# AN INVESTIGATION OF ACTINIDE INTERACTIONS WITH HUMIC SUBSTANCES

by

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# DECLARATION

The material presented in this thesis is the result of independent research carried out between October 1990 and October 1993 at the Scottish Universities Research and Reactor Centre, East Kilbride and the Institute of Terrestrial Ecology, Merlewood, Grange-over-Sands. Any published or unpublished results of other workers have been given full acknowledgement in the text.

# ABSTRACT

Saltmarsh and intertidal areas in SW Scotland and NW England receive inputs of natural and anthropogenic radionuclides from a number of sources. Discharges of low level liquid radioactive waste from the Sellafield nuclear fuel reprocessing plant, with maximum releases in the 1970s, have resulted in contamination of the offshore sediment in the proximity of the discharge point with the anthropogenic nuclides, $^{238}$Pu, $^{239}$Pu, $^{240}$Pu and $^{241}$Am and $^{237}$Np. Dispersal and redeposition of the contaminated sediment has resulted in continuous input of these nuclides to soils and sediments in coastal areas over the past 40 years which will continue for the forseeable future. Natural decay series radionuclides are subject to similar deposition processes following their discharge from a phosphate refinery at Whitehaven. Additionally, natural decay series radionuclides occur at enhanced levels in saltmarsh sediments of the Solway Firth as a consequence of deposition from natural uranium mineralisations. Previous studies, involving sequential extraction of components of soils and sediments, have highlighted the importance of the organic fraction in binding significant proportions of actinides present in these environments.

This thesis presents a study of three locations - a saltmarsh in SW Scotland, a floodplain soil (R. Esk, W Cumbria) and a highly organic soil on the bank of the R. Esk, W Cumbria. The saltmarsh sediments and the Esk soils vary in the degree of marine inundation experienced and provide contrasting environmental conditions which in turn affect actinide geochemistry. Gel filtration chromatography, FTIR, UV and Fluorescence spectroscopy, alpha spectrometry and ICP-MS were used to investigate the influential role of the organic fraction, and in particular humic substances, in determining actinide behaviour. Specifically, these techniques were used to investigate humic properties including structural characteristics and actinide binding ability.

An important part of this work involved the evaluation of traditional extraction methodology and, following the observation that humic substances were irreversibly altered during the separation of humic and fulvic acids, both in terms of their actinide binding capacity and chemical characteristics, the application of

new methodology was developed to minimise these effects.
Conclusions from this study are as follows:

i) the sum of properties of humic acid and fulvic acid is not equal to those of humic substances;

ii) humic substances from diverse locations are compositionally different reflecting the variations in the source of the precursor material;

iii) humic substances comprise only a minor component of the total soil mass but account disproportionately for a significant amount of the actinide binding capacity the soil or sediment;

iv) gel chromatographic separation of humic substances provides fractions which are less heterogeneous than the bulk material ;

v) gel fractionation is unsuitable for size determination of humic materials since certain humic components are retarded by the gel resulting in their elution at greater volumes than proposed purely on the basis of size;

vi) characterisation of fractions of humic substances showed that they vary in chemical composition and in their actinide binding capacity. This provided evidence of discrete associations of actinide elements with fractions of humic substances indicating that a single parameter describing their interaction with the bulk humic material is inadequate;

vii) characterisation of fractions also shows that actinide associations with humic material are influenced by the composition and the degree of diagenetic alteration of the humic material.

In summary, this study has provided evidence of discrete associations of actinide elements with fractions of humic substances and that different components of the humic fraction have widely varying actinide binding capacities. The chemical characteristics of these molecules are vitally important in determining the mobility of these species in the environment.

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# INTRODUCTION

## 1.1 OVERVIEW

This thesis presents a comparative study of the actinides, Th, U, Np, Pu and Am, with soil humic substances from coastal and intertidal areas. Soils in intertidal and floodplain areas around the NE Irish Sea are contaminated with both anthropogenic radionuclides and natural decay series radionuclides originating from nuclear and non-nuclear industrial sources. These areas are of importance agriculturally, recreationally and as nature reserves and the behaviour of radionuclides in this environment following their onshore transport is of importance with respect to human radiation exposure (Pentreath et al., 1980). The presence of long-lived pollutant radionuclides in soils has given rise to concern about their long-term geochemical behaviour, mobility and bioavailability. In addition to their intrinsic importance as pollutants, the presence of relatively high concentrations of actinides facilitates studies of their behaviour in environmental systems, which can provide a better understanding of the processes of mobilisation, transport and retardation of these nuclides. Such studies are of wider importance and provide information which is vital in applications such as the assessment of the performance of planned radioactive waste repositories (Chapman and Smellie, 1986; Côme and Chapman, 1986; Hooker, 1991; Kim 1991; Berry and Bond, 1990; Miller et al., 1994).

Previous work investigating the relative importance of soil components with respect to actinide binding has strongly implicated the organic fraction in surface soils from SW Scotland and W Cumbria. It is well known that natural organic matter exerts a strong influence on the biogeochemistry of the actinides and other trace elements (Degens *et al.*, 1977; Rashid, 1971; Halbach, 1980; Ephraim et al., 1989; Berry and Bond 1991; Bermond and Bourgeois, 1992). Actinide associations with terrigenous and aquatic organic materials have been shown to be significant in intertidal and saltmarsh areas subject to inputs of natural and anthropogenic nuclides (McDonald, 1992; Allan et al., 1991). In particular, the key interest is in humic substances which have been shown to be important in the binding and chemical behaviour of high proportions of metal species present in soils, sediments and waters (Breger and Deul, 1956; MacCarthy and Suffet, 1991; Boyle et al., 1977; Evans, 1989; Dissanayake, 1991; Williams, 1993). The nature of humic interactions with metal species is, however, poorly characterised due to the heterogeneity and chemical diversity of humic macromolecules and the potentially broad range of complexes formed with metal species.

The work presented here comprises the modification of traditional methodology, characterisation of the humic fraction from soils and a comparison of nuclide distributions within the humic fraction.

## 1.2 THE ACTINIDE ELEMENTS

### 1.2.1 Occurrence and Environmental Distribution of the Actinides

This study involved an investigation of the interactions of actinides, U, Th, Np, Pu and Am, with humic substances, so the discussion of the properties of the actinide elements will be restricted to these nuclides for the purposes of this study. Since the discovery of the transuranium elements, there has been much research devoted to studying the chemical properties of the early actinides, atomic numbers 90 to 95, due to their distinct chemical behaviour and observed deviations from predicted behavioural trends arising from complexation reactions, both in laboratory experiments and in the environment. A full discussion of actinide chemistry is found in Katz et al. (1986). Other reviews of the behaviour of Pu, Am and Np appear in Choppin and Stout (1991) and Kauffman (1990).

Of all the actinides, only certain isotopes of U and Th are sufficiently long-lived to be primordial (Table 1.1).

| Nuclide | $t_{1/2}$ | Nuclide | $t_{1/2}$ | Nuclide | $t_{1/2}$ |
|---|---|---|---|---|---|
| $^{227}$Ac * | 21.773y | $^{234}$U * | 2.45x10$^{5}$y | $^{239}$Pu | 2.41x10$^{4}$y |
| $^{228}$Th * | 1.913y | $^{235}$U ** | 7.037x10$^{8}$y | $^{240}$Pu | 6.53x10$^{3}$y |
| $^{230}$Th * | 8.0x10$^{4}$y | $^{238}$U ** | 4.47x10$^{9}$y | $^{241}$Pu | 14.4y |
| $^{232}$Th ** | 1.41x10$^{10}$y | $^{237}$Np | 2.14x10$^{6}$y | $^{242}$Pu | 3.76x10$^{5}$y |
| $^{234}$Th * | 24.10d | $^{238}$Pu | 87.74y | $^{241}$Am | 432.7y |
| $^{231}$Pa * | 3.28x10$^{4}$y | | | $^{243}$Am | 7.38x10$^{3}$y |

**Table 1.1 Half-Lives of Selected Isotopes of Actinides Found in the Environment. (* denotes naturally occurring nuclides ; ** denotes primordial nuclides) from Katz *et al.*, 1986**

U and Th are ubiquitous trace constituents of most minerals and their concentrations in crustal rocks are controlled by geological processes such as fractionation during igneous rock formation (Ivanovich and Harmon, 1992). U and Th become increasingly soluble at higher temperature and pressure and in igneous rocks, they are found predominantly in early-melting phases. Similarly, for metamorphic rocks, there is a dependence on the initial rock composition and effects of migration of nuclides (fluid loss) during metamorphism (Ivanovich and Harmon, 1992).

The transuranium elements are too unstable to have persisted as primordial elements although trace amounts of $^{237}$Np, $^{239}$Np and $^{239}$Pu are found in U-rich ores due to neutron capture and β-decay processes by U where the neutrons originate from (α,n) and (γ,n) reactions involving U and Th (Choppin et al., 1995). Possible formation reactions are shown below :

$^{238}\text{U}\ (\text{n}, \gamma)^{239}\text{U} \rightarrow {}^{239}\text{Np} \rightarrow {}^{239}\text{Pu}$ ;

$^{238}\text{U}\ (\text{n},2\text{n})\ ^{237}\text{U} \rightarrow {}^{237}\text{Np}$;  $^{235}\text{U}\ (\text{n},\gamma)(\text{n},\gamma)\ ^{237}\text{U} \rightarrow {}^{237}\text{Np}$

The probability of such reactions is however reduced by the neutrons being insufficiently energetic or their capture by elements in the ore with high neutron-capture cross-sections.

A further possible source of neutrons is from the spontaneous fission of $^{238}$U although the half life for fission is extremely long (approximately 8 x $10^{15}$ y) resulting in about 70 fissions per second in 1 kg of $^{238}U$ compared with the simultaneous emission of 45 x $10^9$ $\alpha$-particles.

Although they have an extremely low natural abundance, transuranium elements are found in the environment from industrial and weapons testing origin (Table 1.6). Global fallout originating from atmospheric testing of nuclear weapons during the period 1959-1963 has given rise to relatively uniform, low level contamination whereas more recent, localised industrial releases have resulted in restricted areas of higher levels of contamination.

### 1.2.2 Chemistry of the Actinides

Filling of the 5f orbitals for consecutive elements results in contraction of atomic radii along the actinide series which is most significant for elements with atomic number greater than 95. This results in a convergence of chemical properties for the elements following Am due to the high stability of the +3 state for subsequent members of the series. The ability of actinides to exhibit high valences (+3, +4, +5, +6, +7) is the result of the ease with which bonding electrons are lost to give the stable electronic configuration of radon. The most stable oxidation states for the early actinides (Th, Pa and U) are those involving the loss of all f electrons. Known oxidation states for U, Th, Np, Pu and Am are shown in Table 1.3. The solution chemistry of the actinides is complicated by the co-existence of multiple oxidation states and hence several ionic species for each actinide under a given set of conditions. For example, Pu can exist simultaneously in the +3, +4, +5, and +6 states, with the distribution between them being determined by solution $E_h$ and pH conditions and the presence of ligands. Following the normal pattern for polyvalent cations, lower oxidation states of Pu are stabilised by more acidic conditions while higher oxidation states become more stable as the basicity increases (Choppin, 1983; Choppin *et al.*, 1986).

| Element | Oxidation State | Electron Configuration of Most Stable State |
|---|---|---|
| **Th** | (+3) **+4** | $5f^0$ |
| **Pa** | (+3) +4 **+5** | $5f^0$ |
| **U** | +3 **+4** +5 **+6** | $5f^0$ |
| **Np** | +3 **+4** **+5** +6 +7 | $5f^2$ |
| **Pu** | **+3** **+4** **+5** **+6** (+7) | $5f^4$ |
| **Am** | **+3** +4 +5 +6 | $5f^6$ |

**Table 1.2 Stable Oxidation States for Selected Actinide Elements (highest stability indicated in bold) from Katz *et al.*, 1986**

Under pH and redox conditions commonly occurring in natural systems, the higher oxidation states for Np (+6, +7), Pu (+7), and Am (+6) are unstable. This trend of instability of higher oxidation states continues for the actinides following Am, due to greater shielding of the f electrons. Actinide species display great versatility in their potential interactions with a range of ligands. Their ability to form a myriad of complexes is a result of the availability of not only 6d orbitals but also 5f, 7s and 7p orbitals which are of comparable energy and overlap spatially. However, for any given oxidation state, the relative contribution of 6d, 5f, 7s, 7p orbitals is indeterminate. The nature (covalent or electrostatic) of interactions is also uncertain.

The actinide ions are highly electropositive and interact strongly with polar solvents such as water. Metal ions initially cause polarisation and then dissociation of hydrating water molecules. The relative acidity of a metal ion is a function of its charge and size, with smaller size and higher charge resulting in higher acidity. For the actinide elements, the variation in acidities of the metal ions in different oxidation states results in marked differences in their interactions with the hydrate water molecules. Actinide ions in the +3 and +4 states exist as simple cations; their high charge enables them to split a water molecule in spite of their large size and form $M(OH)_x$ species. Actinide ions in the +5 and +6 state are so strongly acidic, due to their smaller size and higher charge, that they can extricate the oxygen from the hydrate water molecules and so form the species, $MO_2^+$ and $MO_2^{2+}$, respectively. Although the M-O distances vary to some extent due to valence changes and the nature of other ligands, measured values show that for the actinides U to Cf this bond is highly covalent (eg. Np-O distance measured to be 1.85 or 1.96 Å, Katz, *et al.*, 1986)

The reaction stages involved in the hydrolysis of actinide +4 and +6 ions are shown in the equations below.

$$M^{4+} + ax\ H_2O \rightarrow M(OH)_x^{(4-ax)+} + axH^+$$

$$M^{6+} + 2H_2O \rightarrow MO_2^{2+} + 4H^+ \ ; \ MO_2^{2+} + ax\ H_2O \rightarrow MO_2(OH)_x^{(2-ax)+} + axH^+$$

The stability of individual actinides with respect to hydrolysis is related to size and effective charge of the actinide ion. For example, the formal charge, +1 and +2 respectively, indicated for the $MO_2^+$ and $MO_2^{2+}$ ions is less positive than that on the central metal ion and this results in the order of stability :

$$MO_2^+ > M^{3+} \approx MO_2^{2+} > M^{4+}$$

The $M^{4+}$ ion is the most highly charged cation known to exist in solution and is the most prone to hydrolysis. The $MO_2^{2+}$ ion has an effective charge of the order of that on the $M^{3+}$ ion so both species are relatively acidic and prone to hydrolysis. The $MO_2^+$ ion is only mildly acidic and is hydrolysed to a lesser extent. In the absence of complexing ligands, all actinide species in solution are prone to hydrolysis and, under varying conditions, form polymeric hydroxy species.

As indicated in Table 1.3 there are chemical differences between the actinide elements in the +4 state. For appreciable concentrations of actinide +4 ions, $Th^{4+}$ is unstable at pH>0.5 whereas $U^{4+}$ is only fully hydrolysed at much higher pH (>2.9) and ionic strength.

$U^{4+}$ and $Pu^{4+}$ are equally prone to hydrolysis whilst $Np^{4+}$ is more stable than would be predicted on the basis of size and charge. However, most hydrolysed cations, where present in non-trace amounts, are highly insoluble and $Np^{4+}$ ions are no exception.

| $M^{4+}$ | I = 0.5M | I = 1M | I = 2M | I = 3M |
|---|---|---|---|---|
| Th | | 4.23 | | 5.0[b] |
| U | 1.47 | 1.56 | 1.68 | |
| Np | | | 2.30 | 2.00 |
| Pu | 1.60 | 1.51 | 1.73 | |

**Table 1.3 Hydrolysis of Actinide +4 Ions At Different Ionic Strength (Constants p$\beta$ refers to the acid dissociation equilibrium $M^{4+} + H_2O \rightleftharpoons M(OH)^{3+} + H^+$ at 25°C where the medium was (Na, H)$ClO_4$ or [b](Na, H)Cl) from Katz *et al.* 1986)**

Actinide 5+ ions are particularly stable for Pa and Np. $NpO_2^+$ and $PuO_2^+$ are predicted to dominate in solution phase interactions of these elements in environmental waters (Pentreath 1982; Choppin *et al.*, 1986; Choppin *et al.*, 1990; Choppin, 1991; Orlandini, 1986).

Hydrolysis of the actinyl ions follows the unexpected order:

$$UO_2^{2+} > NpO_2^{2+} > PuO_2^{2+}$$

and this trend is due to other, presently unknown, factors that influence the stability of the actinides with respect to hydrolysis in the absence of complexing ligands in aqueous solution.

Of the +6 ions, $U^{6+}$ is most stable with respect to reduction, with $Np^{6+}$ and $Pu^{6+}$ being more readily reduced and $Am^{6+}$ being extremely unstable with respect to reduction. This property makes $Am^{6+}$ an exceptionally strong oxidising agent and its existence is only maintained in solution under extreme conditions. There is a related marked decrease in the strength of the metal-oxygen bond with increasing atomic number from U to Am. The actinyl ions are stable entities; once formed the bound oxygen atoms can be removed only by reduction of the actinides to the +4 state.

