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Examination of soil contaminated by coal-liquids by size exclusion chromatography in 1-methyl-2-pyrrolidinone solution to evaluate interference from humic and fulvic acids and extracts from peat

T.J. Morgan [a], A.A. Herod [a,*], S.A. Brain [b], F.M. Chambers [b], R. Kandiyoti [a]

[a] *Department of Chemical Engineering, South Kensington Campus, Imperial College of Science, Technology and Medicine, London SW7 2AZ, UK*
[b] *Centre for Environmental Change and Quaternary Research, GEMRU, University of Gloucestershire, Francis Close Hall, Swindon Road, Cheltenham, Glos GL50 4AZ, UK*

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Abstract

Soil from a redundant coke oven site has been examined by extraction of soluble materials using 1-methyl-2-pyrrolidinone (NMP) followed by size exclusion chromatography (SEC) of the extracted material. The extracted material was found to closely resemble a high temperature coal tar pitch. Standard humic and fulvic acids were also examined since these materials are very soluble in NMP and would be extracted with pitch if present in the soil. Humic substances derived from peat samples and NMP-extracts of peats were also examined. The results show that the humic and fulvic substances were not extracted directly by NMP from peats. They were extracted using caustic soda solution and were different from the peat extracts in NMP. These results indicate that humic and fulvic acids were soluble in NMP in the protonated polyelectrolyte form but not in the original native polyelectrolyte form. The extraction of soil using NMP followed by SEC appears to be a promising method for identifying contamination by coal-based industries.

Keywords: Contaminated soil; Coal-liquids; Humic substances; Size exclusion chromatography; Characterisation

1. Introduction

The present work has investigated the contamination of soil of a redundant coke-works by coal-liquids using size exclusion chromatography in 1-methyl-2-pyrrolidinone (NMP) as eluent and extraction solvent. However, the possibility that humic substances in the soil might interfere with the identification of extracts as coal-derived, has been addressed in detail.

Two recent papers [1,2] have noted that much of the organic material of soils, sediments and natural waters remains beyond the range of methods of characterisation normally applied to such samples. Gas chromatography and other analytical methods where volatilisation is necessary before detection have been commonly used. The debate about the nature of humic substances has suggested that there may be no large molecules and the apparent presence of large molecules is caused by aggregates of small ones [3–5]. The alternative position, that there really are large molecules in humic substances, has received some support from aqueous SEC studies, although the problem remains that the polymers used to calibrate the mass scale of SEC have not been demonstrated to be relevant to the humic substance structure. Since the structure of humic substances remains unknown, there can be no easy resolution of this problem [6–8].

There is, however, evidence in sediments of large molecular mass protein-containing material that can survive early diagenesis [9] and could therefore contribute to humic substances; analysis by electrophoresis indicated that some proteins up to a mass of about 200 kDa could survive into long-term preservation in sediments by modification into

* Corresponding author. Fax: +44 2075945604.
E-mail address: a.herod@imperial.ac.uk (A.A. Herod).

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large and insoluble macromolecular complexes which could not be readily degraded by bacteria. In addition, the analyses of International Humic Substance Society [10] humic and fulvic acids derived from peats, water and coal indicate the presence of proteins that may derive from similar, protein macromolecular structures.

A separate indication [11] is that the isolation of humic acids from peat using NaOH solution causes breakdown of the peat structure to yield the products detected as humic and fulvic acids. The effect on elution times of the addition of LiBr to dimethylformamide for the SEC of sulfonates [12], may be considered to show that the apparently large molecules (which eluted before the exclusion limit of SEC) could be dissociated by the salt and moved to later elution times, although the study concluded that the ionic addition ruined the solvent properties. Similar disaggregation claims have been made for aqueous SEC [13] where the addition of organic anions to the eluent led to increased elution times, despite reducing the solvent powers; Ralph and Catcheside [14] showed that an increase of ionic strength of the mobile phase led to an increase of elution times for alkali-solubilised brown coals in aqueous SEC.

