JOURNAL OF MASS SPECTROMETRY, VOL. 32, 328–330 (1997)

JMS Letters

Dear Sir,

Electrospray Mass Spectrometry of Groundwater Organic Acids

Humic substances are naturally occurring organic compounds found in all aquatic and terrestrial environments. They occur as a result of the breakdown and recombination of biogenic materials and consist primarily of three operationally defined fractions termed fulvic acids, humic acids and humin. The classification of humic material is based on solubilities: humic acids are the fraction of humic substances that are insoluble in water at pH 1, fulvic acids are the fraction soluble at all pH conditions and humin is insoluble under all pH conditions.[1]

Humic substances do not consist of a single component or even a simple mixture. The acidic components have received a considerable amount of attention in recent times in an attempt to understand their structure and the role they fulfil in the various environments in which they occur.[2, 3] Humic material has been shown to have a role in soil fertility, the complexing and transport of metal ions, the chlorination of drinking water and the solubilization of pesticides in soils.[3]

The humic substances found in soil and water cover the complete range of organic compounds that are derived from biogenic materials and encapsulate acids, bases and neutrals. The primary sources, from which they are derived include plants, animals and microorganisms and hence humic substances contain a broad range of organic compounds and functional groups. Owing to the complex nature of humic substances, the determination of structural characteristics has proved difficult.

The area of groundwater humics has received little attention, with the majority of work on humic substances being carried out on extracts from soils, rivers, lakes and oceans. This has partially come about as a result of the inaccessibility of the samples for collection and because the concentration of organics is low, hence large water samples are required to obtain small amounts of material. There has been a major study on groundwater organic acids by Thurman,[4, 5] where it was found that of the groundwater dissolved organic carbon, ~20–60% are humic substances. A simple characterization concluded that the aquatic humic substances were of a similar molecular mass (500–5000 Da), lighter in colour, more aliphatic, lower in carbohydrates, higher in carbon and lower in oxygen than most surface humic substances.[5] Thurman[5] speculated that the organics may have originated from terrestrial plants and surface water through flow or from in ground carbon sources such as coals.

The current methods primarily used for analysis of humic substances include NMR spectroscopy,[6] IR spectroscopy,[7] chemical degradation, pyrolysis/mass spectrometry (MS),[8] pyrolysis/gas chromatography/MS[9] and in some cases a combination of these methods.[10, 11] There have been laser desorption mass spectrometric studies of fulvic acids[12, 13] which showed ions in the region of 200–1000 Da. In the study of groundwater organic acids presented here, the technique of electrospray mass spectrometry (ESMS) was applied to these compounds for the first time.

The material studied consisted of organic acids obtained from the groundwater of a sandmining lease near Newcastle, Australia. The traditional chemical and spectroscopic methods for the characterization of humic substances were applied to these samples and the results of these studies will be the subject of a separate paper.

Four sites were ultimately used during the study period, two from control spears and two from a dredge pond located on the mining lease. The data presented in this paper were from a control spear sample. Up to 20 l of the groundwater were collected in glass aspirator flasks. The water was then pressure filtered under nitrogen through a 0.42 μm cellulose acetate membrane to remove particulates and adjusted to pH-2 with concentrated HCl.

A modified method of Thurman and Malcolm[14] was used primarily for the separation and purification of the organic acids. XAD-7, a non-ionic adsorption resin, was purchased fresh (Aldrich) and was extensively cleaned to minimize column bleeding. The procedure involved passing the eluent solution from the XAD-7 column directly through a cation-exchange resin. This produced a solution of free organic acids containing humic and fulvic acids, which was then freeze-dried to obtain the pure, dry organic acids as a single fraction. The modificaton from the method of Thurman and Malcolm[14] involved the removal of the gel chromatographic step and the sample was not split in to humic and fulvic acid fractions. The analysis of blanks from the XAD column indicated there was negligible bleed originating from the column, which was the reason for the gel chromatographic step. The sample was not split as it was found that the humic acids comprised only 2% of the organic acid material.

ES mass spectra were recorded using a Quattro II triple-quadrupole mass spectrometer (Micromass, Altrincham, Cheshire, UK) fitted with an ES source. Samples were dissolved in methanol at a concentration of 1 mg ml^{-1}. The mobile phase for the ES source was methanol and was provided by a Hewlett-Packard (Palo Alto, CA, USA) 1090 LC at a flow rate of 10 μl min^{-1}. The resolution of the mass spectrometer was set to produce a peak width (FWHM) of 0.7 Da, which was sufficient to distinguish singly from doubly charged ions.