In any aquatic system, in the absence of high concentrations of organic complexants, the relative order of increasing solubility of actinide ions is inversely related to their particle reactivity which follows the sequence:

$$Th^{4+}, U^{4+}, Am^{3+} \ll Pa^{5+}, Np^{5+}, Pu^{5+} \ll U^{6+}$$

Complexation can, however, alter the relative stability of different oxidation states of the actinides. The stability of the oxidation states of Pu is expected to follow the normal patterns for actinide ions in aqueous solution but such general trends can be negated by the presence of other ligands in solution. For example, the presence of organic ligands can reverse the relative stabilities of the +3 and +4 oxidation states (Foreman and Smith, 1957 : in Choppin, 1983).

From this limited discussion of actinide behaviour it is apparent that :

1. only certain oxidation states for each actinide are important in environmental systems

2. Pu, U and Np can exist in higher oxidation states whereas Am and Th are stable only in the +3 and +4 states respectively and this strongly influences their behaviour in the environment

3. complexants in natural systems can significantly alter the stability of actinide species and their influence on the biogeochemical behaviour of the actinides is discussed in section 1.4.5.

## 1.3 OCCURRENCE, DISTRIBUTION AND ASSOCIATIONS OF ACTINIDES IN THE IRISH SEA AND SURROUNDING AREAS

In an environmental context, the presence of natural and anthropogenic radionuclides, in particular the actinides which are predominantly α-emitters, provides a potential hazard to man (Pentreath, 1980). A number of sources of actinides have contributed to their inventory in the Irish Sea, including British Nuclear Fuels plants at Springfields, Chapelcross and Sellafield and the Marchon Chemical Plant at Whitehaven, but it is well documented that Sellafield is by far the largest source of anthropogenic radionuclides to this environment.

### 1.3.1 Sellafield

Since 1952, the British Nuclear Fuel reprocessing plant at Sellafield in Cumbria has discharged low-level radioactive waste (Fig. 1.1), which includes the transuranics Np, Pu and Am, into the NE Irish Sea as exemplified in Table 1.4. Table 1.5 indicates the decay characteristics of these radionuclides.

| Nuclide | Annual Discharge (1974)(TBq) | Authorised Limits (TBq) | Annual Discharge (1992)(TBq) | Authorised Limits (TBq)* |
|---|---|---|---|---|
| U | 10936kg | - | 633.0kg | - |
| Np-237 | | - | 0.18 | - |
| Pu-α | - | | 0.94 | 7.0 |
| Pu-241 | 1700 | - | 25.3 | 170.0 |
| Am-241 | 120 | - | 0.54 | 3.0 |
| Total α | 170 | 225 | 1.6 | 10.0 |

**Table 1.4 Selected Annual Discharges from BNF Sellafield in W Cumbria (* Authorised Annual Discharges as of 1990)**

| Nuclide | Half Life (Years) | Decay Mode | Exposure Risk |
|---|---|---|---|
| $^{238}U$ | $4.47 \times 10^9$ | α | Internal |
| $^{237}Np$ | $2.14 \times 10^6$ | α | Internal |
| $^{238}Pu$ | 87.74 | α | Internal |
| $^{239}Pu$ | $2.41 \times 10^4$ | α | Internal |
| $^{240}Pu$ | $6.57 \times 10^3$ | α | Internal |
| $^{241}Pu$ | 14.7 | γ | External |
| $^{241}Am$ | 432.2 | α<br>γ | Internal<br>External |

**Table 1.5 Decay Characteristics of Selected Radionuclides from Katz *et al.*, 1986**

Reprocessing involves a series of chemical separation processes in which spent U fuel rods are treated to separate residual U (>96% by mass), Pu (1% by mass) and highly-active waste (<3% by mass). The separation processes, following the removal of the fuel cladding, involve dissolution of the U fuel rods in nitric acid. Waste products are removed from solution and the U and Pu subsequently separated by further chemical procedures. The current reprocessing plant began operation in 1964, replacing the earlier plant, its main function being to reprocess fuel from Magnox reactors. Figure 1.1 shows temporal trends in the discharge of Pu, Am and and fission product, Cs over the time period 1952-1993. Figure 1.2 shows the total alpha and total beta activity discharged annually, together with annual authorised discharge limits.

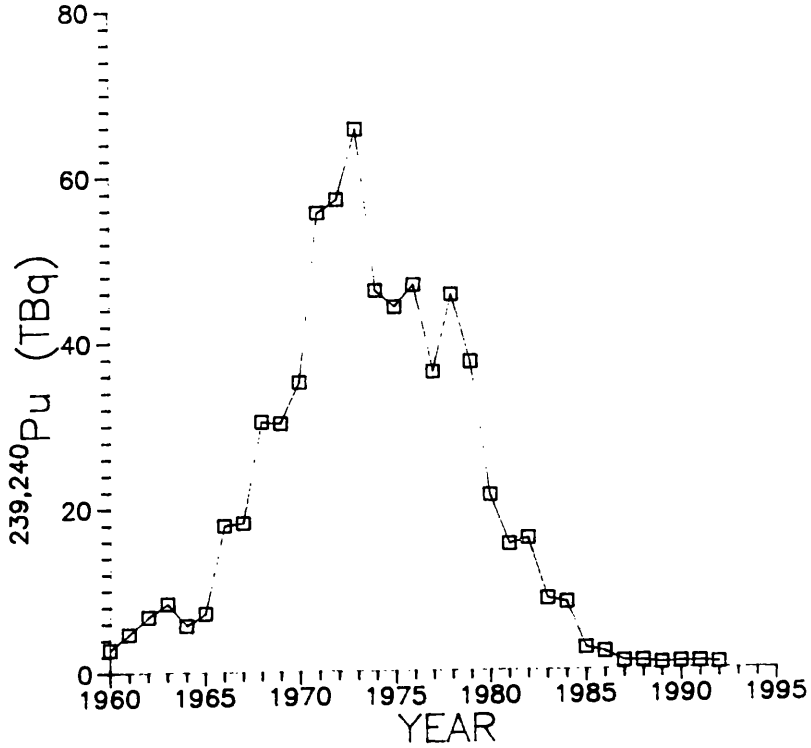


**Figure 1.1 Discharges of $^{239,240}$Pu from BNF plc Sellafield from 1952-1993.**

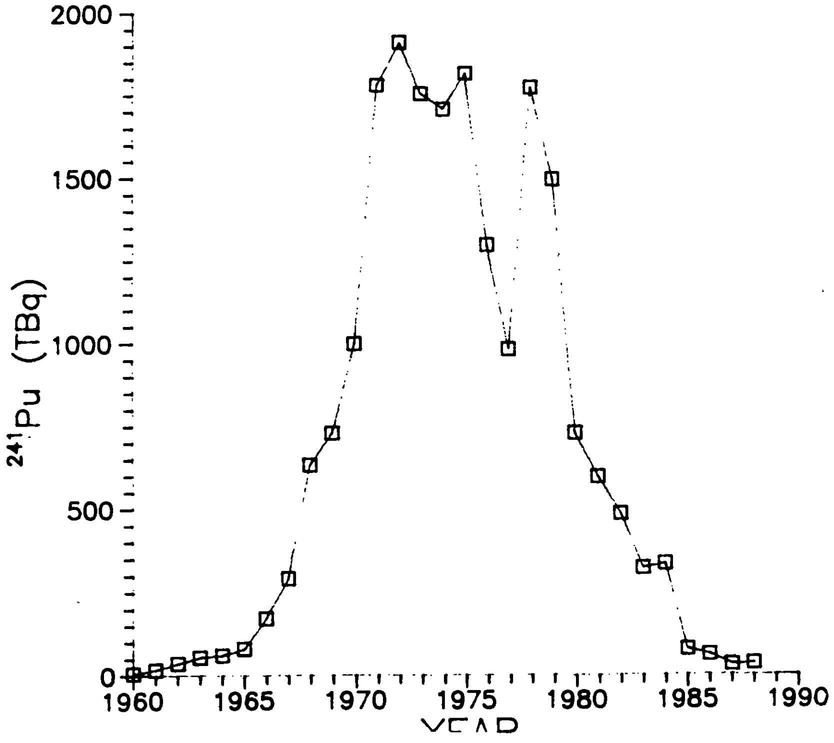


**Figure 1.1 Discharges of $^{241}$Pu from BNF plc Sellafield from 1952-1993.**

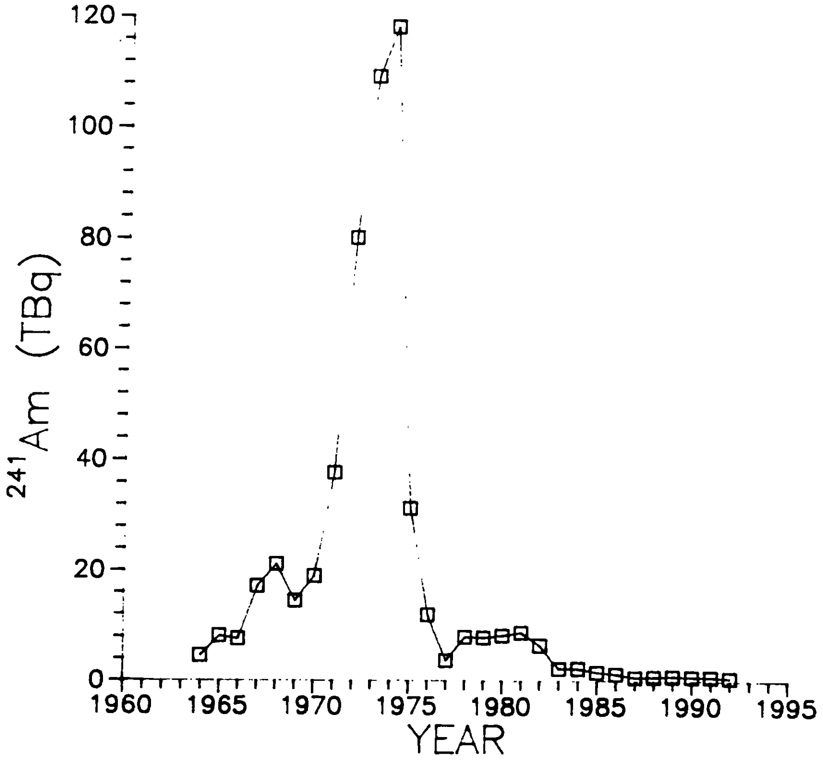


**Figure 1.1 Discharges of $^{241}$Am from BNF plc Sellafield from 1952-1993.**

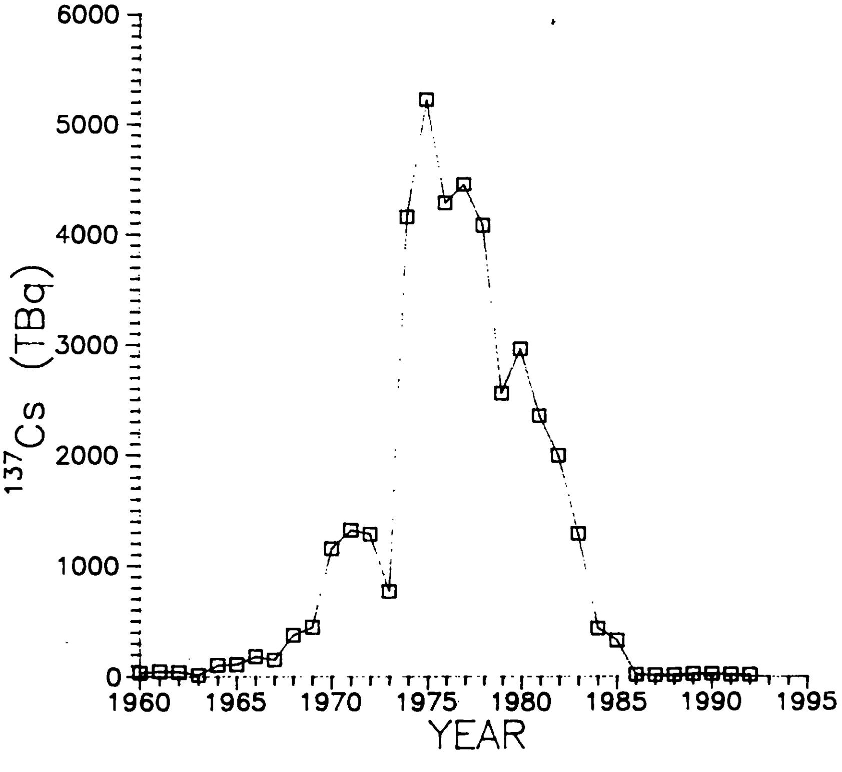


**Figure 1.1 Discharges of $^{137}$Cs from BNF plc Sellafield from 1952-1993.**

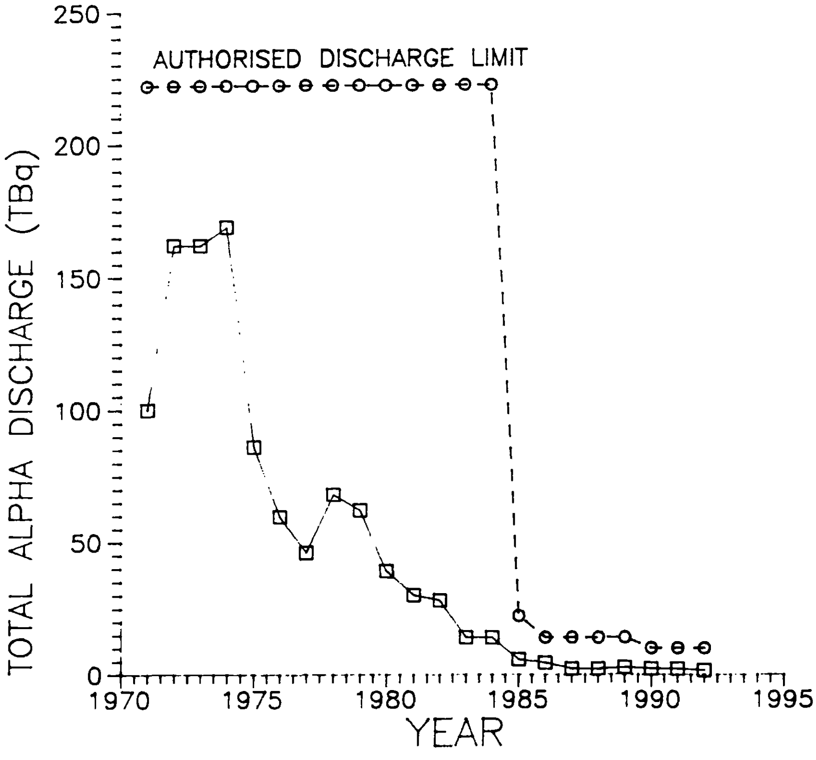


**Figure 1.2 Total Alpha Activity Discharged from BNF Sellafield**

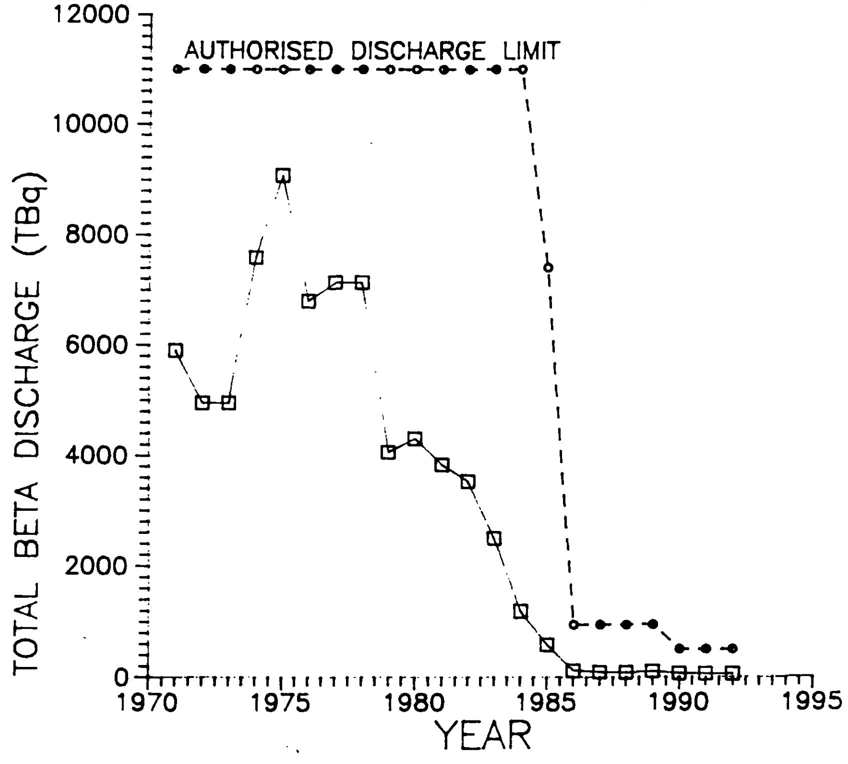


**Figure 1.2 Total Beta Activity Discharged from BNF Sellafield**

Maximum discharges in the mid-1970s are indicative of the Magnox programme reaching full capacity. Subsequent improvements in waste treatment technology have resulted in the reduction by two orders of magnitude in the discharge of the actinides.