Similar discussions regarding the molecular structures of coal-derived liquids have suggested that the addition of salts to the NMP eluent in SEC could disaggregate the apparently large molecules of coal-liquids and return them to their correct (longer) elution times [15,16]. The action of salts has been separated from SEC by using planar and column chromatography [17,18]; the action of salts in SEC using NMP as solvent is to ruin the size mechanism for coal-liquids by promoting surface interactions. In NMP at the dilutions involved, aggregate formation in coal-liquids can be discounted [19].

We have solved the problem of detecting molecules beyond the range of gas chromatography, in part at least, in that we have developed chromatographic methods by which we can observe molecules that are much too large to be detected by gas chromatography. The methods follow from the use of 1-methyl-2-pyrrolidinone (NMP) as an eluent for size exclusion chromatography [20]. This solvent dissolves large aromatic and polar molecules, although not aliphatics, and is able to elute very large molecules from size exclusion chromatography columns. These methods have been applied to many different sample types: solvent extracts of coals and wood, biomass tars, kerogens, soots, petroleum vacuum residues and amber extracts [21]. The calibration of the SEC method using NMP as eluent and polymer standards as calibration molecules has been shown to provide an accurate estimate of molecular mass up to at least 3000 u, for fractions of coal tar pitch of narrow polydispersity, by comparing MALDI mass spectra with SEC elution times of the fractions and polymers [19,22,23].

All this work indicates that it is possible to examine the molecular mass range beyond that of gas chromatography but we are not yet at the point when we can specify molecular structures in the ‘unknown’ region, beyond a mass of 3000 u or so and in the excluded region of SEC. However, the behaviour of three-dimensional spherical particles (molecules and colloidal particles) indicates that they elute in the excluded region of both Mixed-A and -D columns. In this region the molecular mass or particle density has no relevance for the elution behaviour that is dependent only on diameter [19]. An additional problem is that oxygenates elute earlier than hydrocarbons [19] and for instance pyrogallol elutes before rubrene of mass 532 and therefore the calibration using polysaccharides might overestimate the molecular mass of humic and fulvic acids; although their oxygen contents are similar (between 30 and 45% for humic and fulvic acids and 49% for polysaccharides) [10], the humic materials are polyelectrolytes and in the protonated form soluble in NMP, that may affect the hydrodynamic volumes in NMP solution compared with those of polysaccharides. No suitable standards are available to check this point.

Literature reports of attempts to examine humic and fulvic acids by MALDI-MS and LD-MS [24–27] have given weak spectra consistent with the detection of only very minor quantities of the sample. Electrospray ionisation with FT-ICP MS [28] has indicated three possible scenarios concerning the molecular mass of Suwannee river humic acid: (1) the material is composed of low molecular masses which appear larger in other techniques, (2) the humic acid is made up of large molecules which fragment in the ion source or (3) the acid consists of large molecules (5000–10,000 u) that are incapable of acquiring sufficient charge to be detected. It seems that option (1) may be the most likely: aqueous SEC has been coupled to electrospray MS [6] for the analysis of Suwannee River humic and fulvic acids and molecular ions up to m/z 2000 were the highest mass ions detected. Suwannee River fulvic acid examined by electrospray ionisation [29] detected ions up to m/z 2500 and by tandem mass spectrometry showed the presence of carboxylic acid groups. None of these mass spectrometric examinations of humic substances has clearly defined the upper mass limits of these materials [7,8] because of the limitations of the electrospray process.

In the present work, we have applied the methods developed on the types of samples listed above, to soil contaminated with coal-liquids and to standard humic and fulvic acids obtained from the International Humic Substance Society as well as to extracts from peats in NMP and to a humic acid prepared from one peat. The aims of the work were two-fold, one to look at soil contaminated by a coal-based industry and two, to observe how humic substances behaved in the SEC system using NMP solvent. These results show that the presence of humic materials does not confuse the identification of soil contamination as from coal-liquids. Also, the difference between humic and fulvic acids prepared by standard methods in solution in NMP and the material of peat directly extractable into NMP is clear. Humic and fulvic acids are released from the humic substance by the extraction method; they appear to elute from SEC relatively early for the molecular masses for humic acids in the literature [6–8].

