Structural characterisation of groundwater hydrophobic acids isolated from the Tomago Sand Beds, Australia

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Received 22 March 2004; accepted 7 October 2004
(returned to author for revision 28 June 2004)
Available online 8 December 2004

Abstract

Groundwater hydrophobic acids isolated from the Tomago Sand Beds, Australia have been structurally characterised using multiple analytical techniques. Temporal variability was observed during the study period (1996–2003) and data for two representative samples isolated in 1996 and 2002 are presented. The sample isolated in 2002 was a highly aromatic fulvic acid with branched aliphatic functional structures and high oxygen content. It differed from structural concepts which suggest that groundwater hydrophobic acids isolated from organically lean aquifers are predominantly aliphatic in nature, with a low oxygen content resulting from extended degradation processes. Data are consistent with the hydrophobic acids having inputs from both surface recharge and mineralised sedimentary organic carbon. The sample isolated in 1996 was significantly more aliphatic, indicating that a change in microbial activity/community has occurred at the site.

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1. Introduction

Groundwater is an important source of potable water worldwide and its contamination with organic and inorganic compounds is a major concern. The dissolved organic carbon (DOC) in groundwater contains a significant amount of humic substances (HSs) derived from the breakdown of biogenic matter (Thurman, 1985a,b). HSs bind and transport beneficial and pollutant species such as metal ions and organic compounds in a range of aquatic and terrestrial environments (Koopal et al., 2001), and in groundwaters they have been shown to increase the solubility of pesticides and form mutagenic compounds during the chlorination of drinking water (Johnson-Logan et al., 1992; Koivusalo and Vartiainen, 1997). They have a significant geochemical role and understanding the structural features of HSs has been identified as a basic requirement for comparison of samples, understanding their chemistry and the determination of their environmental and ecological importance (Frimmel and Abbt-Braun, 1999; Nikolaou and Lekkas, 2001). As an example, Leenheer et al. (2001) recently characterised the dissolved organic matter (DOM) and its reactivity towards chlorine in reclaimed...
and receiving groundwater of Los Angeles County, CA to assess its safety for use as drinking water.

The structural features of HSs in groundwater have received relatively little attention compared with samples from other aquatic and terrestrial environments. Thurman (1985a) provided a review of the first major study of groundwater HSs which established their chemical, physical and spectroscopic properties, and several unique characteristics were found compared to surface water HSs. The content of DOC in groundwater was generally low (>5 mg L\(^{-1}\)) and the isolated HSs contained low amounts of humic acid, aromatics and oxygenated functional groups. The exception was when the source was located in organic rich sediments containing kerogen, where DOC, humic acid and aromaticity were exceptionally high. Several other studies have been conducted on the specific characterisation of groundwater HSs or have included groundwater samples in broader aquatic HS characterisation (Wassenaar et al., 1990; Kim et al., 1990; Gron et al., 1996; Artinger et al., 2000; Alberts et al., 1992; Pettersson et al., 1989, 1994). These confirm that groundwater DOC is structurally unique as a result of extended exposure to adsorption and degradation processes. Adsorption during infiltration and movement tends to remove the humic acids, lowering DOC and aromaticity, while degradation results in the loss of oxygenated functional groups. Groundwater HSs have also been characterised as part of studies on landfill leachates, contaminated sites, the mineralisation of sedimentary organic carbon (SOC), drinking water studies and DOC budgets (Zwiener et al., 1999; Wong et al., 2002; Wassenaar et al., 1991; Thorn and Aiken, 1998; Cronan and Aiken, 1985; Leinweber et al., 2001; Kumke et al., 1999; Christensen et al., 1998; Calace et al., 2001; Backau et al., 2000; Nanny and Ratasuk, 2002). Given that the much of the literature does not deal specifically with background structural characterisations or Australian sites, data for these areas remain somewhat limited and inconsistent. In particular, the variability in the chemical, physical and spectroscopic techniques applied makes inter-study comparisons difficult.

The Tomago Sand Beds near Sydney, Australia are a remnant dune system and shallow sandy aquifer and have been mined for mineral sands and used to supply drinking water. The groundwater geochemistry was radically altered during mining activities, leading to the potential contamination of water supplies for nearby populations with species such as iron and arsenic. DOC levels were also observed to increase with mining activities and it was thought that organic acids may be involved in the release and transportation of the inorganic contaminants. The objective of this study was to conduct a comprehensive background structural characterisation of the groundwater hydrophobic acids isolated from groundwater at the Tomago Sand Beds and compare the findings with other studies. The hypothesis was that the hydrophobic acids at this Australian site are structurally unique and have a significant role in the groundwater geochemistry. Structural changes in the hydrophobic acids were observed as the study progressed from 1996 to 2003 and thus data are presented for two representative samples isolated in 1996 (GW96) and 2002 (GW02).