Low active liquid waste arises from the reprocessing plant and the fuel storage ponds and is discharged into the Irish Sea through a pipeline which extends one and a half miles from shore. The reprocessing plant liquid waste is contained in sea tanks prior to release and the effluent from these dominates the total discharge inventory for Pu. Pentreath *et al.* (1984) reported that about 99% of the Pu from this source appeared in the reduced, particle associated form, whilst approximately 50% of the Np appeared in the oxidised form. The partitioning of Np and Pu between the solid and aqueous phases varied between the pond water and the reprocessing waste and this has been related to the observed differences in oxidation state for released Pu and Np. In particular, there was a higher proportion of dissolved Pu in the pond water relative to that of the sea tanks which is due to the prevalence of +5 and +6 oxidation states in the pond water filtrate (Pentreath *et al.*, 1984). Np is strongly oxidised in the pond water filtrate but also to an extent in the sea tank filtrate prior to neutralisation and Fe floc formation. Overall, approximately 100% of the Am, and >90% of the Pu is immediately particle associated following release, with their subsequent dispersion being controlled by the redistribution of the particulate phase of the sediment. In particular, a bank of fine sediment close to the discharge point contains a large proportion of the released radionuclides (Smith *et al.*, 1980; Pentreath *et al.*, 1984). The long-lived nature of these nuclides has resulted in their continued presence at enhanced levels in Irish Sea sediments. Table 1.6 shows the environmental inventories of these nuclides in comparison with global fallout and Chernobyl inventories.

| Nuclide | BNF plc Waste Radionuclides (Total to 1990) | | Atmospheric Nuclear Weapons Tests | | Radionuclides Released During Chernobyl Reactor Accident in 1986 | |
|---|---|---|---|---|---|---|
| | Activity (Bq) | Mass (kg) | Activity (Bq) | Mass (kg) | Activity (Bq) | Mass (kg) |
| $^{137}Cs$ | $3.0x10^{16}$ | 9.4 | $1.3x10^{18}$ | 406 | $2x10^{16}$ | 6.2 |
| $^{239,240}Pu$ | $6.8x10^{14}$ | 253 | $8.9x10^{15}$ | 3900 | | |
| $^{241}Am$ | $8.9x10^{14}$ | 7 | $3.0x10^{15}$ * | 24 * | | |

**Table 1.6 Environmental Inventories of Anthropogenic Radionuclides (* Estimated inventory for $^{241}$Am in 1990 produced by the decay of $^{241}$Pu released during atmospheric nuclear weapons tests)**

The inventories for $^{241}$Am incorporate both documented discharges and ingrowth of this nuclide from decay of discharged $^{241}$Pu, a short-lived β-emitter.

The dispersion of these radionuclides is influenced by the movement of water and sediment in the proximity of the discharge point (MacKenzie *et al.*, 1987). The flow of water in the NE Irish Sea is northwards to the Solway Firth and the North Channel (Pentreath *et al.*, 1984). There is a strong dependence on aqueous/solid phase partitioning which governs the mechanism of transport of different radionuclides. The dispersal of these nuclides has been confirmed by measurement of concentrations of up to $4.5x10^3$ $Bqkg^{-1}$ of $^{239,240}$Pu and $3.3x10^3$ $Bqkg^{-1}$ of $^{241}$Am in coastal sediments in W Cumbria (McDonald, 1993). It has been shown that the dominant mechanism of Pu and Am supply to intertidal areas of the Irish Sea involves redistribution of contaminated marine sediment originating from the deposit of fine marine sediment in the proximity of the discharge point (Hunt, 1985; MacKenzie and Scott, 1993).

The point source nature of the Sellafield discharge, together with particle-associated transport of these nuclides, has resulted in the contamination of surrounding coastal and floodplain areas at levels much in excess of those arising from global fallout. Figure 1.3 shows sediment profiles for Pu, Am and Cs for a sediment core collected in 1990.

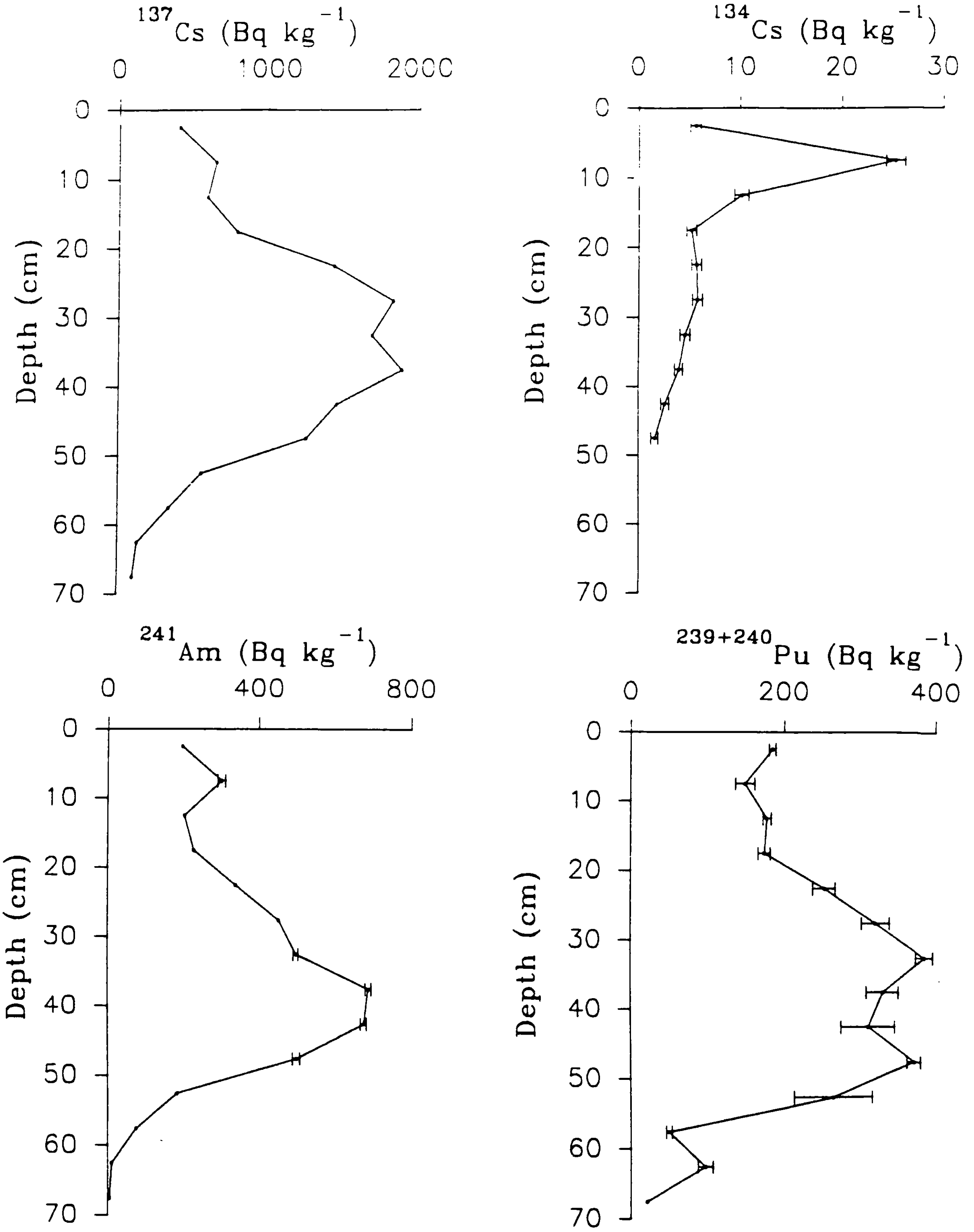


**Figure 1.3 Sediment Profiles for Pu, Am and Cs in Saltmarsh Sediments collected in 1990 (Allan, 1993)**

Continuation for the foreseeable future of onshore transport, in conjunction with dilution of the contaminated sediment, will result in decreasing concentrations of radionuclides in the deposited material but increases in the total radionuclide inventories in accumulating floodplain deposits of the Irish Sea. Short-lived radionuclides that are unsupported by a parent nuclide or by further discharges provide a short term hazard. The long-lived nature of $^{237}$Np, $^{238}$Pu, $^{239,240}$Pu and $^{241}$Am (see Table 1.5) means that only small decreases in environmental inventories of these nuclides will be observed in the short term (decades). $^{241}$Pu with a half-life of only 14y decays to give $^{241}$Am and so increases the activity of $^{241}$Am with time (Livingston and Bowen, 1975; Day and Cross, 1981). The ingrowth of $^{237}$Np from $^{241}$Am provides an additional source of Np in the environment. $^{237}$Np has also been discharged from the Springfields fuel fabrication plant and the Sellafield reprocessing plant. The activity of $^{237}$Np that has been discharged from Sellafield is uncertain and is documented only as a component of total activity due to $\alpha$-emitters in the total discharge. Moreover, little environmental data exist for the distribution and behaviour of Np due to its low concentrations in most soils and sediments (Pentreath and Harvey, 1981; Assinder, 1991). Np is less particle reactive than Pu and Am and empirical results suggest that transport of Np may follow a pattern more similar to Cs than other actinides (Hursthouse, 1990; Hursthouse, 1991; Assinder, 1991). The ratios of Pu and Am respectively to Np decrease with increasing distance from the discharge point and, in particular, Np concentrations in the marine sediment decrease with increasing distance northwards. These observations suggest that Np is more soluble than Pu and Am and this is consistent with measured $K_d$ values calculated from the partitioning between the solid and aqueous phase (Hursthouse, 1991; Assinder, 1991). With increasing time, the radiological importance of $^{237}$Np will increase due to significant levels of discharge from the Sellafield and Springfields plants and its longevity together with its ingrowth following the decay of $^{241}$Am. Figure 1.4 shows the relative importance of nuclides, with increasing time, in terms of human exposure. Figure 1.5 shows the critical group internal and external exposure for the Sellafield discharge.

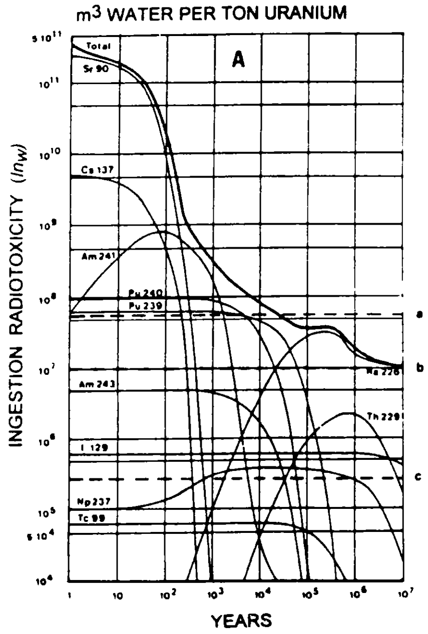


**Figure 1.4 Radiotoxicity of the Dominating Nuclides in Spent Fuel : Ingestion (from Choppin *et al.*, 1995)**

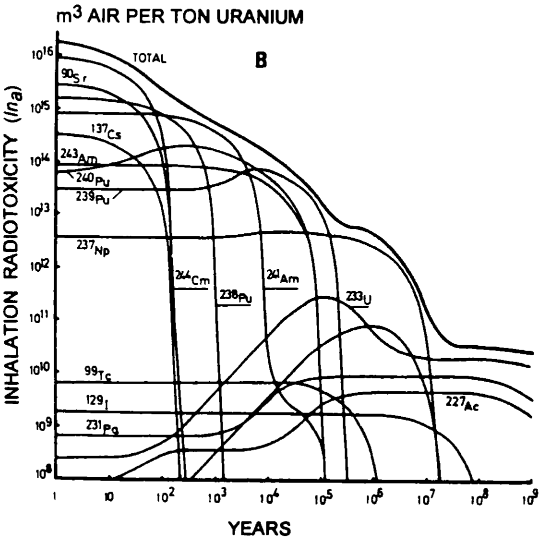


**Figure 1.4 Radiotoxicity of the Dominating Nuclides in Spent Fuel : Inhalation (from Choppin *et al.*, 1995)**

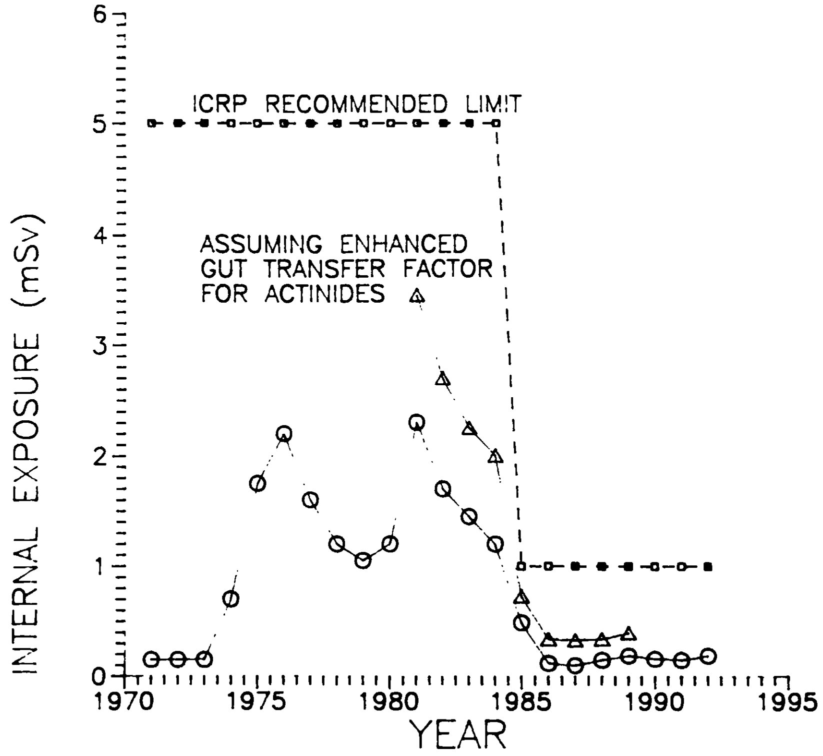


**Figure 1.5 Internal Exposure of the Critical Group as a Result of Discharges of Radionuclides from the Reprocessing Plant at Sellafield**

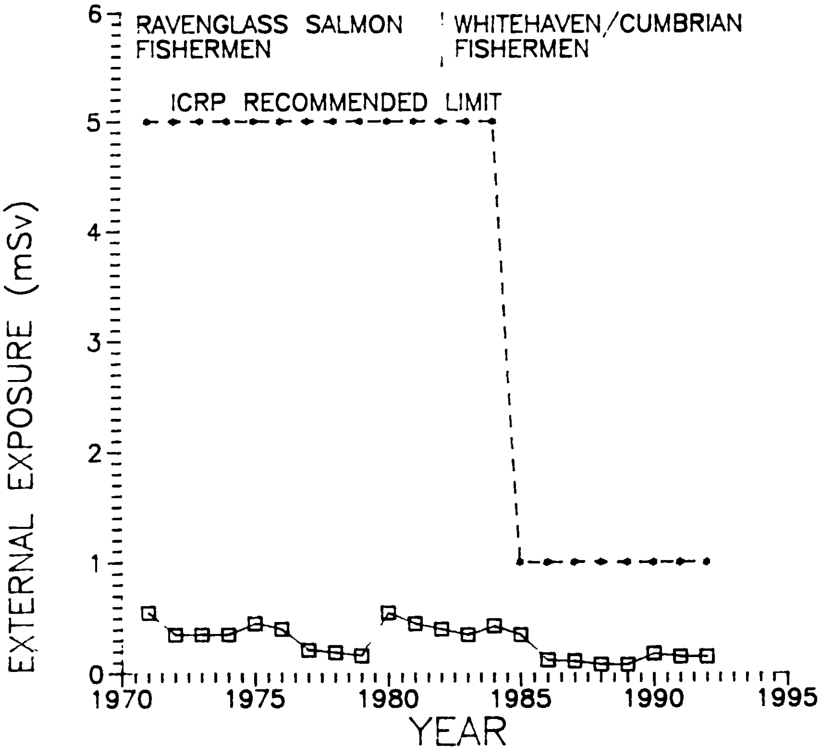


**Figure 1.5 External Exposure of the Critical Group as a Result of Discharges of Radionuclides from the Reprocessing Plant at Sellafield**

Reductions in discharges are expected to give rise to lower exposure levels which are within present authorised limits. The exposure calculated on the basis of maximum discharge levels exceeds the present limits by a factor of four. Particulate transport and resuspension mechanisms mean that actinide supply to accumulating sediments and intertidal areas will continue on a long term basis. This indicates a continuing and potentially increasing potential radiation risk and highlights the importance of further work to elucidate environmental influences on actinide behaviour.

Following their deposition in a terrigenous system, mechanisms of interaction and processes governing movement of the actinides within the soil or sediment are of vital importance in determining their bioavailability.

Other factors influencing actinide distribution in coastal soils and sediments include variations in vegetation and topography which affect the deposition process and hence particle size composition at a given location. Diagenetic processes in sediments can result in changes in actinide association with increasing time after deposition. Importantly, actinide interactions with, and their distribution amongst, soil and sediment components will subsequently control their soil/sediment solution behaviour and hence toxicity.

### 1.3.2 Albright and Wilson Phosphate Refinery

Discharges of natural radionuclides into the Irish Sea are also significant. Natural radionuclides from anthropogenic sources include aquatic discharges from Sellafield, run-off from agricultural land, leakage from coal mines, but most significantly in the Irish Sea, waste releases from the Albright and Wilson Marchon chemical plant at Whitehaven. The plant, sited 2km north of St. Bees Head, manufactured phosphates from sedimentary rocks from 1954-1992. Phosphate ores often contain high levels of naturally occurring U and Th and their decay products. Refining of imported ore at the Albright and Wilson Plant at Whitehaven has led to the discharge of natural decay series radionuclides into the Irish Sea increasing their concentrations in the local environment and providing a second industrial source of actinides. Discharges via a pipeline on the north side of St. Bees Head and spillage of ore during unloading in Whitehaven harbour have resulted in enhanced levels of natural radionuclides, mainly U, in surface sediments and high levels of $^{226}$Ra in solution (McCartney *et al.,* 1990). The annual discharges of U from the Marchon works are in vast excess of those from Sellafield. On average, 30 tonnes per year were discharged over the period 1954-1992 in comparison with maximum annual discharges of 4 tonnes from Sellafield. The Marchon discharges are estimated to have generated a total environmental inventory to 1992 of approximately $1.9\text{x}10^{13}$Bq of $^{238}$U and a similar activity of $^{226}$Ra, assuming secular equilibrium and no differential retention of daughters during processing.