Table 1
Humic and fulvic acid standards and reference materials used

Material	Acid type	Reference number
Soil	Humic acid standard	1S102H
Peat	Humic acid standard	1S103H
Peat	Humic acid reference	1R103H
Suwannee river	Humic acid standard	1S101H
Leonardite coal	Humic acid standard	1S104H
Soil	Fulvic acid standard II	2S102F
Peat	Fulvic acid standard II	2S103F
Suwannee river	Fulvic acid standard	1S101F

2. Experimental

2.1. Samples

Soil from a redundant coke oven was obtained and consisted of black, stony material collected from the surface of the coke oven site. Selected lumps were washed with NMP to provide one extract solution, and a quantity of material was crushed and extracted to prepare a second extract solution by ultrasonic agitation in NMP.

Humic acids were purchased from the International Humic Substances Society, Department of Soil, Water and Climate, University of Minnesota, St. Paul, MN, USA. Eight materials were obtained and are listed in Table 1.

Peat cores were retrieved from a blanket mire in the Faeroe Islands. The cores were taken in 1995 from Stremoy Island. The samples used in this analysis were sub-samples from 0.5 cm thick samples taken at various vertical depths, measured from the surface of the mire. The four samples used were at depths of 56, 58, 92 and 97 cm. The samples were also extracted into NMP solution using ultrasonic agitation.

A further peat sample, from Fenns' Moss, Shropshire (this was a bulk sample and as such cannot be defined as from a particular depth within the mire) was dried under an infra red lamp and then ground to −710 μ. One gram of the ground peat was heated with 500 ml of 8% NaOH for 1 h. The resulting solution was filtered and the liquid acidified with HCl to pH 1. The addition of the acid caused a precipitate to form that was isolated by filtration. The solid obtained in this way was used in this analysis (designated Fenns' Moss fraction 3) [30,31]. In addition the peat sample (Fenn's Moss) was extracted in NMP using ultrasonic agitation; the NMP and the humic extracts were examined as described below.

2.2. Size exclusion chromatography

The chromatographic system has been described previously [19,22]. Briefly, a polystyrene/polydivinylbenzene packed Mixed-D column (5 μm beads; Polymer Laboratories, UK) was operated at 80 °C with a Perkin-Elmer LC 250 isocratic pump. The eluent 1-methyl-2-pyrrolidinone (NMP) was pumped at 0.5 ml min^{-1}. Three detectors were used: a Perkin-Elmer LC 290 variable wavelength UV-absorbance detector, set at 450 nm, an Applied Biosystems diode array detector set at 280, 300, 350 and 370 nm and a Polymer Laboratories (Shropshire, UK) ELS 1000 evaporative light scattering detector. The UV detectors respond to aromatic material, and the solvent, NMP, is not considered a good solvent for aliphatics, particularly alkanes. The ELS detector responds to material of mass greater than that evaporating with the solvent of boiling point 202 °C and may be considered to be more nearly quantitative in response to large and small molecules than the UV detectors. In addition, a Polymer Laboratories Mixed-A column (300 mm × 7.5 mm i.d.) has been used with a flow rate of 0.5 ml min^{-1} and the same set of UV and evaporative light scattering detectors. This system was operated at room temperature.

Both columns were calibrated [19] using standard polystyrenes from Polymer Laboratories with masses up to 15 million; polymethyl methacrylates were found to elute with the same relation between elution time and molecular masses as the polystyrenes. Polysaccharides eluted with a different relation between mass and elution time [19]. The linear range of the Mixed-D column calibrated by polystyrene standards was from about mass 100 up to mass 200,000, with a different relation between mass and elution time up to the void volume for polystyrenes at 5 million mass units. The Mixed-A column calibration was approximately linear (log_{10} molecular mass versus elution time) for polystyrene and polymethyl methacrylate standards of masses from 1000 up to 15 million units. Polysaccharides and pyrogallol eluted earlier than expected from the polystyrene calibration [19]. Samples and standards were dissolved by mixing with the solvent, NMP, and placing the mixture into an ultrasonic bath. Undissolved material was removed by filtration through a 0.6 μm pore-sized filter; solutions were also left to stand for several hours.