2. Site description

A detailed description of the study area can be found in Prosser and Roseby (1995) and a brief summary is given here. The Tomago sand beds are located 16 km north of Newcastle, Australia. They are characterised by a dune system of reworked Pleistocene marine sands that have developed podzol soil profiles since stabilisation (12,000 BP). Vegetation is dominated by dry sclerophyll forests with low lying areas containing swamps. The sands are dominated by quartz and an extensive aquifer underlies the beds, with the water table depth ranging from 1 to 5 m. The podzol soils are predominantly humic in nature at low relief and iron/iron-humic at high relief. The depth of the B horizon varies with the water table and ranges from 0.5 to 3 m. The sands contain economically viable quantities of heavy minerals and these were mined for 30 years (production has ceased). The groundwater is used a source of potable water by the local water corporation for nearby populations and potential contamination resulting from mining has been an ongoing issue of concern. A monitoring programme for the groundwater is ongoing.

3. Experimental

3.1. Background data

Background data for the study site were obtained by personal communication with Peter R. Wieland, Department of Chemistry, Macquarie University, Sydney. The data were extracted from the records of the groundwater monitoring programme at RZ Mines Pty Ltd, Newcastle. Additional data obtained from Peter R. Wieland were part of a separate research study of the site.

3.2. Samples, sampling methodology extraction procedures

Samples were taken from a control piezometer located at UTM 56H 372940 1371020 (RZ Mines Pty Ltd identification: location 3502, piezometer number 2). The sampling site was a control one unaffected by mining activities, with piezometers set at depths of 3.6,
groundwater fulvic acids were averaged for comparison (Wassenaar et al., 1990; Thurman, 1985a; Kim et al., 1990; Frimmel and Abbt-Braun, 1999; Artinger et al., 2000; Alberts et al., 1992; Pettersson et al., 1994). Stable isotope analysis for sulfur and carbon were performed at the Stable Isotope Science Laboratory, Department of Physics and Astronomy, University of Calgary, Canada.

3.4. Carboxyl group content

Carboxyl group content was determined by titration to pH 8 using the method of Christensen et al. (1998). Titrations were conducted using a Radiometer Copenhagen TTA80 titration assembly, PHM82 standard pH meter and a GK2401C pH electrode. Carboxylic acid content was not determined for GW96 due to limited sample quantity. Phenol content was not determined as titration has been shown to give variable results above pH 8 due to hydrolysis reactions (Averett et al., 1994).

3.5. Infrared spectroscopy

Infra-red (IR) spectra were recorded on a Perkin–Elmer Paragon 1000PC FT-IR Spectrometer. Samples were dried at 60 °C under vacuum for 24 h and analyzed as 1% w/w KBr discs. Spectra were recorded over the range 4000–400 cm<sup>-1</sup> using 16 scans at a resolution of 2 cm<sup>-1</sup> and were smoothed after acquisition.

3.6. <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy

<sup>1</sup>H Spectra were recorded on a Bruker DPX400–400 MHz spectrometer using a WATERGATE pulse sequence for suppression of the water resonance (Liu et al., 1998). Samples were dissolved in D<sub>2</sub>O and 10,240 transients were collected with a spectral width of 5,995 Hz, an acquisition time of 2.73 s and pulse delay of 2 s. The solvent peak at 4.6 ppm was irradiated so protons in this region were not observed. Peak assignments were based on the literature (Wilson, 1987; Malcolm, 1990; Leenheer et al., 1998).

Conditions used for Solid-State Cross Polarisation Magic Angle Spinning (CPMAS) <sup>13</sup>C NMR spectroscopy are detailed in Leenheer et al. (2003). Insufficient sample prevented a spectrum for GW96 being recorded. Peak assignments were based on the literature (Malcolm, 1990; Nanny and Ratasuk, 2002).

3.7. High-pressure size exclusion chromatography

High-pressure size exclusion chromatography (HPSEC) is an established method in the analysis of HSs for determination of molecular weight and size (Cozzolino et al., 2001; Conte and Piccolo, 1999). Analysis was performed using a Shimadzu LC10AD pump, a
Perkin–Elmer LC295 UV–Vis detector and a 600 × 7.8 mm Polysyep GFC P 3000 column with a Polysyep P 3000 guard column (Phenomenex). The eluent was 0.05 M phosphate buffer at pH 7 and the analytes were dissolved in the eluent at a concentration of 0.2 g L⁻¹. Standards used for calibration were polystyrenesulfonates (LabService Analytica s.r.l., Italy) with molecular weights of 6.78, 16.8, 32 and 130 kDa. A calibration curve relating molecular weight to elution time was obtained by plotting the natural logarithm of the molecular weights [ln(Mᵢ)] of the polystyrenesulfonate standards versus time. Linear regression was used to obtain the relationship between ln(Mᵢ) and time. The injection volume was 100 μL and spectra were recorded at 250 nm for standards and 280 nm for samples. The different wave-lengths have been shown to have a minimal effect (O’Loughlin and Chin, 2001). Spectra were acquired using Perkin–Elmer PE Turbochrom 4.0.1 software.