Since 1992, refined phosphate ore has been imported and discharges of natural radionuclides have ceased (Keating, 1993). However, the long-lived nature of certain nuclides including $^{238}$U and $^{230}$Th indicates their continued presence in the environment for the foreseeable future. Enhanced levels of natural radionuclides, and in particular $^{230}$Th, have been observed in sediments along the Solway Coast in SW Scotland (Ben Shaban, 1985). Past discharges from the phosphate refinery, therefore comprise a potential additional source of actinide elements to the Solway sediments chosen as a sampling location and are of relevance in the context of this study.

### 1.3.3 Natural Uranium Mineralisations

A further local, non-marine source of natural radionuclides to soils and sediments in coastal areas of SW Scotland is from a series of mineralised U veins occurring along the edge of the Criffel Pluton (Miller and Taylor, 1966). The largest of these is located close to a natural rock arch known as The Needle's Eye. The Criffel Pluton was intruded some 400 million years ago into Silurian metasedimentary rocks while the age of the U vein is estimated to be about 200 million years (Halliday *et al.*, 1980). The vein material consists of pitchblende, a form of uraninite, together with brittle bituminous hydrocarbons, pyrite, chalcopyrite, and native bismuth in a quartz, calcite, dolomite gangue. The U occurs mainly in the leachable pitchblende although some exists in secondary minerals due to redistribution following weathering of the rock (Hooker, 1991).

In front of the cliffs, forming the edge of the pluton, are post-glacial floodplains, known locally as Merse areas, and intertidal mudflats. These sediments are Quaternary deposits 1.5-2m thick, mainly of quartz-rich silts, with variable amounts of clay and humic debris. The Netherclifton Merse, a sampling site in this study, is cut by the tidal channel of the Southwick Water into which many small fresh water streams flow from the base of the cliff and from inland.

Groundwater flowing through fractures in the cliff in the vicinity of the U vein is quite strongly oxidising, causing preferential dissolution of soluble members of the U decay series from exposed minerals. The groundwater draining from the cliff is enriched in U and this is instrumental in the translocation of U into organic rich Quaternary sediments and the floodplain silts of the mudflats (Basham *et al.*, 1989, Roberts *et al.*, 1988, Milodowski *et al.*, 1990). The oxidising nature of this water means that U is predominantly in the oxidised form, $UO_2^{2+}$ with 15% of the U in solution being organic associated (MacKenzie *et al.*, 1991). Conditions immediately below the cliff are highly reducing and the high organic content of the soil contributes strongly to the removal of U from solution. There is a low organic association of U in the cliff groundwater but, not surprisingly, a marked increase in organic association in the coloured soil pore water. Run-off from streams to the saltmarsh area results in the transport of U from the cliff to the Merse where it is redeposited in association with humic materials or Fe/Mn oxyhydroxides (MacKenzie *et al.*, 1991).

The floodplain areas are also subject to periodic inundation from the sea with resultant deposition of sediment and the sampling site has been subject to fast accumulation of sediment over a period in excess of twenty years (Allan *et al.*, 1991). The presence of enhanced levels of natural decay series (and anthropogenic) actinides in sediments at the marine edge of the Merse is predominantly attributed to deposition of contaminated Irish Sea sediments whilst acknowledging that the natural uranium veins become a more important source of naturally occurring radionuclides, particularly with increasing distance inland towards the vein.

### 1.3.4 Radionuclide Associations in Soils and Sediments

A discussion of the nature and characteristics of soil and sediment constituents can be found in Greenland and Hayes (1978). Compositional and structural variations of soils and sediments exert a major influence on their ability to retain radionuclides and the distribution within the solid phase as illustrated in Table 1.7.

| **Soil or Sediment** | **U and Th Distribution** |
|---|---|
| Clays/Sediments | Th strongly associated with clays and heavy minerals |
| Mineral Soil | U and Th strongly associated with Fe/Mn oxyhydroxides |
| Organic Soils/Peat | U and Th strongly complexed by the humic fraction. Peat or humic substances may influence the formation of U minerals |

**Table 1.7 Associations of U and Th in Soils and Sediments**

Sequential leaching techniques are often used to determine solid phase associations within soils and sediments and have consistently highlighted the importance of the organic fraction for many metals including the actinides (Cook *et al.*, 1984; Livens, 1985; Livens *et al.*, 1987; MacKenzie *et al.*, 1991; McDonald *et al.*, 1992). This method, which applies a series of reagents to remove notionally discrete components of the solid phase together with associated metals, provides information relating to actinide associations with components of soils and sediments (Ure, 1991). Criticism of sequential leaching methods has been made on the basis of laboratory experiments which indicated that results depended on the order of extraction of the various soil components (Wilkins *et al.*, 1986; Kheboian and Bauer, 1987; Mudge *et al.*, 1988). These studies involved the addition of metals to the soil sample prior to sequential extraction of components. Tessier *et al.* (1979) showed, however, that the use of sequential leaching methods on samples which had not been subject to metal addition in the laboratory (ie. potentially unaltered environmental metal associations) produced reproducible results for a specific soil or sediment type and concluded that sequential leaching does provide a useful means of studying solid phase associations of radionuclides in the environment.

Sequential leaching is limited in its usefulness by the lack of mechanistic detail regarding actinide interactions with any operationally defined component and the non-identification of the proportions or nature of the extracted component involved in binding. The non-homogeneity of the organic fraction and vast differences in the binding capacity of molecules from which it is comprised are not apparent from the sequential leaching technique. It simply gives a broad indication of the importance of an undefined portion of the organic fraction. Further characterisation of the organic fraction has led to the knowledge that humic substances can account for a significant proportion of the total organic-actinide binding and indeed also actinide binding by the soil matrix. The importance of the humic fraction can be put in context when it is considered that it may comprise as little as 1-2% of the total soil mass but frequently accounts for >50% of Pu and Np binding (Hursthouse, 1990; McDonald *et al.*, 1992) and up to 94% of U (MacKenzie *et al.*, 1991).

For both soils and sediments, a strong relationship between particle size and radionuclide content has been demonstrated. For example, Livens and Baxter (1987) showed that for a sand, a conifer soil, a gley and an intertidal sediment there was preferential concentration of radionuclides in the finer size fractions (<2μm). This is reflected in the non-uniform distribution of radionuclides in sediments in coastal areas of SW Scotland (Allan,1993). Topography and vegetation influence the particle size distribution at a number of locations along this coastline.

Differences in particle size distribution will therefore influence the behaviour of the radionuclides and this information is consequently important in the context of this study. The finer marine particulate material may contain organic material and radionuclide associations in this material prior to deposition would also be of great value. There is some information presently available regarding the radionuclide associations in offshore sediments which suggests a 20% association of Pu with organic matter (McDonald, 1992). However, there is less information regarding the radionuclide associations in suspended particulates in incoming tidal waters and the nature of the changing associations observed on and following deposition in saltmarsh sediments. A major objective of this study has therefore been to characterise radionuclide associations with the organic fraction and the changes occurring with increasing time from deposition of natural and anthropogenic radionuclides in these environments.

## 1.4 HUMIC SUBSTANCES

### 1.4.1 Introduction

Historical reviews of the study of humic substances by Stevenson (1982) and Schnitzer and Khan (1972) suggest that the earliest documented attempts to isolate these materials were made by Achard in 1786 who, using an alkaline solution, extracted an organic component from peat which resulted in a dark amorphous precipitate upon acidification. Schnitzer and Khan (1972) also note the observations made by Wallerius (18th Century) who proposed that a component of soil organic matter had the capacity to adsorb water and plant nutrients. Further study led to the knowledge that a stable component with these properties was derived from organic degradation products of plant and animal matter and, more recently, the organic macromolecules comprising this component were classified as humic substances (Kononova, 1966).

Present consensus suggests that humic substances are a highly complex, heterogeneous mixture of organic macromolecules with high proportions of functional groups distributed non-uniformly along an aliphatic and/or aromatic backbone. Humic substances comprise only part of the total organic fraction of soils, sediments and water, making up approximately 50% of the organic material in a well-formed soil (Rashid, 1985) and up to 80% of dissolved organic material in aquatic systems (Higgo *et al.*, 1992). Conventionally the humic portion of the organic fraction, which is extractable in alkali, can be subdivided into humic acid and fulvic acid on the basis of acid/alkali solubility. Humic acid is only soluble under alkaline conditions and is precipitated on the addition of acid, whereas fulvic acid is soluble under acidic and alkaline conditions (Mulder, 1862; Stevenson 1982). Humic material which is insoluble in alkali is termed humin (Mulder, 1862; Stevenson, 1982). This fraction may simply contain humic material strongly linked to mineral matter causing it to be insoluble in alkali (Stevenson, 1982) or may be highly degraded humic material with low functionality ie. a coal precursor (Killops and Killops, 1993). The high functionality and polyelectrolyte behaviour of extractable humic material is responsible for its importance in metal binding in the environment. Its structure is, however, poorly characterised and its interactions with metals ill-defined, so the properties and behaviour of humic material in soils, sediments and water are topics of continued study.

The presence of organic matter in soil has a number of beneficial effects on its biological, physical and chemical properties and affects the activities of microflora and microfaunal organisms. The initial decomposition of organic material by soil microorganisms releases inorganic species (eg. N, P and S compounds) and small organic molecules (eg. amino acids) required for plant growth and so contributes to soil fertility. Following its formation, humic material, due to its porous texture, increases the proportion of pore spaces in clay soils by the formation of clay-humic aggregates (Stevenson, 1982). This leads to improved drainage and aeration of the soil by maintaining large pore spaces through which water can enter and percolate downwards. Humic material is also responsible for increased water retention in sandy soils where it binds the sand grains, thereby reducing pore spaces (Schnitzer and Khan, 1972). In addition, humus increases the ability of a soil to resist erosion through effects of surface aggregate stability, surface sealing and crusting and compaction (Gabriels and Michiels, 1993; Stevenson, 1982). The formation of aggregates promotes soil granulation and the resultant soil particles are less easily lost by the movement of water. Humic substances therefore play a dual role in improving the physical structure of a soil in that they facilitate the movement/retention of water and nutrients whilst promoting stability of the upper soil profile. Moreover, their ability to form soluble complexes with certain important trace metals, including Cu, Mn and Zn, potentially increases the mobility (Smith, 1991) and bioavailability of metals (Sholkovitz *et al.*, 1978; Sholkovitz, 1976; Picard and Felbeck, Jr., 1978). The humic fraction is also known to reduce the toxicity to plants and indirectly animals, of metal species such as Al by the formation of insoluble complexes. As a consequence of its complexation characteristics, soil humic material has been extracted and marketed commercially as a soil conditioner, stabiliser and fertiliser and more recently to complement other plant feeds by promoting transport of added nutrients to the plant roots. Humic material plays an indirect role by affecting the uptake of added micronutrients by plants. Humic substances are also known to affect the performance of agricultural chemicals by altering their bioactivity, persistence and biodegradability (Stevenson, 1982) and may modify the required application rate of such chemicals.

In aquatic systems, certain ill-defined, high molecular-weight organic compounds, displaying properties similar to humic substances, have several important effects on the growth of phytoplankton. Although nutrient levels and solar energy govern primary productivity, humic-like compounds display growth regulatory effects complementary to these primary effects (Wershaw, 1991). Marine humic compounds are termed "biological conditioners" as a result of their role in influencing bioavailability by the formation of soluble metal chelates. In this respect, humic materials in soil and aquatic systems play a similar role. However, the composition of marine organic matter varies significantly from terrigenous derived organic material as discussed in section 1.4.4.

In this study, the characteristics of humic material of terrigenous origin are compared with humic material from sediments which are highly influenced by marine conditions and subject to inundation of organic and inorganic particulate material of marine origin. The composition of marine suspended particulate material is seasonally influenced both in terms of particle size distribution and variations in primary productivity and may therefore contain varying amounts of marine derived organic material.

### 1.4.2 The Composition of the Humic Fraction of Soils and Sediments

Investigation of the composition of humic substances with a view to understanding their chemical characteristics has been an important area of research since the mid-18th century (Berzelius, 1760; Mulder 1840; Kononova, 1966; Schnitzer and Khan, 1972; Stevenson, 1982; Aiken, 1985; Hayes, 1991). Whereas the chemical characteristics of many other natural organic molecules including biological macromolecules (eg. proteins) are well known, those of humic substances remain poorly understood due to their diversity and complexity (Hayes *et al.*, 1991; Aiken *et al.*, 1985; Stevenson *et al.*, 1982).

Humic substances consist of a heterogeneous mixture of organic macromolecules for which no single structural formula will suffice (Schnitzer and Khan, 1972; Stevenson and Butler, 1969). It is unlikely that any two molecules will have precisely the same structural configuration or array of reactive groups. Consequently, individual functional groups or combinations of functional groups in humic substances do not give rise to unique chemical properties (eg. acid, alcohol etc.) due to the diversity of chemical environments existing in these materials. The averaging of their chemical behaviour is the distinguishing feature between large humic macromolecules and small organic compounds or structured biomolecules (Sposito, 1989). Table 1.9 highlights the main components of a typical soil organic fraction. In general, the structure of non-humic compounds is characteristic for each class or homologous group (Killops and Killops, 1993). For example, proteins comprise amino acid sub-units which are linked via peptide bonds in a non-random manner and possess primary, secondary, tertiary and quaternary structure. The chemical characteristics of these large organic biomolecules arise from uniform and highly specific combinations of sub-units. Following the initial decomposition of organisms upon death, proteins are found in most marine sediments. The chemical environments of amino acids and, more specifically, elemental nitrogen do not differ markedly from their precursors in the living biomass. These functional groups are also present in humic compounds. Soluble amino acids present in soils are incorporated into humic substances but in a wide range of chemical environments and the resultant structures differ strongly from the organic structures from which they originated (Kemp and Mudrochova, 1973). Although nitrogen in most organisms is predominantly found in the form of amino acids in protein structures, amino acid units account for only a proportion of the nitrogen content of humic substances. Significant amounts of nitrogen contained in humic macromolecules cannot be traced to known precursor compounds (Stevenson, 1982; Anderson, 1991). Humic substances are, therefore, complex conglomerates of a variety of known and unknown sub-units which do not constitute uniform macromolecules (Tegelaar *et al.*, 1989; Killops and Killops, 1993).

### 1.4.3 Preservation of Soil and Sedimentary Organic Matter

The main contributors to non-living organic material in marine and terrigenous systems are listed in Table 1.8. Variations in the relative proportions of plant and animal matter exist between marine and terrigenous systems. Whereas in marine systems the ratio of plant to animal matter is approximately 50:50, in terrigenous systems the predominance of plant material is expressed in a ratio of 99:1. Bacteria and fungi are also of considerable importance not only as decomposers of organic material but also as contributors to the biomass. For example, in marine systems, although the biomass of bacteria is small, where primary productivity is high, the contribution of bacteria to deposited sedimentary organic material exceeds that of zooplankton. Fungi, although of low importance in marine systems play a greater role in terrigenous systems and this is discussed in greater detail in the following paragraphs.

| **Marine** | **Terrigenous** |
|---|---|
| phytoplankton | higher plants |
| bacteria | soil animals |
| zooplankton | bacteria |
| (higher plants) | fungi |
| fungi | |

**Table 1.8 Graded Contributors to Soil and Sedimentary Organic Material**

In order to understand the importance of these differences in contributing species, it is necessary to consider the chemical composition of organisms. All organisms are similar in that they are made up of three main (geochemically important) compound classes : carbohydrates, proteins and lipids and the remaining text in this section deals with the initial results of degradation of these general groups. The classes of compounds shown in Table 1.9 display varying solubilities and reactivities.

| **Class of Organic Compound** | **Components** | **Composition** |
|---|---|---|
| **debris** | plant and animal remains | |
| **partial decomposition products** | the three main classes of compounds are carbohydrates, lipids and proteins; lignin | |
| **carbohydrates** | polysaccharides eg. starch, cellulose | monosaccharides ie. simple sugars eg. glucose |
| **lipids (substances produced by organisms that are insoluble in water)** | phospholipids, polyesters, steroids, fats, oils, waxes, resins, photosynthetic pigments | hydroxy fatty acids and alcohols ; steroids structurally different and contain four carbon rings interlocked |
| **proteins** | fibrous proteins -supporting tissue<br>globular proteins - enzymes, hormones, antibodies, storage and transport units (eg. haemoglobin) | amino acids |
| **lignin** | found only in higher plants ; plays a similar role in plants to fibrous proteins | substituted phenols |

**Table 1.9 Non-humic components of Soil Organic Matter**

The residence times of individual compounds in soils can vary from several weeks for easily decomposable tissues up to several thousand years for chemically stable organic matter (Bowen, 1979). The smallest, most highly soluble, total decomposition products have the shortest residence time in the solid phase, whilst other hydrophobic, partial decomposition products have longer residence times in the soil matrix. Macromolecular components of plants such as proteins and polysaccharides are broken down into their water-soluble, constituent amino acids

(Stevenson, 1982) and monosaccharides (Killops *et al.*, 1993) respectively. These smaller, soluble compounds can be assimilated by the living biomass. Alternatively, amino acids may be decomposed by microorganisms with the release of $NH_3$ which may also be assimilated by living organisms or react chemically with partially altered lignin components or humic macromolecules. Similarly, certain lipids are transformed into assimilable components. Natural polyesters are rapidly broken down by enzymes which show a specificity for primary alcohol esters prevailing in these structures. Lignin is a highly abundant natural polymer second only to cellulose and survives early diagenetic reactions (Michaelis *et al.*, 1989; Benner *et al.*, 1990; Fustec *et al.*, 1988). Lignin and cellulose in higher plants play a similar role to fibrous proteins in organisms. Vascular plants have lignin structures in their cell walls which provide the rigidity of the plant stem during its lifetime. Lignin precursors (eg. coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) are synthesised within plants from D-glucose via a shikimic acid pathway. Condensation of varying amounts of these precursors (depending on the plant type) results in the formation of lignins. These lipids are commonly found in combination with carbohydrates or proteins eg. lignocelluloses and lipoproteins. Death of a plant followed by microbial degradation results in the release of lignin structures, constructed from phenolic and other aromatic units which may only be partially degradable chemically and microbially. Lignocelluloses and insoluble, non-hydrolysable aliphatic biopolymers (originating from waxes in protective leaf coatings in higher plants) are in general more resistant to chemical and microbial degradation. Lignin may be degraded by fungi to give polyphenolic compounds which are thought to comprise a significant proportion of the precursors of terrigenous humic substances (Fustec *et al.*, 1988). In particular, white rot fungi use lignin as their preferred source of both carbon and energy. Microbes capable of degrading lignin do so by cleaving ether linkages between phenolic units (Michaelis *et al.*, 1989). Various additional minor sources of phenolic compounds in soils have been identified and include uncombined phenols in plants, glycosides and tannins.