2.3. UV fluorescence spectroscopy

The procedure has been described in detail elsewhere [32]. The Perkin-Elmer LS50 luminescence spectrometer was set to scan at 240 nm min^{-1} with a slit width of 2.5 nm; synchronous spectra were acquired at a constant wavelength difference of 20 nm. A quartz cell with 1 cm path length was used. The spectrometer featured automatic correction for changes in source intensity as a function of wavelength. Emission, excitation and synchronous spectra of the samples were obtained in NMP; only synchronous spectra have been shown and the spectra have been presented in peak-normalised mode. Solutions were diluted with NMP to avoid self-absorption effects: dilution was increased for the contaminated soil extracts until the fluorescence signal intensity began to decrease; in the humic samples and peat extracts, the fluorescence intensity was very low and sample solution was added to the cell to increase the concentration to obtain synchronous spectra significantly more intense than the background signal of the fresh solvent alone, which was itself of very low intensity.

3. Results and discussion

3.1. Size exclusion chromatograms

The calibration of the SEC columns has been discussed in detail [19]. The low mass range of the polystyrene calibration using the Mixed-D column provided a good estimate of masses of standard polycyclic aromatics and other compounds up to mass 1000 [19]. Narrow time fractions isolated from a pitch sample using either a preparative SEC column or the analytical column [19,23,33], gave peak mass values (m/z) by MALDI mass spectrometry that corresponded closely with the polystyrene calibration masses for the times of collection, up to masses of 3000. This calibration provided for the first time, using two independent methods for estimating peak mass of fractions, a good agreement between techniques.

Hence, the polystyrene calibration provides a good estimation of molecular mass of unknown materials at relatively low masses, below 3000 u. At higher masses, the situation is less clear. However, Islas [33], has measured masses of fractions of coal tar pitch excluded from the porosity of the Mixed-D column by static light scattering methods and found values of the order of one million mass units even after the application of "pessimistic" corrections to the measured values to allow for anisotropy of the materials [34]. Similarly, for the Mixed-A column, small standard molecules elute within 1 or 2 min of the extrapolated polymer calibration line [19], where, in addition, the behaviour of spherical standard molecules (soot ~40 nm diameter, colloidal silicas of diameters 22, 12 and 9 nm and fullerene of diameter 1 nm) has been investigated. For both Mixed-A and -D columns, the spherical standards elute within the excluded region and show a relation between $\log_{10}$ diameter and elution time. Oxygenates eluted earlier than expected as shown by polysaccharides and pyrogallol and for the polyelectrolytic and highly oxygenated humic substances, the calibrations are liable to overestimate their molecular masses.

Fig. 1a shows the SEC chromatogram on the Mixed-D column of material extracted from a stony lump of soil. Fig. 1b shows the SEC chromatogram on the Mixed-A column of material extracted from the crushed sample. Both chromatograms show a bimodal distribution with the early, excluded peak of relatively low intensity compared with the second peak, and separated by a valley of zero intensity. These chromatograms are very similar to the equivalent chromatograms for coal tar pitch and it seems highly probable that the contamination at this site is by a high-temperature coal tar pitch.

In order to avoid the possibility of contamination of the coal-derived extract with materials such as humic substances, a set of standard materials from the IHSS was obtained as shown in Table 1.

The behaviour of humic acids and fulvic acids from soil and peat in SEC, using the ELS detector in NMP and the Mixed-A column, is shown in Fig. 2a and b; the two fulvic acids elute at slightly longer times than the humic acids. All of the standards elute within the range of times from 13 to 17 min with no significant signal at smaller masses, equivalent to longer times. These results indicate that the distribution of molecular masses within the humic and fulvic acid samples were similar to each other, with higher peak masses in the humic acids compared with the fulvic acids. Fig. 3 shows the equivalent data for some of the humic and fulvic acids using the Mixed-D column. The peaks for humic acids lie earlier than those for fulvic acids, as for the Mixed-A column, but all of the peaks lie before the exclusion limit of the column at about 10.5 min which is equivalent to a polystyrene mass greater than 200,000 u. The data from both columns are in good agreement in their indication of relative masses of humic and fulvic acids, but the resolution of large molecules by the Mixed-D column was poorer than that by the Mixed-A column, as expected. Estimates of mass using the polysaccharide calibration suggest masses of humic and fulvic acids to be 124,000 and 66,000, respectively; given the mass determinations indicated by ESI-MS [6–8] these values can be seen as overestimates by an unknown factor. However, SEC indicates the standard humic and fulvic acids to be quite different in elution behaviour to the coal-liquids extracted from soil.