Data were processed manually by exporting the chromatograms as time versus signal ASCII data files into Microsoft Excel® and only time and signal data corresponding to the major peak of interest were retained. For each time slice a corresponding molecular weight, Mᵢ, was calculated using the linear regression equation fitted to the calibration data. The weight fraction, wᵢ, of analyte in any time slice was calculated by dividing the detector voltage response (baseline subtracted) by the sum of detector voltage responses for each analyte (i.e., wᵢ = intensity/Σintensity). 

3.8. Vapour pressure osmometry

Vapour pressure osmometry (VPO) was performed using a Westcor, Vapro 5520 vapor pressure osmometer. Samples were analyzed using the method of Aiken and Malcolm (1987) and benzoin was the calibration standard.

3.9. UV–Vis spectroscopy

UV absorbance was determined on a Varian, Cary 300 Bio UV–Vis Spectrophotometer using the procedure of Chen et al. (1977). Absorbance values were recorded at 365 and 250 nm for calculation of E₂/E₃ ratios and at 280 nm for calculation of molar absorbance.

3.10. Thermochemolysis with TMAH

Offline tetramethylammonium hydroxide (TMAH) thermochemolysis was performed using the method of del Rio et al. (1998). The internal standard was eicosane at a concentration of 25 ng L⁻¹. Analysis was performed using a Fisons Instruments GC8000 gas chromatograph coupled to a MD800 mass selective detector. The oven was held at 40 °C for 2 min, then ramped at 10 °C min⁻¹ to 100 °C, then ramped at 4 °C min⁻¹ to 300 °C and held for 2 min. Mass spectra were obtained by scanning from 35 to 500 Da in 1 s. The capillary column was a 50 m BP1 one (SGE, Australia) with a 0.5 μm film thickness. Compounds were identified by comparison of the spectra with a NIST 92 library.

3.11. Electrospray ionisation mass spectrometry

Electrospray ionisation mass spectrometry (ESI-MS) was performed using the methods of McIntyre et al. (1997, 2002). Samples were dissolved in 75:25 methanol:water at a concentration of 1 mg mL⁻¹. Conditions for the acquisition of spectra are given in McIntyre et al. (2002). Neutral loss spectra were obtained with the same parameters used for product ion spectra but with a mass range of 100–700 Da, a scan time of 3 s and quadrupole resolution settings to achieve unit mass resolution.

4. Results and discussion

The background data for the piezometer are summarised in Table 1. The pH of the groundwater is acidic and is controlled by the oxidation of sulfur and/or sulfide to sulfate in the oxic zone, with the low content of clay minerals minimising buffering of the system. Increasing sulfate and decreasing iron and sulfide levels with time indicate a change in microbial activity/composition associated with dissolution of pyrite and reduction of sulfate (Massmann et al., 2003). The pH has decreased with time, consistent with a reduction in bicarbonate production associated with microbial mineralisation of SOC during sulfate reduction and acid production from pyrite dissolution. DOC concentration has declined with time, indicating a reduction in the microbial mineralisation of SOC or reduced DOC recharge from surface environments. The decrease in concentration of iron and sulfur is consistent with the former (Backau et al., 2000). Oxidation/reduction potential (ORP) values indicate a reducing environment, although the periodic low levels of dissolved oxygen (DO) reflect the sampling depth being proximal to the water table. Conductivity (Cond.) is constant and indicates that the spear has not been affected by mining activities.

Table 1 is incomplete as data were derived from several sources. Trends at this piezometer were observed in adjacent control piezometers set at different depths (data
confirmed their validity. All values were within normal variation for the site which has been monitored for 30 years. Variation in the parameters could not be correlated with rainfall and season but a major influence on the system is the high lateral flow rate of groundwater (1 m week\(^{-1}\)) which results in the rapid dispersal of groundwater and surface recharge. The background data indicate this is a typical groundwater system.

### 4.2. DOC composition

The concentration of DOC in the groundwater in 2002 (Table 2) is consistent with previous studies that have reported concentrations in soil solutions which range from 20 to 50 mg L\(^{-1}\) in the O/A horizons, decreasing to >3 mg L\(^{-1}\) in groundwater as infiltration occurs (Wassenaar et al., 1991; Thurman, 1985a,b; Pettersson et al., 1994). This reduction is generally attributed to extended exposure to adsorption and degradation processes. The DOC concentration in 1996 was considerably higher (Table 2). The background data indicates a higher level of microbial sulfate reduction in 1996 and are consistent with an elevated DOC being the result of microbial dissolution of SOC.