It is therefore proposed that derivatives of the lignin precursors, cinnamyl, coumaryl, coniferyl and sinapyl alcohols are probable precursors to terrigenous humic substances (Stevenson, 1982; Hedges *et al.*, 1988).

Other major sources of phenolic compounds are known to be microorganisms (Kononova, 1966) and, in estuarine environments, macroalgae (Carlson and Mayer, 1983). Microorganisms can synthesise phenols and other small organic molecules which are subsequently excreted and form part of the soil organic pool. Macroalgae are important during the summer months in estuarine areas in the exudation of phenolic compounds (Carlson and Mayer, 1983). In marine environments, humic material contains predominantly methyl and dimethyl phenolic compounds which are not derived from lignin (Michaelis *et al.*, 1989) and it is suggested therefore that they are derived from the microorganisms or algal sources.

In conclusion, only a small portion of the total biomass is preserved in soils and sediments (< 0.1%) but clearly the composition of the organic fraction of soils and sediments is a result of the selective preservation of certain components of the original material. The overall composition is influenced by

1. the input material,
2. microbial populations and
3. environmental conditions.

### 1.4.3 Formation of Humic Substances

Humic substances comprise more than half the organic fraction of most soils and sediments and up to 80% of dissolved organic matter in aquatic systems but, despite their abundance, the processes involved in their formation are ill-defined. It is thought that humic substances are predominantly produced by abiogenic reactions that link together relatively low molecular weight aliphatic and aromatic, hydrophilic and hydrophobic compounds. In marine systems, the low molecular weight compounds are derived mainly from the degradation of the biopolymers, proteins and carbohydrates.

In terrigenous systems, low molecular weight compounds, including substituted phenolic compounds deriving from lignocelluloses (Gõni and Hedges, 1992; Bailly and Raboanary, 1986; Fustec *et al.*, 1988; Zech *et al.*, 1989; Shindo and Huang, 1982; Kogel- Knaber *et al.*, 1991), and partially degraded lignin compounds are implicated as the main precursors of soil humic substances. The formation of soil humic substances from phenolic units as described by Flaig (1960) is outlined in Figure 1.6.

lignocellulose $\xrightarrow{\text{degrad.}}$ lignin $\xrightarrow{\text{degrad.}}$ phenols
oxid.,
demethylation,
enzyme
quinones
polym. with N compounds
HUMUS (dark coloured polymers)

**Figure 1.6 Formation of Humic Substances (Flaig, 1960)**

The importance of aliphatic and aromatic sub-units as contributors to humic substances depends on their inherent resistance to degradation but is also related to their continued presence in the extant biomass. The source of the organic material influences the degree of aromaticity and, in general, soil humic substances have higher proportions of aromatic precursors (Steurmer and Payne, 1976, Alberts and Filips, 1989) whereas floodplain and intertidal sediments, subject to marine inundation, have an additional source of more highly aliphatic organic material. The principal source of natural organic matter in seawater is planktonic and bacterial excretion and degradation whereas the terrestrial biomass and hence natural soil organic matter is principally provided by higher plants (Buffle, 1988) (Table 1.10).

| Component | Higher Plants<br>(% dry weight) | Plankton<br>(% dry weight) |
|---|---|---|
| **Protein** | 5 | 25-50 |
| **Lipid (non-lignin)** | low | 5-25 |
| **Lignin** | 15-25 | - |
| **Carbohydrate** | 30-50<br>(mainly cellulose) | 40 |

**Table 1.10 Composition of Sources of Organic Matter in Marine and Terrigenous Environments (Killops and Killops, 1993)**

The predominantly aliphatic nature of marine humic material has been associated with the importance of algae or microbial degradation in these systems (Hatcher *et al.*, 1980; Hatcher *et al.*, 1981; Minai and Choppin, 1992) and is consistent with the lower proportions of lignin compounds in these environments (Minai and Choppin, 1992; Rashid, 1985). The resultant differences in composition are discussed further in section 1.4.4.

In terrigenous systems, however, the importance of aliphatic units in the formation of humic compounds has often been underestimated since they often comprise only a small fraction of the total biomass. Relative increases in concentration of two or three orders of magnitude for minor, stable aliphatic constituents may occur during early stages of diagenesis (Tegelaar *et al.*, 1989).

Although, as described above, a predominantly abiogenic synthetic route is proposed, the importance of microbial synthesis of humic materials has also been investigated. Microbial synthesis of specific phenolic compounds is well known and their incorporation into humic substances is indicated by the detection of resorcinol derivatives (3,5 dihydroxybenzoic acid) among phenolic units isolated from humic substances (see section 1.4.4). In addition, it is proposed that certain microorganisms can synthesise higher molecular weight polyphenolic compounds. Laboratory based syntheses of compounds with properties similar to those of natural humic compounds have been undertaken by a number of workers including

Bailly *et al.* (1986). Studies using simple phenolic compounds to produce para-humic compounds concluded that the formation of humic macromolecules can result from the following routes :

i) a chemical reaction involving phenolic monomers or

ii) the use of phenolic compounds either energetically or as part of a secondary metabolism by microorganisms.

Other laboratory based studies suggested an alternative abiotic route in which the oxidative polymerisation of phenolic compounds by $MnO_2$, an effective oxidising agent, is important in the formation of humic substances under oxygen depleted conditions (Shindo and Huang, 1982; Huang, 1989).

A single polymerisation reaction involving specific polyphenolic compounds is, however, too simplistic an approach to the synthesis of natural humic substances and cannot account for their inherent heterogeneity and stability. Other precursor molecules and, in particular, N-containing compounds are known to be involved in the final stages of humic synthesis and may partially account for the observed longevity of humic compounds in soils, sediments and waters. As discussed in section 1.4.2, a proportion of N occurring in humic compounds cannot be accounted for by any known precursor molecules (Anderson, 1991) and this, again, emphasises the complexity of the natural processes involved in the synthesis of humic materials. Non-nitrogen containing synthetic humic molecules have also been studied. For example, Almendros *et al.* (1989) studied the spectroscopic characteristics of synthetic carbohydrate derived humic-like polymers in order to elucidate structural features using a simpler system and therefore provide further information regarding formation processes. Complex polysaccharides not found in higher plants may represent products of microbial metabolism. In soils, their preservation may be enhanced by incorporation into humic substances via ester linkages. Flaig (1988) debated the importance of such compounds in terms of their contribution to the composition of the humic fraction in soils and sediments.

Overall, humification is a non-uniform process and the rate, and potentially the mechanism, of the formation processes will be affected by temperature and availability of precursor units. Seasonality factors may also lead to differing concentrations of these precursor units over a period of time. The heterogeneity of the soil or sediment matrix, in terms of variable inputs and degree of degradation of the organic material may also influence the availability of precursor units in any micro-environment within these systems (Gõni and Hedges, 1992). In conclusion, the relative importance of different processes, precursor molecules and the precise steps involved in the formation of humic substances in any environmental system remain uncertain (Stevenson, 1982; Bailly and Raboanary, 1986).

### 1.4.4 Composition of Humic Substances

The existing information regarding humic substance formation processes has been surmised from structural determination of humic substances which has been approached using a number of techniques (Alberts *et al.*, 1992; Almendros *et al.* 1989; Grant, 1977; Posner, 1963; Hatcher and Spiker, 1988; Kim 1990; Falk and Smith, 1963; Schnitzer *et al.*, 1958; Schulten and Schnitzer, 1993). These techniques fall into two main categories, namely degradative (thermal and chemical) and non-degradative (spectroscopic) which are discussed in turn below.

#### 1.4.4.1 Elemental Analysis

Elemental analysis of bulk humic materials (ie. humic acids, fulvic acids and humins) is achieved by the total breakdown of the humic molecules using microanalysis techniques which determine the relative proportions of carbon, nitrogen, hydrogen, oxygen and sulphur. Humic materials from different locations vary in their elemental composition, although all are composed principally of C and O with smaller amounts of H and N and trace levels of S (and P) (Rashid, 1985). In accordance with previous sections (see table 1.9 and 1.10), the relative proportions of these elements are dependent on a number of factors including the source of the organic matter and environmental conditions (Rashid and King, 1970; Malcolm and MacCarthy, 1991; Miodragović *et al.*, 1992; Fründ *et al.*, 1989).

In general, the trends indicates that C/H and C/N ratios can facilitate partial characterisation of the humic fraction of a soil or sediment. For example, terrigenous humic acids have values of C/H greater than 10 whilst marine derived material has values less than 10. Table 1.11 gives the elemental composition (% mass) of humic acids from soils and sediments experiencing a range of environmental conditions.

| **Humic Material** | **%C** | **%H** | **%N** | **%O+S** | **C/H** | **C/N** | **$\delta^{13}C$** | **$E_4/E_6$** | **Ref.** |
|---|---|---|---|---|---|---|---|---|---|
| **Sediment** | | | | | | | | | |
| **Marine Basin** | 51.84 | 6.56 | 5.17 | 40.11 | 7.9 | 10.0 | -21.8 | 5.65 | Nissenbaum and Kaplan 1972 |
| **Estuary** | 54.37 | 6.04 | | | 9.4 | | -27.3 | | Nissenbaum and Kaplan 1972 |
| **River** | 52.64 | 6.04 | 5.00 | 41.6 | 8.7 | 10.5 | -26.5 | | Nissenbaum and Kaplan 1972 |
| **Soil** | | | | | | | | | |
| **Chestnut** | 61.74 | 3.72 | 3.92 | 30.62 | 16.6 | 15.8 | | 3.8 | Kononova 1966 |
| **Chernozem** | 62.55 | 2.78 | 3.32 | 31.35 | 22.5 | 18.8 | | 3.5 | Kononova 1966 |

**Table 1.11 Elemental Composition of Humic Acids from Soils and Sediments (adapted from Rashid, 1985)**

### Carbon

Comparison of humic acids from well-developed soils with those isolated from estuarine sediments shows that C content is generally considerably lower for marine humic compounds (Alberts and Filip, 1989). These differences are attributed both to the differences in the source organic material and to prevailing Eh, pH, O content and ionic strength in the marine environment.

### Nitrogen

Higher N contents in marine humic acids are attributed to the source material dominantly being derived from phytoplankton which are rich in nitrogen due to their high protein content, although lower marine plants may also be rich in N. A high proportion of the N in estuarine sediments is derived from amino acids. In particular, aromatic amino acids in estuarine humic acids are more abundant than in soil derived counterparts, although total amino acid contents need not show similar trends (Alberts and Filip, 1989). C/N ratios are in general lower for marine than terrigenous humic acids.

### Hydrogen

The hydrogen content in humic acids from surface sediments is again influenced by the initial source of organic matter. The inherent aliphatic nature and high protein content of marine debris under restricted aerobic conditions favour their preservation and promote their incorporation into humic structures and this is reflected in lower C/H ratios in the marine environment..

### Oxygen

The oxygen content of marine humic acids is again relatively (to C) higher than for their terrigenous counterparts, reflecting not only the input material but also the indigenous conditions which favour to some extent the preservation of humic macromolecules with higher proportions of (particularly oxygen containing) functional groups.

| | % C | % H | %N | %O |
|---|---|---|---|---|
| **Soil Humic Acid**[a] | 60.4 | 3.7 | 1.9 | 33.6 |
| **Lake Sediment Humic Acid**[b] | 53.7 | 5.8 | 5.4 | 35.1 |
| **Soil Fulvic Acid**[c] | 50.9 | 3.3 | 0.7 | 44.8 |
| **Water Fulvic Acid**[d] | 46.2 | 5.9 | 2.6 | 45.3 |

**Table 1.12 Elemental Composition of Humic and Fulvic Acids ([a] Riffaldi and Schnitzer, 1972; [b] Schnitzer and Gupta, 1964; [c] Schnitzer and Desjardins, 1962; [d] Khan and Schnitzer, 1972; table modified from Schnitzer and Khan, 1972)**

In general, elemental ratios for fulvic acids also provide a similar trend in compositional differences relating to the predominant source of the organic material but also indicate characteristic differences between humic and fulvic acids.

C/H ratios

C/H ratios for soil humic acids are in general higher than those for fulvic acids and are consistent with the experimentally observed higher aromatic content of humic acids (Killops *et al.*, 1993). The C/H ratios for both soil humic and fulvic acids are substantially higher than those of the respective materials isolated from marine systems (Rashid, 1985) (Table 1.12).

C/N ratios

It has been observed that the C/N ratios for fulvic acids are in general higher than those for humic acids. The C/N ratios of some marine fulvic acids are however lower than would be expected on the basis only of source material and are similar to those of terrigenous humic acids. These interesting observations continue to lead to further research to define the composition and origin of this material

(Alberts and Filip, 1989) and also the chemical environment and mechanism of incorporation of N into humic materials.

C/O ratios

Further differences between humic and fulvic acids are also apparent from their respective elemental oxygen contents. Fulvic acids, in general, have higher oxygen contents than humic acids from similar origins and this has often been linked with the higher solubility of fulvic acids (Stevenson, 1982; Rashid, 1985) (Table 1.12).

Many recent studies have investigated the transfer of terrigenous derived organic matter, including terrigenous humic material, to riverine, estuarine and marine systems (Hedges, 1984). It was proposed that inputs of terrigenous organic material into aquatic systems have resulted in the incorporation of specific aromatic compounds, derived from higher plants, into aquatic humic material (Ertel *et al.*, 1986; Jasper *et al.*, 1990). However, in terms of elemental composition, fulvic and humic acids isolated from estuarine systems appear to resemble more closely marine humic matter than their terrigenous counterparts (Table 1.11). Elemental analysis *per se* provides inconclusive evidence of sources of organic material.

### 1.4.4.2 Chemical and Thermal Degradation

Chemical or thermal degradation of humic substances is often used in laboratory studies in an attempt to identify sub-units present in humic structures (Alberts *et al.*, 1992). The two main chemical degradative routes involve the use of oxidising or reducing agents although each has associated disadvantages. The Oxidative techniques involve cleavage of carbon-carbon bonds but conditions may be so severe that the resulting products cannot lead to the identification of the source of the material. Alternatively, reductive techniques are milder and more selective and so provide less structurally altered sub-units. However, milder reagents often lead to lower yields of degradation products for subsequent analysis and therefore, results are less representative of the composition of the total humic material (Stevenson, 1982).

Degradation techniques have been employed to identify specific compounds including amino acids, carbohydrates, acids, alcohols and hydrocarbons (Meyers-Schulte and Hedges, 1986; Cowie and Hedges, 1992; Cowie *et al.*, 1992) and are used to determine the nature and importance of various precursor units and sources.

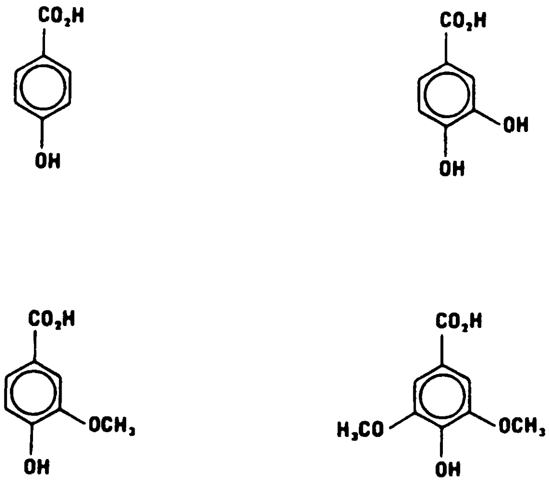


**Fig.1.7 Alkaline CuO Degradation Products of Lignin**

For example, such techniques have been used to identify phenolic subunits which are derivatives of specific components of higher plants. Vanillic, syringia, p-hydroxy benzoic acid, gueniaryl and syringyl propionate subunits (Figure 1.7) have been identified in humic substances and are indicative of a contribution from lignin residues. In addition, these structures were not detected in humic substances formed in a lignin-free environment (Burges *et al.*, 1963).