Fig. 4 shows the data for the four Faeroes peat extracts on the Mixed-A column. However, the portion of peat soluble in NMP is very small and whereas the humic and fulvic acid solutions were black, the solutions of the peat extracts were

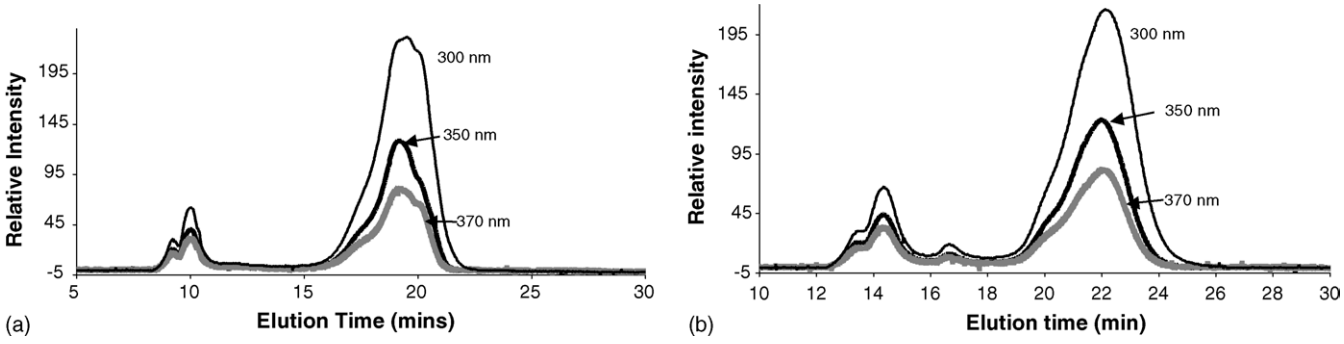

Fig. 1. (a) SEC chromatogram of soil extract from one stony sample, Mixed-D column and (b) SEC chromatogram of extract from the crushed soil sample, Mixed-A column.

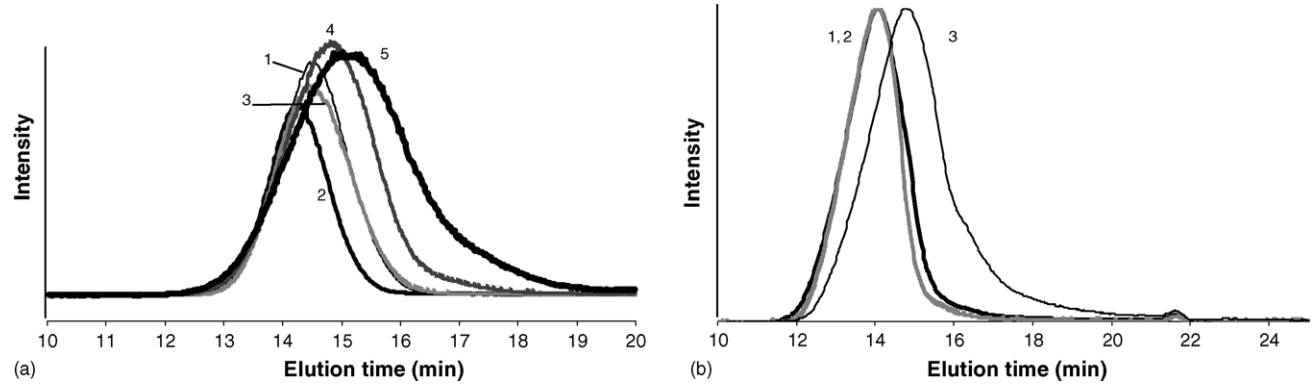

Fig. 2. IHSS humic and fulvic acids on Mixed-A column. (a) Peat standard humic (curve 1), peat reference humic (curve 2) soil humic (curve 3), peat fulvic (curve 4), soil fulvic (curve 5) and (b) Leonardite and Suwannee river humic acids (curves 1 and 2) and Suwannee river fulvic acid (curve 3) by ELS detection; arbitrary intensity units.