Previous studies have reported the hydrophobic acid content of groundwater in organically lean aquifers (%DOC as hydrophobic acids) as 5–58% with a predominance of fulvic acids (Wassenaar et al., 1990; Thurman, 1985a; Gron et al., 1996; Artinger et al., 2000; Pettersson et al., 1994). Data for GW96 are consistent with this; however, for GW02 the hydrophobic acid content is above the upper end of the range. Higher levels of microbial sulfate reduction in an organically lean aquifer have been shown to produce a greater proportion of hydrophilic acids in the DOC (Wassenaar et al., 1991); this explains the lower value for GW96 and suggests that inputs of surface DOC may be more significant for GW02. The hydrophobic acid content as related to DOC content is not consistent with the findings of Wassenaar et al. (1990) and Thurman (1985a) who showed that humate content increases with DOC content in groundwater. This is also attributed to a change in microbial activity/community which has altered the total DOC inputs over the study period. The low humic acid content of both samples is consistent with removal by adsorption and degradation processes as water infiltrates and migrates through the ground and also reflects the organically lean composition of the aquifer.

### 4.3. Elemental and isotopic composition

The average values of elemental composition from previous studies (cf. 3.3, 1 s.d. given in brackets) for carbon content, hydrogen content and H/C ratio were 53.9% (2.1), 4.95% (0.54) and 1.11 (0.12), respectively (n = 75). Oxygen content and the O/C ratio were 37.6% (2.0) and 0.53 (0.5) (n = 72). Nitrogen and sulfur contents were 1.07% (0.35) (n = 73) and 2.97% (0.68) (n = 48).

Compared to reported values the oxygen content of GW02 is high. The lower oxygen content of reported samples has been attributed to the removal of carboxyl groups from fulvic acids during microbial and chemical degradation processes (Pettersson et al., 1994). A lower hydrogen content and H/C ratio indicate that GW02...
has a less aliphatic character. Groundwater fulvic acids are suggested to have a lower aromatic content due to removal of humic acids by adsorption and degradation. In surface environments where these processes are less extensive, elemental compositions of fulvic acids are similar to that presented for SRFA. The C, H, O data for GW02 is closer to that of SRFA and suggests that the sample is relatively immature and/or may still receive some allochthonous DOC. If the DOC is derived in situ, this suggests that it has been oxidised, resulting in a higher oxygen content, or that the character of the sedimentary organic matter is unique. Dating (14C) of the samples and characterisation of the sediments at the site have not been done and thus conclusions with regard to DOC age/origin are not yet possible. The higher sulfur content of GW02 compared to SRFA is characteristic of groundwater environments and is indicative of hydrogen sulfide/colloidal sulfur produced by microbes during sulfate reduction being incorporated into the hydrophobic acids. Average N and S values of reported values are comparable to GW02 and typical for groundwater HSs.

The elemental composition of GW96 is significantly different from that of GW02 and the reported values given above. Limited data were obtained due to the limited amount of sample available, but a lower carbon content and a higher H/C ratio suggest that GW96 contains more aliphatic (possibly oxygenated) compounds than GW02. This is consistent with an addition of low molecular weight organic acids produced during microbial action on aquifer SOC during sulfate reduction, as proposed by Wassenaar et al. (1991), and the background data presented above.

The carbon isotope ratio of −27.6‰ for all three samples (Table 2) indicates that they are most likely derived from higher plant matter (Smith and Epstein, 1971). This is consistent with published data (Wassenaar et al., 1990; Thurman, 1985a). The organic sulfur isotope ratios are consistent with literature values and are consistent with a change in biological activity at the site (Nissenbaum and Kaplan, 1972). GW96 is isotopically heavier, indicating greater depletion in 32S through sulfate reduction by microbes. This is supported by higher sulfide levels observed in the background data prior to 2002. The δ34S values of the dissolved sulfate and sulfide at the site (data not shown) also support this finding and indicate that the microbial community has changed or that microbial activity was occurring at shallower depths and closer to the piezometer, prior to 2002.