A further problem associated with degradative techniques is the possible formation of procedural artefacts (Stevenson, 1982). For example, the stability of aromatic components also results in their resistance to chemical degradation under laboratory conditions and, for example, CuO degradation often leads selectively to aromatic products (Tegelaar *et al.,* 1989). All humic substances contain a significant proportion of aliphatic subunits (Hatcher *et al.*, 1981) and the use of such degradation techniques in the study of humic composition therefore tends to overestimate the aromatic nature of humic substances.

Thermal degradation by pyrolysis techniques yields subunits which can be analysed by GC-MS techniques and has resulted in the identification of higher proportions of aliphatic structures, with phenolic compounds being of lesser importance (Tegelaar *et al.* ,1989). These results are incompatible with the commonly accepted polyphenol theories of humic formation (section 1.4.3) and more realistic formation theories must include stable aliphatic precursor molecules. Studies suggesting that lipids comprise up to 20% of humic substances (Stevenson, 1972) are not substantiated by analytical pyrolysis studies which suggest that lipids, proteins and polysaccharides are minor components of soil humic material. In addition, high proportions of these would not be expected from their low resistance to biodegradation and hence short residence time in the soil matrix. It has been proposed that insoluble, non-hydrolysable highly aliphatic biopolymers which occur in both higher plants and algal cell walls may be responsible for observed aliphatic structures in humic substances. This is substantiated by pyrolytic degradation studies in combination with $^{13}C$ NMR (Tegelaar *et al.*, 1989). Spectroscopic techniques can be used in such studies to complement the information obtained from elemental analyses by further characterising the sources of the organic material, for example, in the detection of the presence of terrigenous material in humic structures occurring in aquatic systems, but also to gain additional understanding of the processes which lead to the formation of humic substances (Norwood, 1988). However, thermal degradation by pyrolysis is limited to the detection of structures that can be volatilised intact (Hempfling and Schulten, 1989). Pyrolysis of carbohydrate units may result in the formation of furan derivatives (Wershaw and Bohner, 1969) and again give a misleading indication of the composition of the humic fraction.

In conclusion, degradative methods employed in structural studies are likely to produce biased quantitative and qualitative results (Frund *et al.*, 1989; Farmer and Pisaniello, 1985).

Non-destructive techniques used in structure determination comprise a range of spectroscopic methods including FTIR, UV/visible, fluorescence and solid state $^{13}C$ NMR spectroscopy (Baes and Bloom, 1989; Alberts, 1975; Chen, 1977; Frund and Lüdemann, 1989; Gessa *et al.*, 1983; Hopkins and Shiel, 1991; Ruggiero *et al.*, 1977; Ruggiero *et al.*, 1979; Stevenson and Goh, 1971; Stevenson and Goh, 1972, Schnitzer, 1971). The aim in such studies is to determine the functionality of humic macromolecules in order to evaluate interpretations based on destructive degradation studies but also to further understanding of the chemical and physical properties displayed in the interactions of humic macromolecules with metals and organic pollutants.

### 1.4.4.3 FTIR Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is a structure determination technique commonly used in conjunction with other spectroscopic methods in the identification of simple organic compounds. It has been used extensively in the attempted characterisation of humic and fulvic acids from diverse origins (eg. Schnitzer et al, 1959, Stevenson and Goh, 1972, Guggenberger, 1989) and is one of the main spectroscopic techniques used in the present study.

FTIR spectroscopy of simple organic compounds permits the identification of different chemical environments of hydrogen and carbon atoms as well as oxygen and nitrogen containing functional groups and, together with complementary spectroscopic information, leads to partial structural elucidation. FTIR spectra of humic and fulvic acids are characteristically broad and give little definitive structural information (Figure 1.8). These complex multicomponent systems contain functional groups in numerous chemical environments and overlap of absorptions at similar wavenumbers gives rise to broad bands and little fine structure. As a result only qualitative and non-specific structural information is obtained from FTIR spectroscopy alone (Ibarra, 1989; Lynch and Smith-Palmer, 1992).

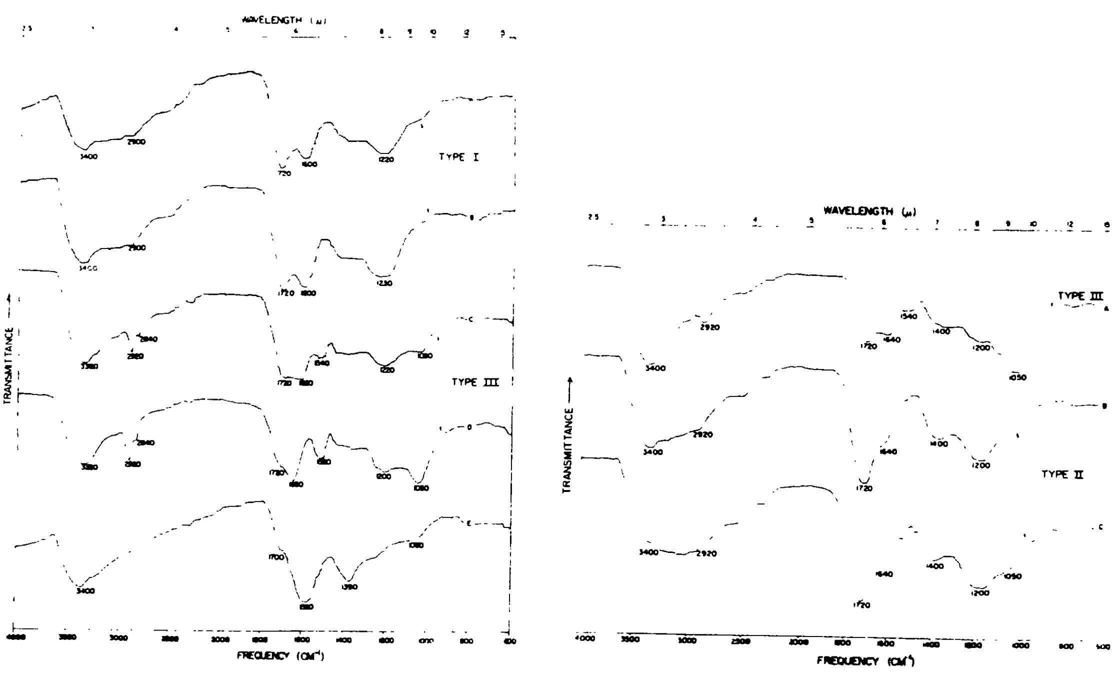


**Figure 1.8 Typical FTIR Spectra for Soil Humic and Fulvic Acids (Stevenson and Goh, 1971).**

It has been suggested that fulvic acids contain less aliphatic C-H groups and more oxygen containing groups than humic acids and this is confirmed by elemental analyses (Table 1.12).

The spectra are, however, not substantially different for humic and fulvic components from diverse origins. It has been suggested that this indicates the presence of structurally similar chemical units (Schnitzer and Khan, 1972) but it is probable that the complexity of the spectra masks the fine structure and further confirms that FTIR spectroscopy alone is insufficient to characterise humic materials (Malcolm and MacCarthy, 1991; MacCarthy and Rice, 1991).

Characterisation of humic and fulvic acids using FTIR spectroscopy has also involved chemical treatment of the materials in order to form specific derivatives (Brooks et al, 1960; Schnitzer and Skinner, 1965; Stevenson and Goh, 1972). Methylation of the oxygen containing functional groups results in changes in the

FTIR spectra of the material and selective methylation has been employed in order to identify the reactivity and proportions of specific oxygen containing groups in humic and fulvic acids (Brooks *et al.*, 1960; Stevenson and Goh, 1972; Kim, 1990). Similar studies investigated the effect of chemical modifications (eg. methylation or acetylation) on metal retention capacity by observing changes in the strength of relevant absorptions in FTIR spectra of the humic material (Barton and Schnitzer, 1963).

### 1.4.4.4 UV/Visible Spectroscopy

Humic and fulvic acids also contain many chromophores which absorb in the UV/visible region but UV/visible spectra of humics are broad and featureless with maximum absorption occurring at 260 nm and absorption intensity decreasing monotonically as wavelength increases (Kim, 1990) (Figure 1.9). However, a slight shoulder in the region 260-300 nm is often observed although the overlap of many different absorptions allows little structural interpretation (Stevenson, 1982, Aiken *et al.*, 1985). It has been proposed that some structural information can be obtained from the slope of the absorption curves, measured by the ratio of the absorbance at 465 and 665 nm, termed the $E_4/E_6$ ratio, which is related to the degree of the condensation of the material (Chen, 1977). Low ratios have been related to a higher degree of condensation and aromaticity and, more recently, higher molecular weight of the material. (Senesi *et al.*, 1989) Humic acids exhibit lower $E_4/E_6$ ratios than corresponding fulvic acids and this has been interpreted as confirmation of the greater molecular weight of humic acids and the presence of aromatic core structures (Table 1.13). An inverse relationship between the value of this ratio and the age of the humic material has also been proposed, suggesting that humic acids are in fact older than fulvic acids (Campbell, 1958 in Stevenson, 1982). However, the increasingly contradictory interpretations of changes in this ratio restrict its usefulness in definitive structural characterisation (Kim, 1990).

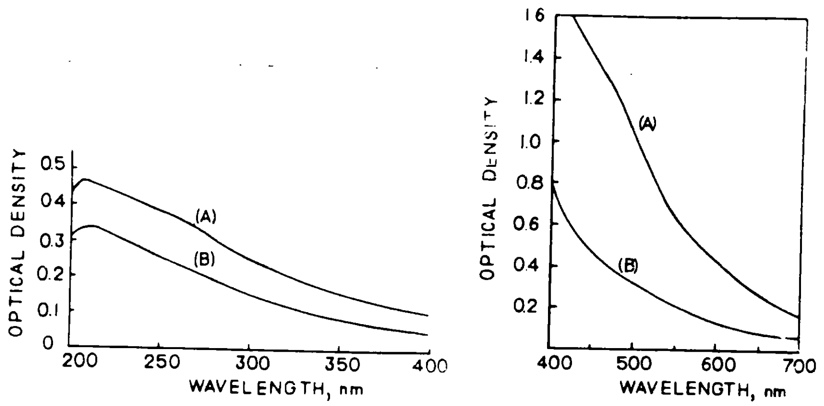


**Figure 1.9 UV/visible Spectra of Humic and Fulvic Acids**

| Acids | $E_4/E_6$ |
|---|---|
| **Humic Acids** | |
| Serozem | 4.0-4.5 |
| Chernozem | 3.0-3.5 |
| Chestnut | 3.8-4.0 |
| **Fulvic Acids** | 6.0-8.5 |

**Table 1.13 $E_4/E_6$ Ratios of Humic and Fulvic Acids (from Schnitzer and Khan, 1972)**

### 1.4.4.5 Fluorescence Spectroscopy

The presence of UV/visible active chromophores has prompted the use of fluorescence spectroscopy in the study of humic substances. The exact nature of the fluorescing groups is, however, far from being clarified and the broad nature of the absorptions is again attributed to the sum of the contribution of different subunits, particularly highly substituted aromatic structures or extended conjugated unsaturated systems capable of a high degree of resonance (Senesi *et al.*, 1989). Fluorescence spectroscopy is claimed to be a very sensitive technique for the study of certain humic characteristics (Provenzano *et al.*, 1989) and variations in the composition of humic material are apparent in changes in positions and intensities of the various peaks (Provenzano *et al.,* 1989).

In addition, fluorescence studies have been used to study metal complexation where, for example, $Eu^{3+}$ and $Am^{3+}$ have been added to give metal-humic and metal-fulvic complexes at concentrations sufficient to exceed the detection limits of this technique (Bertha and Choppin, 1978; Kim, 1991; Buckau *et al.*, 1991; Maes et al, 1991). Buckau *et al.* (1991) suggest that humic and fulvic acid interact in a mechanistically identical manner with these +3 ions and therefore conclude that a single interaction occurs between +3 ions and humic materials. This is important in the context of modelling the behaviour of actinide-humic complexes and will be discussed further in section 1.5.

### 1.4.4.6 NMR Spectroscopy

An example of an NMR spectrum of humic acid is illustrated in Figure 1.10. The resolution of $^{13}C$ NMR spectra of heterogeneous mixtures such as humic substances is insufficient for direct identification of individual molecular subunits (Hemfling and Schulten, 1989). Its application is however valuable, in that it provides a chemical overview of the nature of the humic material. Quantitative results can be obtained by the application of advanced NMR techniques. In particular, CPMAS $^{13}C$ NMR spectroscopic studies can provide quantification of aliphatic (Kogel-Knaber, 1989) and aromatic components of humic extracts (Frund, 1989). Non-degradative analysis by $^{1}H$ and $^{13}C$ NMR spectroscopy certainly gives a more realistic estimate of the degree of aromaticity of humic substances than destructive chemical techniques. Comparative studies of humic substances from diverse origins show that the aromatic content of soil humic substances can be as high as 50% whereas that of peat humic substances is around 15% and that for marine humic substances less than 15% (Hatcher *et al.*, 1980).

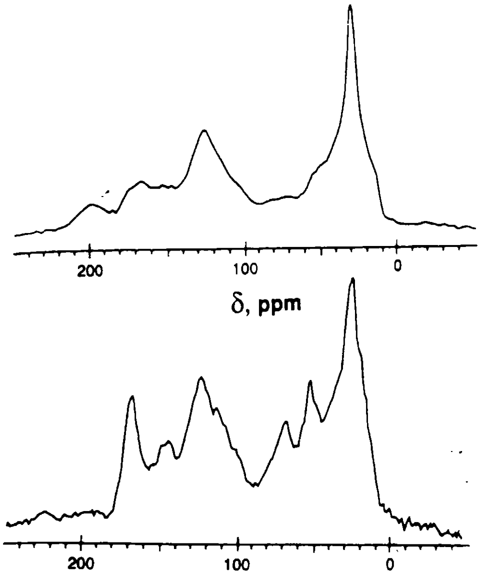


**Figure 1.10 $^{13}C$ NMR Spectra of Humic Acid (Rao, 1994)**

### 1.4.4.7 Gel Chromatography

Gel Filtration Chromatography is a size separation technique commonly used in areas of organic chemistry including protein purification (Barth and Boyes, 1990). Sephadex gels, produced by Pharmacia, have frequently been applied in the study of humic substances in attempts to determine their average molecular weight. The approximate molecular weight distribution of a humic acid was determined by sequential application of the excluded humic material to a range of gels of increasing pore size to produce a range of fractions containing humic acid molecules of more homogeneous molecular weight. The determination of absolute molecular weight using Sephadex gels was attempted by Cameron *et al.* (1958)

using protein or dextran based calibrations. However, these molecules have definitive molecular properties and shapes and Stevenson (1982) suggests that they are poor analogues for humic or fulvic acids.

Sephadex gels have been used in the present study to provide fractions for further analysis. A discussion of the chemical and physical properties of these gels can be found in "Gel Filtration" (Pharmacia, 1993). Gel chromatographic techniques have been applied with varying success to fractionate humic and fulvic acids, in principle on the basis of molecular size (Davis and Gloor, 1981; Posner, 1963, Schnitzer and Skinner, 1968; Swift and Posner, 1971; Stevenson, 1982), and spectroscopic studies have been used to provide information on functionality of macromolecules of different size within the total humic fraction (Posner, 1963, Schnitzer and Skinner, 1968). Gel chromatography using porous polysaccharide gels has been applied to fractionate humic materials to obtain a reduction in the polydispersity of the sample by judicious collection of the effluent (Swift and Posner, 1971). However, two bands observed during gel filtration were both found to be polydisperse (Posner, 1963). Other fractionation techniques eg. ultracentrifugation and ultrafiltration also purport to achieve separation on the basis of molecular size and all three techniques aim to provide a measure of the size distribution within the organic fraction (MacFarlane, 1978; Smith, 1991). However, molecular size determination of humic materials is subject to subtle interferences in each method and reported molecular weights may or may not reflect the true size of the macromolecules (Chin and Gschwend, 1991). Reported molecular weights, using these and other techniques, range from 5,000 to 1,500,000 for humic acids of similar origins (Stevenson, 1982) (Table 1.14).