A negative ion ES mass spectrum of one of the control spear samples is shown in Fig. 1; this spectrum is typical of the samples analysed. The spectrum shows a distribution of single charged ions every second mass-to-charge value from ~200 to 700 Da with a maximum at around 350 Da; there is also a high-mass tail extending to around 1200 Da that is not shown.

The sampling cone potential used to acquire the spectrum shown in Fig. 1 was 35 V. The distribution of ions shifted to lower masses with an increase in the sampling cone potential. The maxima occurred at ~160 Da at a sampling cone potential of 100 V (data not shown). This shift is attributed to fragmentation of the ions within the high-pressure region of the ESI source with higher sampling cone potentials as has been observed with peptides.[15]

There is also a series of peaks within the spectra that are separated by 14 Da, which implies a difference of a CH_2 group. The 2 Da mass difference implies unsaturation in the molecules, which could arise from olefinic bonds or ring structures. The molecular mass distribution found for this sample is approximately the same as that found for fulvic acid analysed by Novotny *et al.*[12]

It is common for carboxylic acids to undergo a loss of mass 44 under collision-induced decomposition (CID) conditions. This has been observed recently for fatty acids produced by negative ion ES ionization.[16] The product ion spectra obtained from CID at a laboratory collision energy of 20 eV produced a characteristic fragmentation pattern for all the ions selected for their CID analysis. A representative CID spectrum of the peak at *m/z* 339 is shown in Fig. 2. The fragmentation pattern shows losses of multiples of mass 44 Da, these masses occurring at *m/z* 295, 251, 207 and 163. There is also a loss of water associated with each loss of CO_2, and this

CCC 1076–5174/97/030328–03 $17.50

Received 26 September 1996
Accepted 15 November 1996

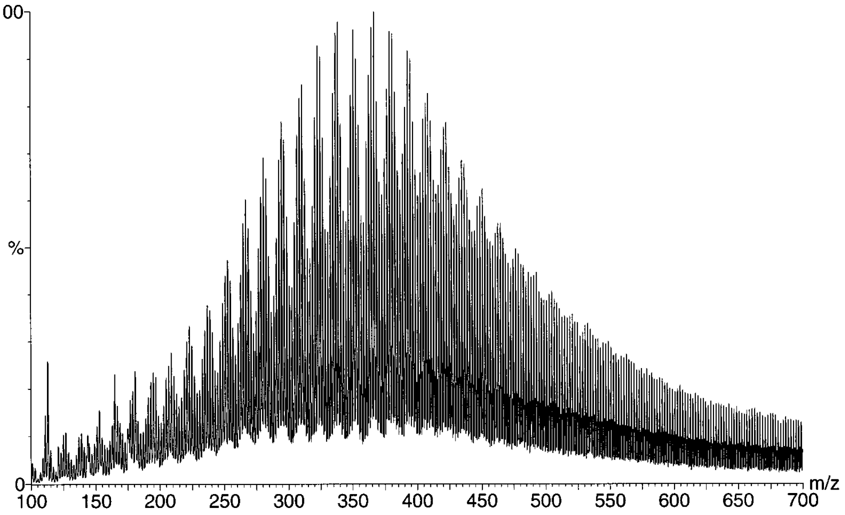

Figure 1. Negative ion ES mass spectrum of a control spear organic acid sample. Capillary voltage, 3.50 kV; sampling cone voltage, 35 V; mass scan rate, from 100 to 700 Da in 4 s. The spectrum is an average of 15 scans and has been smoothed.

was also observed by Kerwin *et al.*[16] for the monocarboxylated fatty acids that they studied. At higher CID energies (30–40 eV) there was a shift to lower mass fragments, particularly fragments that would result from the breaking of the backbone of the molecules. Interpretation of the higher energy CID to give definitive structures is difficult owing to the unlikely possibility that any particular peak in the ES mass spectrum is due solely to a single structural or compositional isomer. However, the fragment ions produced in the higher energy CID appeared to indicate that the compounds were mainly of an aliphatic nature owing to the presence of fragment ion peaks separated by 14 Da in the lower mass region of the CID spectra acquired at a collision energy of 30 eV (data not shown).

The neutral loss experiment, which is readily performed on the triple-quadrupole instrument, allows the study of compounds of similar structure.[17] Neutral loss experiments were performed at a laboratory collision energy of 20 eV. Experiments monitoring the neutral loss of 44, 88, 132 and 176 Da were performed, corresponding to the loss of one to four CO_2 molecules. A representative spectrum for the neutral loss of 88 Da is shown in Fig. 3. The neutral loss spectra indicated that a significant proportion of the ions observed in the negative ion ES mass spectrum were related and that they were polycarboxylic acids. The spectra also showed a shift to higher masses for the parent ions with the number of acid groups lost, that is, with a larger neutral loss, as would be expected.