### 4.4. Carboxyl content

GW02 has a carboxylic acid content which lies between values reported by Thurman (1985b) for fulvic acids in groundwater (5.1–5.5 meq g⁻¹) and surface

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Table 2

Sample information and analysis results for the hydrophobic acids isolated from groundwater (GW02 and GW96) and standard Suwannee River fulvic acid (SRFA)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1996/GW96</th>
<th>2002/GW02</th>
<th>SRFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling date</td>
<td>12/10/96</td>
<td>18/03/02</td>
<td>–</td>
</tr>
<tr>
<td>Volume collected (L)</td>
<td>10 × 3</td>
<td>150</td>
<td>–</td>
</tr>
<tr>
<td>DOC (mg C L⁻¹)</td>
<td>10.1</td>
<td>2.2</td>
<td>–</td>
</tr>
<tr>
<td>Hydrophobic acids isolated (mg)</td>
<td>226</td>
<td>410</td>
<td>–</td>
</tr>
<tr>
<td>% of DOC as hydrophobic acids</td>
<td>36</td>
<td>64</td>
<td>–</td>
</tr>
<tr>
<td>Humic acid content (% wt)</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>–</td>
</tr>
<tr>
<td>C (%)</td>
<td>48.2</td>
<td>52.3</td>
<td>52.4</td>
</tr>
<tr>
<td>H (%)</td>
<td>4.21</td>
<td>4.10</td>
<td>4.31</td>
</tr>
<tr>
<td>O (%)</td>
<td></td>
<td>41.5</td>
<td>42.2</td>
</tr>
<tr>
<td>N (%)</td>
<td>1.32</td>
<td>1.20</td>
<td>0.72</td>
</tr>
<tr>
<td>S (%)</td>
<td>8.42</td>
<td>2.87</td>
<td>0.44</td>
</tr>
<tr>
<td>Ash (%)</td>
<td></td>
<td>2.58</td>
<td>0.46</td>
</tr>
<tr>
<td>H/C</td>
<td></td>
<td>6.62</td>
<td>8.8</td>
</tr>
<tr>
<td>O/C</td>
<td></td>
<td>0.56</td>
<td>0.60</td>
</tr>
<tr>
<td>δ13C (‰)</td>
<td>−27.6</td>
<td>−27.6</td>
<td>−27.6</td>
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<tr>
<td>δ34S (‰)</td>
<td>9.9</td>
<td>1.6</td>
<td>–</td>
</tr>
<tr>
<td>Carboxyl content (meq g⁻¹)</td>
<td>–</td>
<td>5.5</td>
<td>5.7</td>
</tr>
<tr>
<td>Molecular weight (Da) by VPO</td>
<td>–</td>
<td>953</td>
<td>859</td>
</tr>
<tr>
<td>Mₐ (Da) by HPSEC</td>
<td>1773</td>
<td>1810</td>
<td>2438</td>
</tr>
<tr>
<td>Mₚ (Da) by HPSEC</td>
<td>1356</td>
<td>1499</td>
<td>1972</td>
</tr>
<tr>
<td>Polydispersity (Mₚ/Mₐ) by HPSEC</td>
<td>1.31</td>
<td>1.21</td>
<td>1.24</td>
</tr>
<tr>
<td>E₂/E₃ ratio</td>
<td>4.5</td>
<td>4.3</td>
<td>4.4</td>
</tr>
<tr>
<td>Absorbance at 280 nm (L mol OC⁻¹ cm⁻¹)</td>
<td>310</td>
<td>530</td>
<td>420</td>
</tr>
</tbody>
</table>

390

C. McIntyre et al. / Organic Geochemistry 36 (2005) 385–397
waters (5.5–6.2 meq g⁻¹). It is higher than a calculated mean value of 5.2 meq g⁻¹ for groundwater fulvic acids reported by Pettersson et al. (1994) and similar to the 5.7 meq g⁻¹ determined for SRFA (Table 2). These data are consistent with the elevated oxygen content determined by elemental analysis. The high carboxyl group content and oxygen content when compared to literature values again suggest that GW02 is immature and has been exposed to a lesser degree of decomposition or to greater oxidation.

### 4.5. Infrared spectroscopy

Infrared spectra are similar to those reported by Kim et al. (1990) and Alberts et al. (1992) for groundwater fulvic acid (Fig. 1) and show a close resemblance to those of hydrophobic acids isolated from streams, lakes and forest floor solutions (Guggenberger et al., 1994; David and Vance, 1991). The data are typical for terrestrially derived aquatic hydrophobic acids, but also highlight the fairly non-specific nature of infrared spectroscopy. The IR spectra indicate the hydrophobic acids are primarily carboxylic (1720, 3400 cm⁻¹; C=O, OH stretching) with a low content of humic acids and complexed metal ions (1620 and 1400 cm⁻¹).