Contrarily, the application of HPLC to the fractionation of humic materials is reported to separate humics only on the basis of hydrophobic or hydrophilic character (Saleh *et al.*, 1989; Salzer, pers. comm.). It has been shown that the most hydrophilic humic material is first eluted in a sharp band followed by a broad ill-defined band conatining more highly hydrophobic humic material. The fractionation method, per se, does not result in the determination of molecular size of the hydrophilic and hydrophobic components.

| Method | Molecular Weight |
|---|---|
| Gel Filtration (NaOH) | 12,800-412,000 |
| Ultracentrifugation | 53,000-100,000 |
| Small-angle X-ray Diffraction | 200,000-1,000,000 |
| Light Scattering | 65,000-66,000 |
| Electron Microscopy | <20,000 |

**Table 1.14 Molecular Weights of Humic Acids Obtained Using Different Methods (from Stevenson 1982)**

In certain gel chromatographic studies, UV/visible absorbance of fractions at the absorbance maximum was used to monitor the separation process occurring on the gel column and to estimate sample concentration in each fraction (Livens *et al.*, 1991; MacFarlane, 1978). Interpretation of UV/visible spectra is, as described above, impeded by the diversity of functional groups in different chemical environments present in humic fractions. However, it does allow the measurement of some spectroscopic differences between fractions of the humic material following separation on the gel column. Differences between fractions have also been observed using other spectroscopic techniques (Bailly and Margulis, 1968; Schnitzer and Skinner, 1968; Guggenberger, 1989). Spectroscopic investigations of the gel fractionation of varying concentrations of humic substances using water as the eluent showed that the elution pattern varied with the concentration of the humic material (Swift and Posner, 1971). Charge interactions are maximised at low ionic strength so that increasing concentrations of humic substances resulted in greater exclusion of anionic species from the gel which also carries a negative charge (Pharmacia, 1993). Charge effects are found to be minimised by altering the composition of the eluent and can be effectively avoided by the addition of high ionic strength electrolyte to the eluent. Alkaline solutions, including sodium hydroxide (Dubin and Fil'kov, 1968) and sodium borate (Ferrari and Dell'Agnola, 1963), are regarded as being most effective eluents of humic substances, although there are potential problems due to the oxidation of the humic material (Stevenson, 1982; Schnitzer and Khan, 1972). Adsorption of humic material by the gel matrix has also been observed (Dubach *et al.*, 1964; Dell'Agnola *et al.*, 1964; Posner, 1963) although reports vary regarding the proportions of adsorbed material. Many gel chromatographic studies have been concerned with adsorption effects and their possible suppression with reported values as a high as 50% loss of the humic material (Rashid and King, 1969). Reduction of this effect is not achieved by the addition of electrolyte to the eluent and often the reverse is observed. Attempts to define the nature of this interaction have involved the use of smaller, well-characterised organic molecules and reports suggest strong interactions of aromatic, heterocyclic and phenolic compounds with Sephadex gels (Gelotte, 1960; Demetriou *et al.*, 1966; Brook and Housely, 1969; Somers, 1966). These arise from interactions with the ether bonds in crosslinking groups in the gel matrix and were observed by Determann and Walter (1968) who found that as the degree of crosslinking increased, so did the affinity of phenol for the gel. Similar effects were observed for higher molecular weight polyphenolic compounds (Somers, 1966 in Hayes, 1989). Hydrophobic molecules are also likely to be retarded by the gel matrix which offers a more favourable, lower energy environment than the aqueous phase (Eaker and Porath, 1967). Humic macromolecules contain varying amounts of hydrophobic and hydrophilic subunits and aliphatic and aromatic units (Tipping and Woof, 1991; Wershaw, 1989; Hedges, 1988), so fractionation is likely to be influenced strongly by the degree of hydrophobicity and aromaticity of individual macromolecules. Overall, fractionation using Sephadex gel is likely to be partly a chemical separation (Wershaw, 1989). Therefore, discrete elution peaks observed visually and spectroscopically are unlikely to reflect actual molecular weight distributions within, for example, humic acid (Bailly and Margulis, 1968; Schnitzer and Skinner, 1968). For example, Rashid and King (1969) compared separation of marine and soil humic materials on a gel column with water as the eluent and suggested that the average molecular weight of marine humic acid was greater than that of soil humic acid. The low ionic strength of the eluent, however, suggests that interactions with the gel should be high and any differences between the marine and soil humic material would therefore be more likely to be related to compositional differences rather than purely size distribution. This is proposed on the basis of retardation of highly aromatic terrigenous material and potentially the exclusion of more highly anionic, aliphatic marine material from the knowledge of the differences in the chemical composition of these humic materials. Caution is required in interpretation so that artefacts are detected and eliminated in the determination and comparison of molecular weights of humic materials (Chin and Gschwend, 1991). Systems in which gel-solute interactions occur should, obviously, not be used for molecular weight determinations. Swift and Posner (1971) reported that, under conditions where adsorption effects were eliminated and production of artefact peaks (eg. from the use of an eluent of differing ionic strength from the sample medium) were avoided, the measurements of molecular weight were comparable with those obtained by other methods. However, the elimination of adsorption effects is rarely achieved for humic materials extracted from soils and sediments and so discrepancies in reported values of average molecular weight are found in the literature (Stevenson, 1982)(Table 1.14). Swift and Posner (1971) also suggested that the molecular weight distribution observed under these conditions was of the type expected from a continuous distribution of individual components grouped around a single mean value. However, this would appear to be contradictory to results presented by Bulman (1992) where elution patterns suggest a bimodal distribution of humic acid macromolecules (Figure 1.11a). Similar patterns were obtained for fulvic and humic acids by Livens *et al.* (1989) (Figure 1.11b).

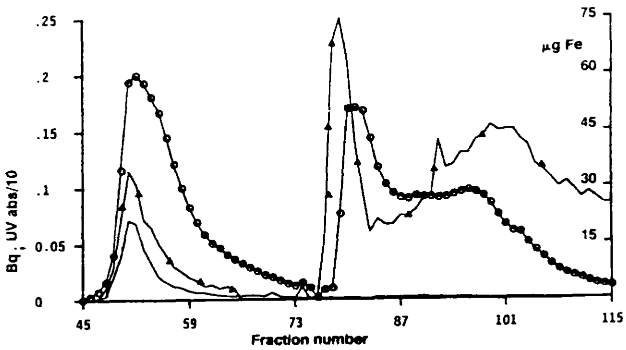


**Figure 1.11a. Elution Patterns Obtained by the Gel Chromatographic Fractionation of a Humic Material (Bulman, 1991)**

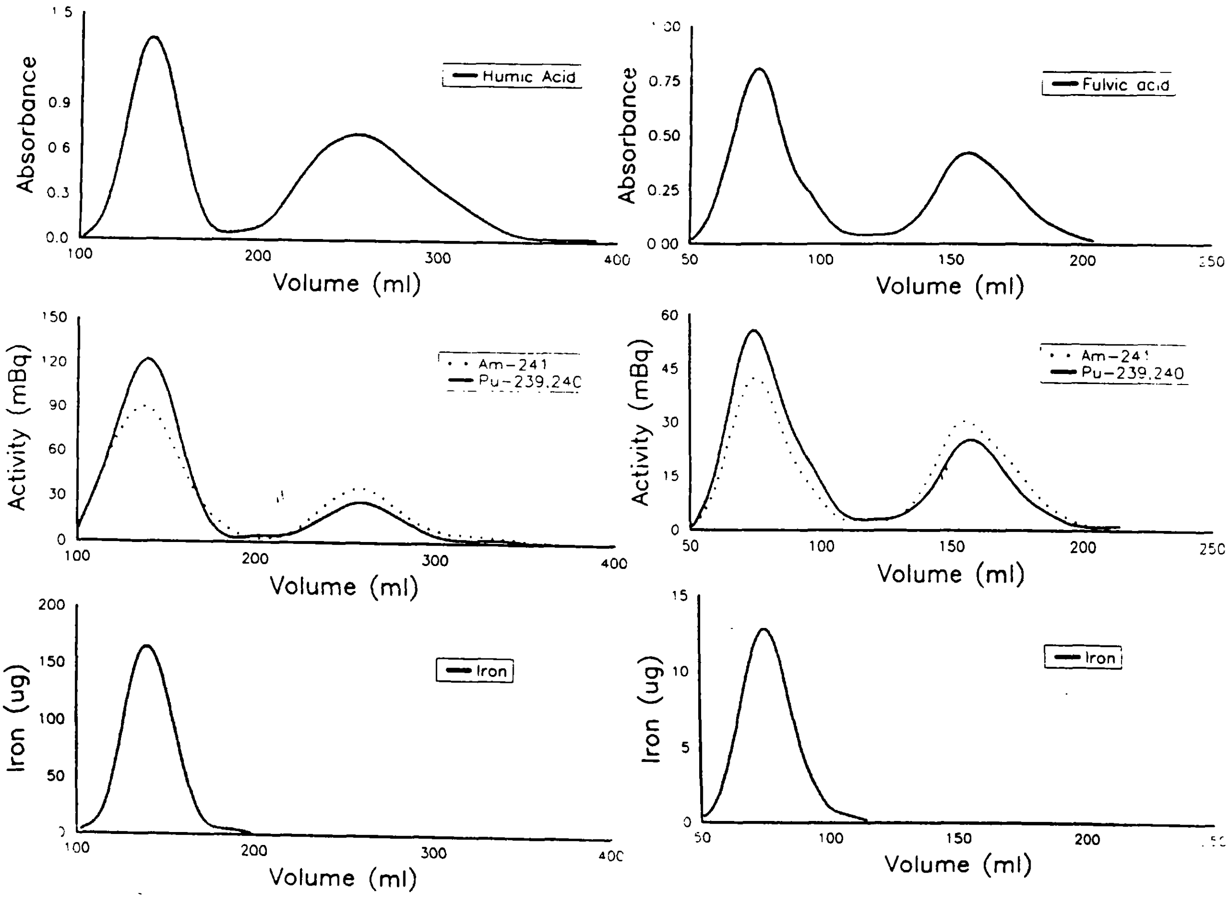
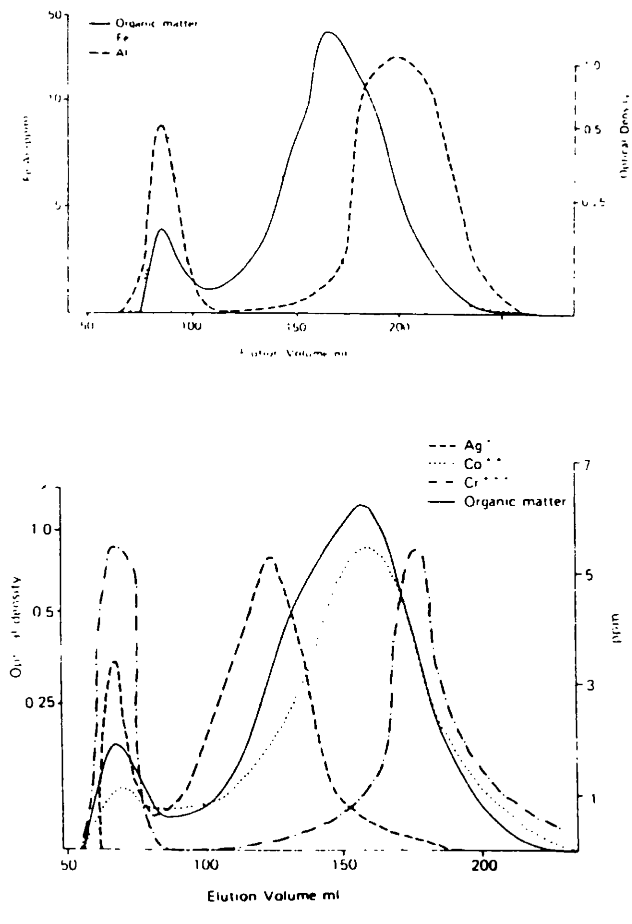


**Figure 1.11b Elution Patterns Obtained by the Gel Chromatographic Fractionation of Humic and Fulvic Acids (Livens, 1989)**

Only a small number of studies have involved the fractionation of bulk humic substances (ie. where humic and fulvic acids have not been separated). The results of such studies (Figure 1.12) are notably different from those presented above (Figure 1.11a and b). Most significantly, the strongest UV absorbance is observed for later fractions and the absorbance for early fractions is markedly smaller than those obtained in the above studies of humic and fulvic acids. Importantly, the metal distribution between fractions of humic substances is dependent on the metal. In other words, different metals interact with different components of the humic material which additionally do not necessarily produce the greatest UV absorbance. This is again in strong contrast to the results presented by Livens (1989) and Bulman (1991).



**1.12 Elution Patterns Obtained by the Gel Chromatographic Fractionation of Bulk Humic Substances (Sequi *et al.*, 1971; Guidi *et al.*, 1972)**

### 1.4.4.8 Gel chromatography and FTIR Spectroscopy

The fractionation of humic and fulvic acids following separation using various gels, including Sephadex and Sephacryl, has been monitored by FTIR spectroscopy and produces a range of fractions with varying composition (Schnitzer and Skinner, 1968; Guggenberger, 1989). Schnitzer and Skinner (1968) showed that fractions of fulvic acid which eluted first from the gel column had stronger absorption bands in the region assigned to carboxyl groups compared with later fractions. They also contended, on the basis of these spectra, that later fractions contained aromatic functional groups although from the broad nature of the spectra and lack of strong absorptions in the fingerprint region (<1000cm$^{-1}$), there is little evidence to sustain this conclusion. The spectra of later fractions do, however, differ from those of earlier fractions.

Guggenberger (1989) used Sephacryl gel to fractionate water-soluble organic matter and characterised the separated organic fractions using both FTIR and NMR techniques. This less chemically treated organic matter gave rise to spectra with characterisable differences. Identifiable low molecular weight components consisted predominantly of carbohydrates and amino acids whereas the larger size fractions contained higher proportions of humic material. The use of water as the eluent can enhance the separation of these components by allowing charge effects to dominate in the separation procedure and may therefore be an advantage in this context. A number of important conclusions were drawn from this study which included :

i) the observation of greater fine structure indicated a reduction in heterogeneity of the material in fractions

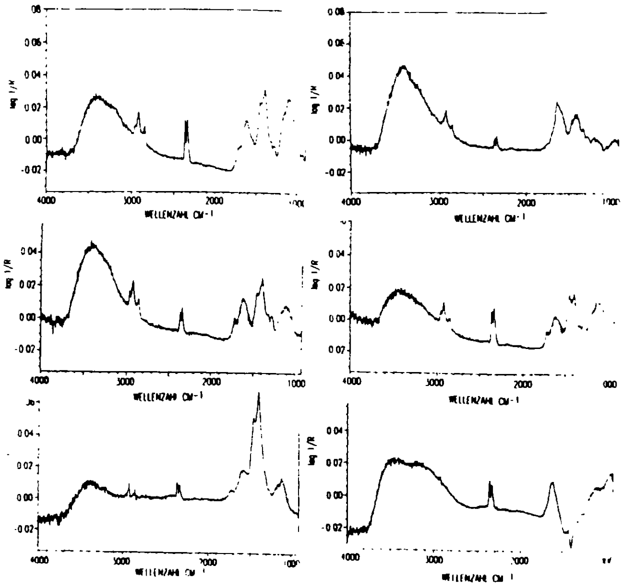
ii) by implication, fractions contained molecules with certain common spectroscopic features

iii) certain spectroscopic features which were weak or absent in the spectra of the bulk material, were observed more clearly in the fractions in which they predominated.

### 1.4.4.9 HPLC Studies of Humic and Fulvic Acids

Fractionation of humic materials using reversed phase HPLC has been reported (Smith, 1991) to give results consistent with separation based on an ion repulsion/size exclusion mechanism. It was, however, suggested that this technique was unsuitable for humic separations on the basis of poor inter-run reproducibility. An investigation of reverse phase HPLC methods for the fractionation of fulvic acid also resulted in the elution of two hydrophilic components before the exclusion volume as a result of poor interaction with the stationary phase. These variable interactions with the separation matrix also resulted in poor inter-run reproducibility (Smith, 1991). Smith noted that interactions with the separation media also occur for ion suppression, thin layer and ion pair chromatographic fractionation limiting the usefulness of these techniques for highly terrigenous materials due to their inherently stronger interactions with gel structures. HPLC studies, therefore, predominantly involve only aquatic humic materials with their higher aliphatic content (Rashid, 1985; Schnitzer and Khan, 1972; Stevenson, 1982).

Salzer (pers. comm.,1994) contends that HPLC separation of humic materials results in the appearance of an exclusion peak containing large, hydrophilic humic components, which would be consistent with the proposed charge repulsion separation mechanism. The combination of HPLC and FTIR techniques has been proposed as a useful method of partial characterisation of these complex organic mixtures (Salzer, 1994 pers. comm.). In HPLC separations, characteristic polar groups can be identified in the eluting fractions of humic substances (Figure 1.13). Results suggest that the leading edge of the exclusion peak is dominated by stronger hydrophilic components such as polysaccharides and proteins, with the more hydrophobic structures of the humic acids being eluted subsequently (Salzer, 1994). These results were consistent with the findings of Smith (1991), which showed that a significant proportion of humic acid was excluded from the gel not on the basis of size from which it was concluded that, although this could have been due to aggregation prior to chromatographic separation, it was more probably due to hydrophobic/ionic repulsion effects.



| | Fraction 1 | Fraction 2 | Fraction 3 | Fraction 4 | Fraction 5 | Fraction 6 |
|---|---|---|---|---|---|---|
| OH stretch | strong | strong | weak | weak | medium | medium |
| CH arom stretch | - | - | | - | | medium |
| CH aliph stretch | medium | medium | weak | weak | medium | medium |
| C=O stretch | weak | weak | weak | medium | weak | |
| C=C stretch | medium | medium | medium | medium | medium | medium |
| [illegible] | strong | medium | very strong | weak | medium | |
| [illegible] | strong | medium | | | medium | medium |

**Figure 1.13 Spectroscopic Differences between Fractions of Humic Acid Obtained by HPLC Fractionation (Salzer, 1994)**

The above studies show that gel chromatography (Guggenberger, 1989) or HPLC (Salzer, 1994), combined with FTIR spectroscopy can provide information on functionality of fractions of humic materials. These studies give indications of compositional differences between humic materials from terrigenous and aquatic system and allow tentative identification of differences in chemical properties of these complex biomolecules (MacCarthy and Suffet, 1991). The chemical structure of humic materials is, however, still a matter of controversy as it is claimed that the procedures used for extraction could lead to significant chemical alteration of the organic compounds (Frund *et al.*, 1989).