The product ion and neutral loss experiments indicated that the organic acids found in groundwater from the study site consisted predominantly of aliphatic polycarboxylic acids. The presence of a peak at every second mass value and the separation of a series of peaks by 14 Da also support this. Two points should be made about what has been observed with this technique. First, the eluent from the XAD-7 column

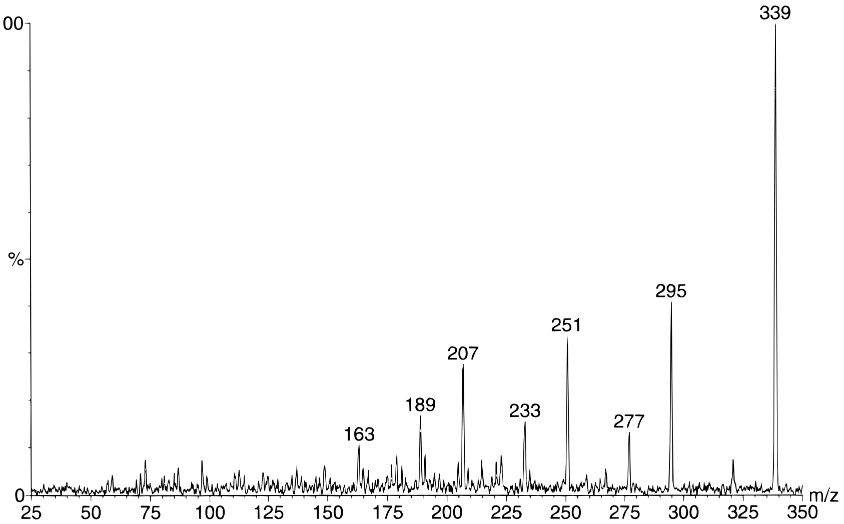

Figure 2. CID mass spectrum of the *m/z* 339 ion from the control spear organic acid sample. Source conditions as in Fig. 1; collision energy, 20 eV; collision gas, argon; collision gas pressure, 1×10^{-3} mbar; mass scan rate, from 20 to 420 Da in 4 s. The resolution of Q1 and Q3 was set to produce a peak width (FWHM) of 0.9 Da. The spectrum is an average of 15 scans and has been background subtracted and smoothed.

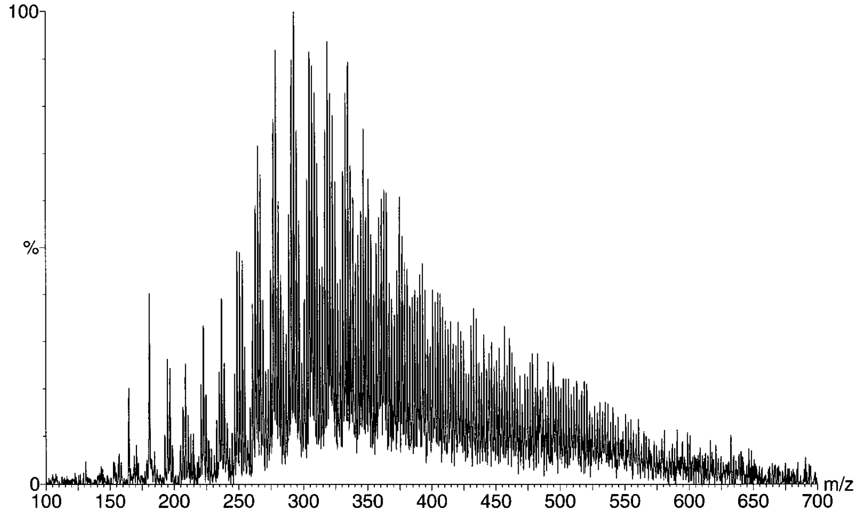

Figure 3. Neutral loss spectrum of the control spear organic acid sample. Neutral loss monitored, 88 Da; source conditions as in Fig. 1; collision energy, 20 eV; collision gas, argon; collision gas pressure, 1 × 10^{-3} mbar; mass scan rate, from 100 to 700 Da in 4 s. Resolution settings for Q1 and Q3 in Fig. 2. The spectrum is an average of 15 scans and has been background subtracted and smoothed.

may not represent the organic acid composition of the groundwater, e.g. the lower molecular mass acids were not retained. Second, there may be compounds present in the sample that do not produce significant negative ions under the ES conditions used.

ESMS could be a valuable tool for analysis of groundwater organic acids in that it can produce an accurate moleclar mass and together with MS/MS techniques can provide some component-specific structural information. Other significant advantages are that ES is readily compatible with the polar solvents used to dissolve the organic acids and no derivatization is required. There are obvious extensions of the technique to the study of organic acids from other sources such as, soils, surface water and coals.

Yours

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