yellow; the SEC profiles of the extracts using the ELS detector were very weak. The profiles using the UV absorbance detectors were even weaker, with signal only a few times greater than noise. Although Fig. 4 shows a partly resolved peak at around 15 min as for humic and fulvic acids, there are in addition, both a peak at about 16 min and a peak at longer elution times, corresponding to smaller molecules. The polymer calibration masses equivalent to the peaks from 15 to 16 min range from 5 to 200,000 u, while the peaks at about 20 min are equivalent to polymer masses of about 500 u. The SEC profiles do not appear to show any trend with depth of sampling; the solution from the 98 cm depth sample was too dilute to give a satisfactory chromatogram.

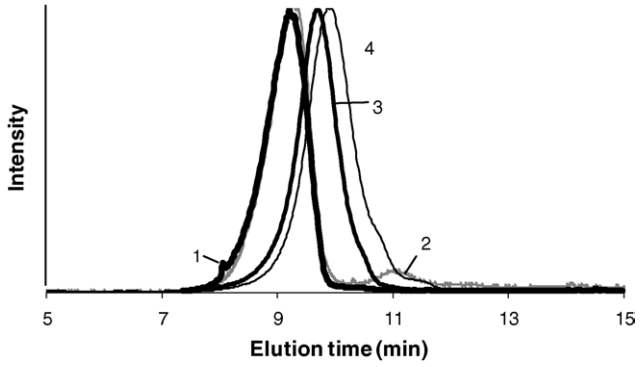

Fig. 3. IHSS humic and fulvic acid standards on Mixed-D column, curves are 1-peat standard humic, 2-peat reference humic, 3-peat fulvic, 4-soil fulvic, by ELS detection; arbitrary intensity units.

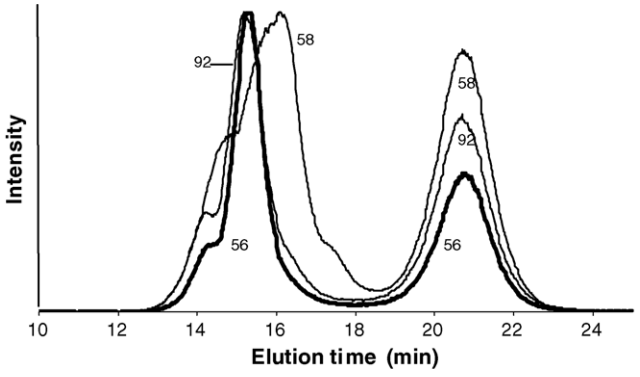

Fig. 4. Extracts from Faeroes peat samples in NMP, Mixed-A column, labels on curves indicate the depth of sampling, by ELS detection; arbitrary intensity units.

Fig. 5a shows the ELS profiles of an extract in NMP of Fenn's Moss peat and of a humic extract from the peat while Fig. 5b shows the same samples by UV-absorbance detection at 300 nm. The NMP extract was very weak compared with the humic material although showing essentially the same features. The slight difference in maxima of the peaks by ELS detection corresponds to small difference of polymer equivalent masses as humic extract 2.7 million and NMP extract 1.9 million. The small peak at about 20 min was from the NMP extract rather than the humic extract. Fig. 5b indicates a greater match of the peaks of NMP and humic extracts with the polymer calibration indicating a mass of about 3 million; the chromatograms were very weak and the noise levels were greater than those by ELS detection. The reasons for the slight difference in behaviour of the two detectors are not clear. Clearly, the NMP extracts from both peats were very much weaker than the humic substances recovered from caustic solution. The NMP extracts behaved differently from the humic and fulvic acids in SEC using NMP as eluent, particularly for the Faeroes peat extracts. These preliminary data suggest that the extraction from these peat samples of humic acids by aqueous base solutions [4,35] might be because these acids are not present as free molecules in the peat sample examined; they cannot be extracted directly by NMP but they might be released by acidification of the alkali solution. The materials in solution in NMP do not appear to be aggregates but might behave as large molecules because of their high oxygen content or polyelectrolytic nature.