### 4.6. ¹H NMR spectroscopy

GW02 has the majority of the observable protons occurring between 1.75 and 3 ppm (Fig. 2), indicating a high content of protons on carbon atoms adjacent to carboxyl groups. The peak at 2.5 ppm indicates a significant proportion of these are in branched aliphatic structures. In unbranched aliphatic structures, protons in this spectral region give a peak at around 2.2 ppm. Although protons of methyl groups attached to aromatic rings may also appear in this region, ¹³C NMR spectra presented in the next section confirm that branched aliphatic structures are dominant in GW02. The spectrum is substantially different from that for hydrophobic acids isolated from a control groundwater, presented by Thorn and Aiken (1998), Well 310), which gave a major peak at 1.1 ppm corresponding to aliphatic methylene groups. It also differs substantially from marine and terrestrial HSs which have been shown to have major peaks in the aliphatic (~1 ppm) and carbohydrate (~4 ppm) regions (Hatcher et al., 1979; Malcolm, 1990). The spectrum more closely resembles that of surface water fulvic acids isolated from streams and lakes, where resonances near 2 ppm are more prominent (Malcolm, 1990; Ma et al., 2001). In particular, the spectrum shows a similar down field shift of protons adjacent to carboxyl groups as observed in the metal binding fraction of SRFA, which was attributed to clustering of the carboxyl groups (Leenheer et al., 1998).

GW96 has a significantly different spectrum to that of GW02, highlighting the change in composition of the hydrophobic acids in the groundwater at the site since 1996 (Fig. 2). More intense peaks at 1.2 and 2.2 ppm indicate a greater content of aliphatic acids, and this is consistent with greater microbial mineralisation of SOC and other data. These may be branched methyl structures rather than linear aliphatic/acyclic compounds, as resonances at 1.6–1.8 ppm due to protons.
β to carboxylic acids increase only very slightly compared to corresponding peaks in GW02.

4.7. CPMAS $^{13}$C NMR spectroscopy

The normal spectrum has a high content of aromatic carbon (130 ppm), which is not consistent with the concept of groundwater humic substances having a low content of aromatic structures due to adsorption of humic acids (Fig. 3). The major aliphatic peak at 43 ppm is indicative of branched aliphatic carbons while the peak at 30 ppm is due to methylene carbons. The low signal at 15–20 ppm due to methyl carbons and the major resonance occurring at 43 ppm indicate that the peak at 2.4 ppm in the $^1$H NMR can be attributed to protons on branched aliphatic groups adjacent to carboxylic groups, rather than to methyl groups attached to aromatic rings. The small shoulder at 105 ppm and the peak at 76 ppm indicate a low content of anomic carbon of carbohydrates and C–O linkages, respectively. Shoulders at 115 ppm and 155 ppm indicate lignin-derived aromatics are present in low quantities. Carboxylic carbons (170 ppm) are moderate in content and normal for aquatic fulvic acids (cf. Malcolm, 1990). The low signal level at 58 ppm also indicates methoxyl carbons of lignin and esters have a minimal contribution. The data are consistent with previous sections and indicate predominantly aryl-branched aliphatic structures with a low content of lignin-derived compounds and C–O linkages (carbohydrates).

The dipolar dephased spectrum indicates that the majority of aromatic and aliphatic carbon (0–160 ppm) is non-quaternary (Fig. 3). Terminal methyl at 15 ppm, quaternary methine at 48 ppm and tertiary alcohols at 80 ppm are evident while a low proportion of the aromatic carbon is protonated. These structures are hypothesised to be indicative of degraded terpenoid structures, as reported by Leenheer et al. (2003). For this site, selective or incomplete absorption processes in the vadose zone of the soil during water infiltration may have resulted in the enrichment of these types of species. The overlying vegetation (Eucalyptus) at the site is rich in terpenoids and is a ready source of precursor terpenoid molecules.

4.8. UV–Vis spectroscopic, molecular weight and molecular size measurements

SRFA has been reported to have a $M_n$ by VPO of 840 Da and a $M_m$, $M_w$ and polydispersity by HPSEC of 1652 Da, 2290 Da and 1.39 (Aiken and Malcolm, 1987; O'Loughlin and Chin, 2001). Chin et al. (1994) determined the molar absorbance at 280 nm to be 389 L cm$^{-1}$ (mol OC)$^{-1}$. The results presented for SRFA in this study correlate well with the above data (Table 2). The $E_2/E_3$ ratio was measured for comparison of aromaticity and average molecular weights as it has been shown to give a stronger correlation than the $E_4/E_6$ ratio (Peuravuori and Pihlaja, 1997).

The molecular weight of GW02 determined using VPO is high compared with values for aquatic fulvic acids reported by Aiken and Malcolm (1987) of 500–950 and 859 Da obtained for SRFA (Table 2). Pettersson et al. (1994) published molecular weights for aquatic fulvic acids determined by HPSEC using polystyrenesulphonate standards. Their results showed that the fulvic acids in deep groundwater were lower in molecular weight than in surface waters and this was attributed to adsorption and degradation processes. The VPO result for GW02 is not consistent with this concept and suggests a lesser degree of exposure to these processes, allowing higher molecular weight compounds to stay in solution.

