydrocarbons in petroleum also has the potential to produce alkylated phenols (e.g. Vogel and Grbic-Galic, 1986; Grbic-Galic and Vogel, 1987). However, the alkylphenols produced could then be consumed by further biodegradation resulting in the complete oxidation to mineralised products which can occur under both aerobic and anaerobic conditions (Howard et al., 1991). Water washing of the oil, which is often associated with biodegradation would selectively remove many of the alkylphenols, especially those of lower molecular weight which are more water soluble (Taylor et al., 1997). Therefore, although it is clear that C₀–C₃ alkylphenol concentrations are controlled by a number of factors, in this present work they show no relationship with oil acidity.

5. Conclusions

This study shows that carboxylic acids are a major group of compounds responsible for the high TAN values of the oils studied, and that biodegradation is the dominant process that produces high concentrations of carboxylic acids in these oils. However, there are examples of undegraded oils with significant TAN values, which suggests that factors other than biodegradation can result in high TAN oils. Such factors may be related to sulphur content which depends on the source rock depositional environment, and possibly the maturity of an oil.

This study supports previous reports in showing that the n-acid concentration of an oil does not significantly influence its TAN, and there is also strong evidence that the C₀–C₃ alkylphenol content of oil does not contribute greatly to oil TAN.

The distribution and concentration of C₃₀–C₃₂ hopanoic acids can be seen to be partially controlled by biodegradation. A trend of increasing concentration of these acids, with increasing biodegradation is apparent for all but the most degraded oil, with the hopanoic acids in the extensively degraded oils being predominantly C₃₂ compounds of the 17β(H), 21β(H) configuration. These hopanoic acids with the “biological” ββ configuration, are thought to be derived from the bacteria responsible for the biodegradation of the oil, and the presence of this thermodynamically unstable configuration in crude oils may provide information on the timing of the biodegradation. Since hopanoids have not been detected in obligate anaerobic bacteria, the presence of the ββ hopanoic acids in biodegraded oils indicates that either the degradation conditions were not strictly anaerobic, or that the degradation involved a type of previously unreported hopanoid containing anaerobic bacteria.

Further work is being undertaken to investigate the effects of source, migration, maturity and environment of degradation on the few oils that plot away from the carboxylic acid concentration / degree of biodegradation versus TAN trend line.

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