3.2. UV-fluorescence spectra

The synchronous UV fluorescence spectra of the material extracted from the contaminated soil was of high intensity,

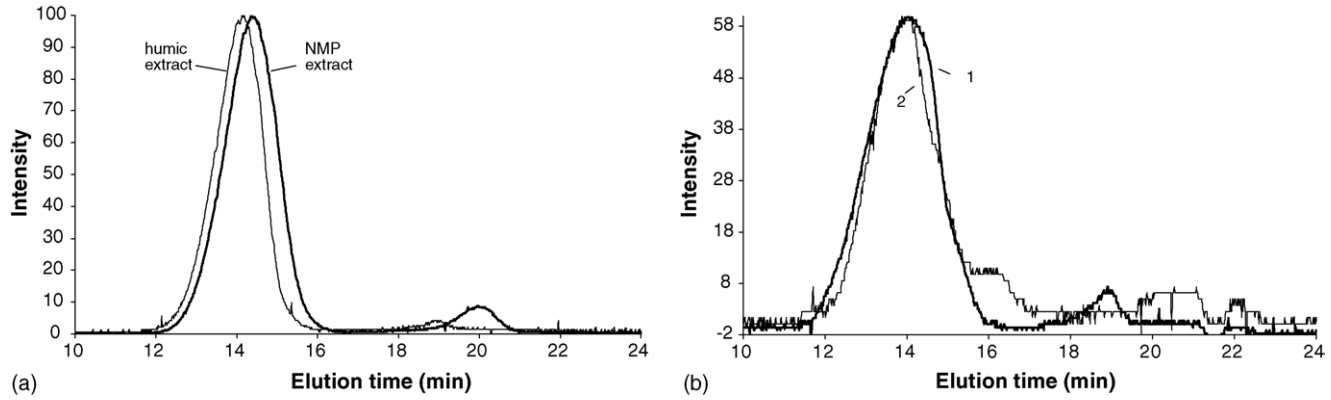

Fig. 5. Fenn's Moss peat, humic extract (curve 1) and NMP extract (curve 2); Mixed-A column, (a) by ELS detection and (b) by UV-A detection at 300 nm.

shown in Fig. 6a and closely resembled that of pitch that has been shown previously [36]. The synchronous spectra of two humic acid standards (Suwannee river 1S101H and a peat standard 1S104H) are shown in Fig. 6b; they compare very well with the spectra issued by the IHSS [10] as data sheets (not shown here). In particular, the peat humic acid shows a maximum intensity at longer wavelengths than the river humic acid, as shown in the data sheets. Although the present spectra are of low intensity, showing low fluorescence, the maxima compare closely with the reference spectra. Fig. 6c shows a comparison of the extract from Fenn's Moss peat in NMP, with the humic extract from the peat, as described above. The humic extract is clearly shifted to longer wavelengths than observed for the solution of peat in NMP, even though the two spectra are of similar, very weak intensities. The UV-F spectra in Fig. 6d of the four extracts in NMP of the Faeroes peat samples show profiles much closer to the Fenn's Moss extract in NMP than to any of the humic acids. The maximum intensity of the synchronous UV-F spectra appear at shorter wavelengths than found for the spectra of