Peuravuori and Pihlaja (1997) have shown that molecular weights determined by HPSEC have a linear relationship to those determined by VPO. HPSEC data for GW02 (Table 2) infer that it has a lower molecular weight than SRFA. However, given the branched, compact nature of the sample as indicated by NMR, this suggests it is more likely to reflect a smaller hydrodynamic size. The polydispersity of GW02 is slightly lower.
then that determined for SRFA and indicates that the sample has a more homogenous composition. This is consistent with the findings of Pettersson et al. (1994) which indicated groundwater fulvic acids were less polydisperse than surface water fulvic acids, which again was attributable to maturity and a greater exposure to adsorption and degradation processes. Molar absorption at 280 nm and $E_2/E_3$ ratio have been shown to be related to aromaticity and inversely related to average molecular weight (Peuravuori and Pihlaja, 1997). The results for these parameters are consistent with $^{13}$C NMR and VPO data and confirm that GW02 is both more aromatic than and higher in molecular weight than SRFA.

The molecular weight for GW96 could not be determined using VPO as in higher ash content resulted its being insoluble in the solvent. Molar absorbance at 280 nm and $E_2/E_3$ ratio indicate that the sample is less aromatic than GW02 and SRFA and that it has a lower molecular weight. The polydispersity is higher and indicates that the sample has a less homogenous composition. These data are consistent with the suggestion of greater microbial sulfate reduction and SOC mineralisation at the site in 1996 producing additional low molecular weight aliphatic acids and an increase in DOC. This also infers that the molecular weight determined by HPSEC for GW96 reflects both the lower molecular weight acids produced by microbes as well as a smaller hydrodynamic size.

4.9. Thermochemolysis with TMAH

The total ion current (TIC) chromatogram for GW02 (Fig. 4) is very similar to that for SRFA presented by del Rio et al. (1998) and indicates that the sample contains terrestrially derived aquatic DOC. TMAH thermochemolysis products identified in Table 3 are dominated by lignin derived compounds with guaiacyl and syringyl structures (Clifford et al., 1995) which are consistent with the overlying angiosperm vegetation. Products arising from tannin and cutan (peaks 14 and 21) are present and, even though the technique is not very efficient for carbohydrates, some markers can be seen as peaks 13 and 38. The total yield of products is <1.0% assuming a response factor of 1 for all compounds; this is consistent with the low content of these biopolymer classes observed using NMR spectroscopy. The data indicate that there is incomplete adsorption of DOC from surface water recharge. Ratios of vanillyl and syringyl acid:aldehyde are used to indicate lignin degradation (del Rio et al., 1998). Although the presence of vanillyl and syringyl aldehyde could not be confirmed, the methyl esters

![Fig. 4. TMAH thermochemolysis GC/MS total ion chromatograms for hydrophobic acids isolated from groundwater (GW02 and GW96). Peak identities are given in Table 3; * denotes internal standard eicosane.](image-url)
of vanillyl and syringyl benzoic acids (peaks 21, 22, 27) are present in significant amounts. This indicates that the lignin components are highly degraded and this is consistent with the high oxygen content. Nanny and Ratasuk (2002) have suggested that C<sub>4</sub>–6 n-alkanedioic acids are products of catechol degradation and microbial fermentation processes. These compounds are present (peaks 1, 2, 5, 8) and indicate some microbial activity is still occurring at the site.

GW96 has a similar TIC chromatogram to GW02 and to SRFA, indicating predominantly terrestrially derived aquatic DOC (del Rio et al., 1998), but with two significant differences. The C<sub>4</sub>–6 n-alkanedioic acids are more intense and peaks corresponding to the methyl esters of fatty acids (peaks 29, 31–38, 40–41) are prominent. The increased quantity of n-alkanedioic acids is consistent with higher levels of microbial degradation of aromatic compounds (Nanny and Ratasuk, 2002) while the fatty acids are consistent with the mineralisation of sedimentary organic carbon and higher microbial input. Grasset and Ambles (1998) demonstrated that lipids with an even carbon number preference are released after the enzymatic degradation of humin. These findings are consistent with other data suggesting a higher level of microbial sulfate reduction and/or SOC mineralisation in 1996. The similar distribution of lignin-derived compounds in both samples suggests they are derived from the same origin.