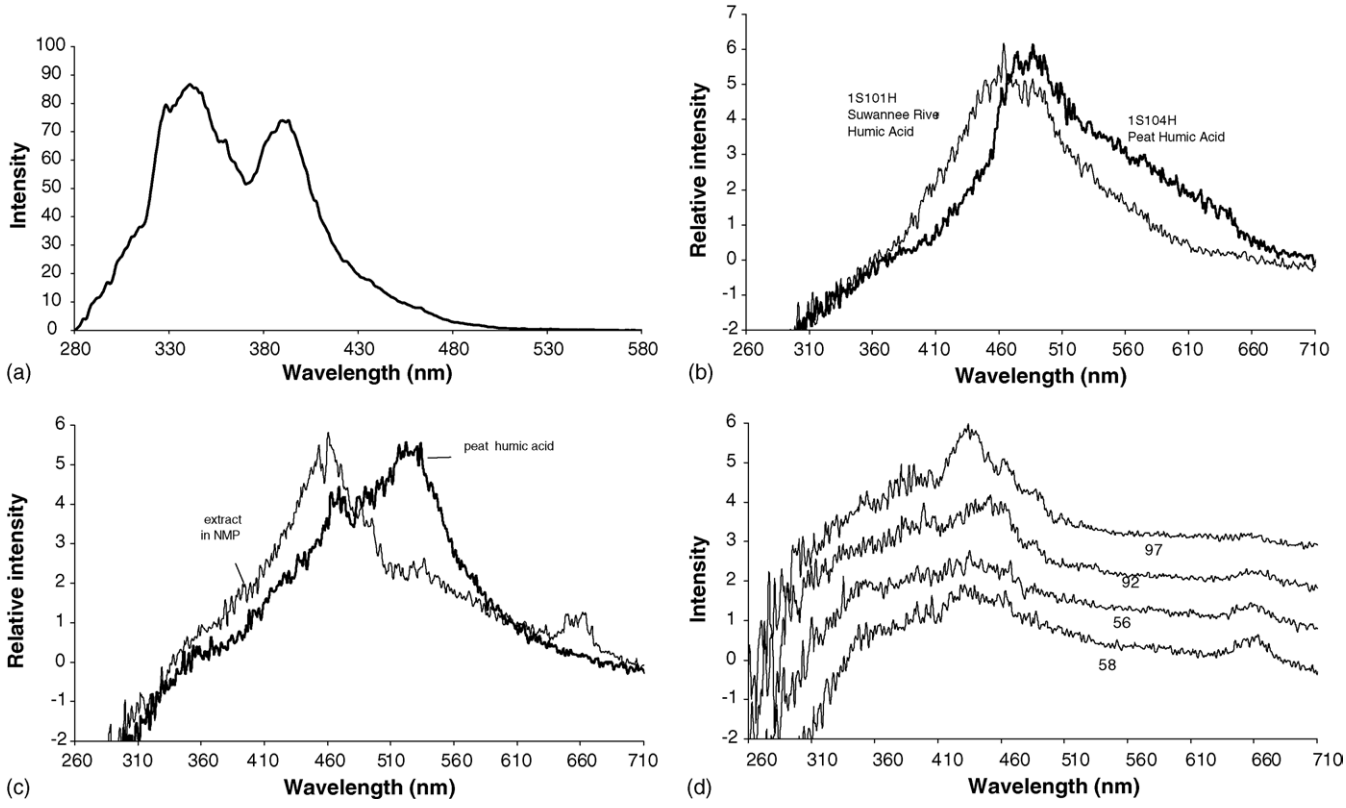

Fig. 6. Synchronous UV-fluorescence spectra of soil extract, peat extracts and humic substances (a) coal-liquid extract from soil; (b) Suwannee river and peat humic acids; (c) Fenn's moss NMP and humic extracts; (d) Faeroes peat extracts labelled with depth of collection (baselines of curves are off-set by successive intensity units as: 58 [0], 56 [1], 92 [2] and 97 [3].

the humic extracts and standard material. These data indicate that the molecules of the extracts in NMP have smaller chromophores than found in the humic materials. These spectra support the suggestion that the humic substances are not present in the original materials as free molecules, but are extracted by reaction with caustic solutions. The spectra of humic substances and extracts of peak are clearly different from that of the contaminating material both in terms of intensity of fluorescence and wavelength of maximum intensity of fluorescence.

4. Conclusions

The size exclusion chromatographic method using NMP as eluent, can distinguish contamination of soil by coal-derived liquids from contamination of the extract by humic substances that may have been in the soil. Humic and fulvic acid standards elute from SEC in a different manner from coal-liquids, suggesting they may either have three-dimensional structures, or elute early because of their highly oxygenated and protonated polyelectrolytic structures, when in solution in NMP. Extraction of peats by NMP and by caustic soda gave samples which were observed to be different by SEC, suggesting that the humic and fulvic acids were not free to dissolve from the peat but were released by the extraction method using boiling caustic soda followed by acidification. The molecular masses of humic and fulvic acids have not been measured in this work and indications from polymer calibrations are liable to be overestimates. UV-fluorescence spectra indicate significant differences between the coal-liquid contamination, the humic substances from peat, soil and coal, and extracts of peat in NMP.

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