4.10. Electrospray ionisation mass spectrometry

Negative ion ESI-MS mass spectra for GW02, GW96 [Fig. 5(a) and (b)] and SRFA (not shown) are typical for aquatic fulvic acids and there are no major differences, reflecting the non-specific nature of the technique at this level. The spectra show a distribution of ions at even mass from m/z 100–700, with groups of ions spaced every 14 Da. Ions spaced at 2 and 14 Da have been shown for SRFA, using high resolution mass spectrometry, to result from structural differences of two hydrogen atoms and CH<sub>2</sub> units such as those found in aliphatic, alicyclic and olefinic structures (Stenson et al., 2002). High-resolution mass data obtained for a sample obtained in 1998 at this site found similar mass differences (unpublished data) and from this finding it is inferred that similar structural entities exist in the samples used in this study. The spectrum for GW02 differs slightly from those of GW96 and SRFA. GW02 contains ions that were less well resolved and with a more uniform intensity, showing that GW02 does contain a different assemblage of compounds, although the exact nature of the compounds the ESI-MS spectrum represents is still unclear (Stenson et al., 2002; McIntyre et al., 2001). The greater resolution between the groups of ions spaced at 14 Da in GW96 is consistent with a higher content of aliphatic acids, as indicated by 1H NMR spectra and elemental analysis.

Tandem mass spectrometric experiments have been used to reveal more specific structural features of fulvic acids (McIntyre et al., 1997, 2002). The product ion spectra for both samples indicate the presence of aliphatic polycarboxylic acids [Fig. 5(c) and (d)]. Major fragments spaced at 44 and 18 Da result from loss of CO<sub>2</sub> and H<sub>2</sub>O from carboxylic acids. Lower mass ions such as 59 Da (acetate) and 73 Da (propanoate) are suggestive of fragmentation of aliphatic structures (Leenheer et al., 2003). The product ion spectrum for GW96 contains low mass ions of greater intensity than GW02, consistent with greater aliphaticity. Neutral loss of 88 Da mass spectra (loss of two CO<sub>2</sub> molecules) was
used to indicate compounds containing at least two carboxylic acid groups [Fig. 5(e) and (f)]. Based on the product ion spectra of individual ions (data not shown) the neutral loss spectrum shows aromatic polycarboxylic acids, consistent with the products of lignin degradation, at m/z 171, 181, 197, 209 and aliphatic polycarboxylic acids predominantly at m/z 200–400. These data indicate that the samples contain a mixture of aryl aliphatic polycarboxylic acids and lignin-derived compounds.

5. Conclusions

Groundwater hydrophobic acids isolated in 2002 from the Tomago Sand Beds contained predominantly fulvic acids with a high content of aromatic and branched aliphatic polycarboxylic structures. The molecular weight and oxygen content are high and similarities to the surface water fulvic acid SRFA were found, suggesting some input from surface DOC still occurs. Low polydispersity and a low content of carbohydrates and lignin-derived compounds indicated that adsorption and degradation processes are a major control on the DOC composition. Although the origin of the hydrophobic acids could not be conclusively determined, it is hypothesised that compounds derived from precursor terpenoid molecules, selectively degraded and adsorbed during infiltration of surface water recharge, may contribute to this fraction. The high lateral flow rate of the system is likely to have minimised the time of exposure of recharge DOC to natural processes. The sample is also interpreted to have at least a minor contribution of compounds from microbially mineralised SOC. The high content of aromatics in the sample is a concern with regards to drinking water supplies as this could produce higher yields of disinfection byproducts during chlorination. The sample is structurally unique, differing from the current concepts of groundwater hydrophobic acids which suggest they should be low molecular weight aliphatic acids depleted in oxygen content, carboxyl groups and aromaticity. The findings show the site is unique with regard to the structure of the hydrophobic acids and that they are likely to have an important geochemical role with regards to drinking water quality.

Limited data obtained for the hydrophobic acids isolated in 1996 indicate that sample has a significant content of compounds with structural characteristics different substantially to those of the 2002 sample. Background data and analysis show DOC levels were elevated at that time and suggest that elevated microbial activity associated with sulfate reduction and mineralisation of SOC led to a greater input of lower molecular weight (possibly branched) aliphatic acids. The observed changes in the structural characteristics and composition of the DOC demonstrate that temporal stability of DOC in control locations cannot be assumed, even over relatively short periods of time. The dynamic nature of the system confirms the groundwater DOC has a significant geochemical role. Dating using 14C, metal binding studies and investigation of SOC at site remain to be done. Determination of the DOC origin (SOC or recharge) would greatly assist in the further interpretation of the structural data.
Acknowledgments

We thank Jerry A. Leenheer of the U.S.G.S. for providing the $^{13}$C NMR spectrum and assisting with its interpretation, Dr. Pellegrino Conti at the University of Napoli, Italy for providing the HPSEC data, Dr. Sinan Ali and Dr. Rita Holland at Macquarie University, Australia for the use of their vapour pressure osmometer and Dick Holroyde of the Hunter Water Corporation, Australia for supplying the rainfall data. We are also grateful to Simon George, José C. del Rio, Ian Bull and an anonymous reviewer for reviews.

Associate Editor—I.D. Bull

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