### 1.4.4.10 Gel Chromatographic Fractionation and Amino Acid Analyses

Soil fulvic acids have been fractionated on G25 Sephadex gels and the fractions analysed for their amino acid content. Results of such studies showed that the distribution of amino acids was fairly uniform. Further studies showed that fractionation on a polyamide column prior to Sephadex G25 gel fractionation resulted in the observation of pronounced differences between fractions in terms of their amino acid content (Sequi *et al.*, 1975). The heterogeneity of humic substances which makes their analyses difficult can therefore be sufficiently reduced by the application of a set of fractionation procedures to obtain useful information regarding the structure of components of these materials.

### 1.4.5 Extraction of Humic Substances from Soils and Sediments

Ideally, extraction of humic substances from any environmental matrix should effect the complete removal of a pure, unaltered humic material (Stevenson, 1982; Aiken *et al.*, 1988). However, Williams *et al.* (1993) expressed concern that techniques used to isolate natural organic complexants may be destructive and may alter the form and the nature of organic compounds which exist under environmental conditions. Characterisation of the extracted material would then be of limited value in identifying possible interactions with radionuclides or other trace elements under natural conditions. Many authors (Williams *et al.*, 1993; Hayes, 1991) conclude that methods have yet to be developed which minimise the alteration of the natural system.

### 1.4.5.1 Alkaline Extraction of Humic Substances

Humic substances are most effectively separated from the bulk soil matrix using aqueous alkaline solvents (Parsons, 1988; Hayes, 1991). The most effective available solvent has been shown to be aqueous sodium hydroxide which isolates in excess of two thirds of organic material from soils and sediments (Stevenson, 1982). Claims by Schnitzer (1958) that aqueous solutions of sodium hydroxide could extract approximately 96% of organic matter from certain soils have not been substantiated by other workers and complete extraction of the organic fractions from soils and sediments, in general, has not yet been achieved (Stevenson, 1982). However, repeated extraction of a sample with alkaline solutions has been shown to result in the maximum recovery of humic material (Stevenson, 1982).

Concern regarding the oxidation of the organic fraction under alkaline conditions was expressed by Dubach *et al.* (1964) and Swift and Posner (1972) and results suggest that this effect is greatest for high ionic strength, high pH solvents (Table 1.14).

| Reagent | pH | $O_2$ Uptake ($mm^3/0.2g$) |
|---|---|---|
| 0.5M NaOH | 13 | 896 |
| 0.5M $Na_2CO_3$ | 10.5 | 56 |
| 0.1M $Na_4P_2O_7$ | 9 | 31 |
| 0.2M Na-citrate | 7 | 39 |

**Table 1.15 Effect of Increasing pH on Oxygen Uptake by Humic Acid : Reaction Time 7 hours (Bremner, 1950 in Stevenson, 1982)**

Stevenson (1982) suggests that uptake of oxygen increases with increasing pH and clearly an order of magnitude reduction in oxygen uptake can be achieved by restricting the pH of the extractant to values < 11 (Gascho and Stevenson, 1968; Bremner, 1950) (Table 1.14).

Many studies have reported the consumption of hydroxide during prolonged periods of extraction in sodium hydroxide (Swift and Posner, 1972; Bremner, 1950; Breger, 1974; Stevenson, 1982). Initially, it was proposed that this effect could be eradicated by extracting under nitrogen in the absence of oxygen and carbon dioxide (Swift and Posner, 1972; Stevenson, 1982) but similar effects have since been observed under these more stringent conditions. Davis and Mott (in Stevenson, 1982) proposed that the stripping and subsequent degradation of weakly associated polysaccharide units could account for the loss of hydroxide, while Bowles *et al.* suggested that hydrolysis of ester groups occurred at high pH values. From the results of recent studies, Antweiler (1991) contends that ester hydrolysis is the predominant mechanism leading to the consumption of hydroxide ions and a consequent decrease in pH. An increase in ionic strength of the extractant from 0.1M NaOH to 0.5M NaOH has also been linked to the promotion of the hydrolysis of the humic fraction as a result of the isolation of greater proportions of fulvic relative to humic acid. Conversely, an increase in temperature of 0.5M NaOH solution from 20° to 60°C resulted in the condensation of humic and fulvic acid molecules and the observation of molecular weights of the residue in excess of 1,300,000.

Powell and Town (1992) proposed that a minimum pH of 8 was required to isolate a representative humic fraction and it is therefore most probable that a representative and chemically less altered humic fraction will be obtained where the pH of the extractant lies in the range 8 to 11.

Tipping and Ohnstad (1984) showed that measurable alteration of the humic fraction in solutions of sodium hydroxide occurred over a time period of several days and reinforced the viewpoint that humic materials should not be exposed to the extremes of pH for prolonged periods.

Additional undesirable effects of sodium hydroxide extraction include the dissolution of humic material together with mineral matter (Greenland, 1971). It has been indicated by some workers that, although 0.1M NaOH extracts more organic material than 0.5M solutions, considerably higher ash contents of the resultant humic material are associated with extraction using the lower molarity alkaline solutions. However, other workers have been unable to confirm these findings. It is however clear that alkaline solutions may mobilise a small proportion of fine clay particulates which may or may not have been associated with humic materials prior to extraction and this may be quantified by determining the ash content of the humic extract. An alternative extractant, tetra sodium pyrophosphate, has been used where solutions were adjusted to pH 7, 8 or 9. It was observed that the amount of organic matter associated with mineral structures increased with increasing pH of the solution.

Traditionally, any mineral component is removed during the purification procedures described in section 1.4.5.3.

### 1.4.5.2 Separation of Components of Humic Substances

Classical fractionation methodologies for humic materials, based on the differing solubilities of their constituent components under varying conditions of pH and electrolyte concentration, have been used in attempts to isolate more homologous fractions of humic substances (Schnitzer and Skinner, 1968; Kononova, 1966). In the 1830s, Berzelius denoted the fraction that was soluble in alkali as humic acids. Those fractions that were also soluble in water were termed crenic and apocrenic acids whilst the inert residue was known as humin. He also determined that crenic and apocrenic acids had the ability to interact with di- and tri-valent metal ions to form salts. In 1840, Mulder modified this classification on the basis of colour to give further subdivisions as illustrated in Table 1.16.

| **Chemical Classification** | **Fraction** |
|---|---|
| insoluble in alkali | humin<br>ulmin |
| soluble in alkali | humic acid - black<br>ulmic acid - brown |
| soluble in water | crenic and apocrenic acids |

**Table 1.16 : Classification of Humic Fractions**

At a later stage, Oden (1910-1920) described crenic and apocrenic acids collectively as fulvic acids.

This brief history highlights the use of simple chemical fractionation to characterise soil humic materials and modifications of these methods have continued to be used to the present day. By definition, humic substances collectively comprise the proportion of organic material that is soluble in alkali and are conventionally subdivided on the basis of solubility into three fractions: fulvic acids are soluble in both alkali and acid, humic acids are soluble only in alkali and the organic residue, which is insoluble in both alkali and acid, is known as humin. Further fractionation, resulting from partial precipitation of the humic acid fraction, is effected by the addition of electrolyte giving soluble "brown" humic acids and insoluble "grey" humic acids. Hymatomelanic acid is defined by some workers as the fraction of humic acid soluble in alcohol, although Stevenson and Butler (1969) contend that it is an artefact resulting from the chemical fractionation process. In simplest terms, classical fractionation on the basis of acid/alkali solubility results in the separation of operationally defined humic and fulvic acids which are less heterogeneous than the bulk humic material, and Schnitzer and Khan (1972) contend that humic and fulvic acids are more suitable starting materials for further characterisation than the unfractionated bulk humic substances.

Alternatively, XAD resins have been used in the isolation (and purification) of humic substances in natural waters. The organic material sorbed onto the column is eluted using dilute acid solutions (Malcolm and MacCarthy, 1991). Similarly, diethyl amino ethyl (DEAE) cellulose has been used to extract humic material from groundwaters (Smith et al, 1991; Stuart et al, 1989). These procedures also involve the separation of humic and fulvic acids by the elution of fulvic acid in acidic solutions and the subsequent elution of humic acid in alkaline solution.

### 1.4.5.3 Purification of Humic Substances

It has been suggested that the extraction of humic substances under aqueous alkaline conditions may also involve the extraction of small amounts of fine clay and loosely bound polysaccharide impurities (Stevenson, 1982) (Section 1.4.3.1). Ash contents of 10 to >30% are commonly observed for humic acids, whilst values of 2-10% are observed for fulvic acids, leading to potential misinterpretation of the importance of the organic material in metal complexation. To eradicate this uncertainty, traditional methodology often involves the purification of the humic and fulvic components using chemical reagents, including dilute HF, 0.1M NaF and 6M HCl. The ash contents of humic and fulvic acids are ideally reduced to <1% in each case by the purification procedures. However, this is often accompanied by significant loss of the humic and fulvic acid and potentially the chemical alteration of the original humic material.

HCl-HF mixtures used by Khan (1971) are considered to be less harmful than HF solutions which contain no hydrochloric acid (Dormaar *et al.*, 1970 ; Lowe, 1969) but it is well known that chemical modification can occur in the presence of dilute HF solutions during the removal of mineral matter from humic acids (Stevenson, 1982). Likewise, hydrolysis using 6M HCl to remove loosely bound polysaccharides may result in significant alteration of fulvic acids. FTIR studies showed that hydrolysis of fulvic acids using 6M HCl, resulted in the formation of a dark, acid-insoluble residue which displayed spectroscopic characteristics similar to humic acids (Stevenson and Goh, 1971). Goh and Reid (1975) also showed that prolonged standing of extracts in acid led to an acid-induced polymerisation and increased the amount of organic matter in the higher molecular weight ranges. The inference that acidic conditions lead to the alteration of the humic material is in agreement with other reports (Farmer and Morrison, 1960; Kosaka *et al.*, 1960). Other studies (eg.Wershaw and Pinckney, 1973) of the physical and chemical properties of humic acids involving the aggregation of humic acid molecules as a function of pH have shown that a fraction of humic acid is irreversibly aggregated at pH<3.5. It must be concluded from this result that the molecular weight distribution of humic acid macromolecules is irreversibly altered at low pH values. Contradictory conclusions, however, were drawn by Mehta *et al.* (1983), who suggested that the average molecular weight is unaltered after drying at 100°C and storage in 1M HCl at room temperature for one month. It is well-known, however, that the effects of exposure to temperatures greater than 40°C are detrimental in studies of humic substances since they irreversibly alter the structure of the material and therefore the effect of storing in acid for any period of time may be expected to have little further effect. The effects of temperature on humic structure have been observed spectroscopically following the heating of KBr discs containing humic material (Stevenson and Goh, 1974).

Recent reviews have concluded that different purification processes lead to humic materials with different compositions and properties (eg. Hayes, 1991) and this observation has resulted in attempts to implement standardised methodology for extraction of 'pure' humic materials and commonplace use of a small number of reference humic materials. The International Humic Substance Society (IHSS) has produced a small number of highly purified reference humic substances which have been used in the study of humic acids by a number of workers (eg. Kim, 1990; Antweiler, 1991). In addition the commercially available humic acid from Aldrich Chemical Company is used in many studies for comparison with humic materials extracted from soils from chosen study locations (eg. Rao, 1994).

Several workers (Antweiler, 1991; Williams *et al.*, 1993; Aiken *et al.*, 1985) have concluded that the use of less destructive isolation techniques is required, particularly where there is a need to extrapolate from laboratory measurements to environmental behaviour. Most recent studies of humic substances, however, have involved the use of conventional alkali-acid solubility separation and purification methodology in order to achieve the extraction of a pure humic substance and to allow direct comparison of subsequent measurements. The value of these studies and those involving the use of reference humic acids (IHSS and Aldrich humic acids) is, however, questionable in the light of the increasing evidence for the chemical alteration of humic and fulvic materials during their extraction.

### 1.4.5.5 Alternative Non-Aqueous Extraction Techniques

In a review of current extraction procedures, Hayes (1991) highlighted the need for a suitable non-alkaline solvent system as efficient as aqueous alkali in the dissolution of a representative humic fraction.

A number of new extraction techniques have been proposed by various authors (Szabo *et al.*,1991; Bulman, 1992) involving the use of non-aqueous extraction media. In comparative studies of different available methodologies it has been suggested that traditional sodium hydroxide extractions are significantly less effective in the extraction of soil humic substances and associated metals than non-aqueous extractants including silylating agents (trimethylchlorosilane and triethylchlorosilane) in the presence of dimethylformamide. Silylation of predominant functional groups is proposed as a mechanism for altering the solubility of the humic macromolecules without modifying their structure or interaction with metal species. These experiments involved the addition of $^{239}$Pu to environmental samples and the alternative methodology resulted in the Soxhlet extraction into dimethyl formamide (DMF) of approximately 70% of $^{239}$Pu in association with humic material from a saltmarsh soil following a reaction with triethylchlorosilane (TECS) (Szabo *et al.*, 1991). In comparison with alkaline extraction of the humic fraction, where 0.1M NaOH was added to the sediment and the stirred suspension heated to 60°C, indicated that only 10% of the added $^{239}$Pu was co-extracted. However, the effects of silylating agents and other non-aqueous extractants on the overall chemical composition of the humic fraction are unknown and perhaps a detailed study of the elemental and functional group composition which, to date, has not been published by the authors, would provide an improved measure of the effectiveness and validity of these methods.

### 1.4.6 Colloidal Behaviour of Humic Substances

During the period 1910-1920 two workers, Oden and Shmuck (in Kononova, 1966), independently showed that humic substances exhibited characteristics that were typical of colloidal particles. In particular, they observed that the coagulation of humic materials by different electrolytes occurred according to colloidal principles. Ong and Bisque (1968) suggested that humic substances may be considered either as true solutions of macro-ions or as negatively charged hydrophilic colloids. The effect of variation of salt concentration on the alteration of the conformation and, where sufficiently high, the coagulation of humic molecules has also been studied by Ong and Bisque (1968) who explained this colloidal behaviour in terms of the Fuoss effect. The presence of salt ions can reduce the intra and inter molecular distances (by masking the charge and hence reducing the repulsive forces between polyelectrolytes such as humic substances) and results in the expulsion of hydrating water molecules, hence the alteration of conformational shape and ultimately coagulation. Alteration of conformation and coagulation of humic materials in a similar manner is also achieved by lowering the pH of the solution. Ultimately, the present view of the colloidal nature of humic substances is that their size and shape in solution is determined by pH and the presence of neutral salts (Stevenson, 1982).

In further support of the colloidal properties attributed to humic materials, Ong and Bisque noted that the order of coagulating ability of metal ions was: $M^{3+} > M^{2+} > M^{+}$ in agreement with the Schultz-Hardy rule. Additional observations relating to the mean critical concentrations of metal ions of different valencies followed the predicted behavioural trends (inverse relationship to the sixth power of the valency).

The treatment of the interactions of metals with humic substances purely in terms of colloidal principles and therefore assuming that the metals are held in the diffuse double layer (ie. 'charge-only' model described in Tipping *et al.*, 1988) is insufficient to characterise completely the associations of metals with these complex biomolecules and this has led to much further study of the properties and complexation characteristics of humic substances.

The following sections describe the structural properties of humic substances which account for their observed behaviour in solution and in particular attempt to rationalise the available information relating to their interactions with metals.

### 1.4.6.1 Functionality of Humic Substances

Due to their structural heterogeneity, the behaviour of humic macromolecules in solution and in their interactions with metal species has been rationalised on the basis of simplified model structures with the consideration only of predominant functional groups.

Polar and ionisable functional groups in humic macromolecules are responsible for their solubility in aqueous solutions. Oxygen is the major heteroatom present, predominantly as carboxyl, phenolic and alcoholic hydroxyl, ketonic and quinoid C=O (Rashid, 1971) and methoxyl groups. For example, experimental determinations of the proportions of functional groups for different humic materials have been compiled and are presented in Table 1.17 below.

| Material | Carboxyl | Phenolic Hydroxyl | Alcoholic Hydroxyl | Carbonyl | Methoxy | Other |
|---|---|---|---|---|---|---|
| Humic Acid | 34-50% | 7-14% | 1-8% | 15-30% | 2-4% | 5-29% |
| Fulvic Acid | 57-75% | 1-10% | 9-20% | 11-17% | 3-5% | 0-10% |

**Table 1.17 Distribution of Oxygen-containing Functional Groups in Humic Materials (Stevenson, 1982)**

The relative abundance of these functional groups contributes to the solubility, reactivity and overall chemical character of the molecule. The distribution of functional groups along the backbone of the humic macromolecule determines the position of areas of polar and non-polar nature, and hydrophilic or hydrophobic character. Due to the three dimensional flexibility of the backbone, the molecule is able to coil and the polarity/charge associated with the functional groups gives rise to numerous weak interactions and sometimes chemical bonds between strands of the same molecule.

#### 1.4.6.2 Effects of Charge on Metal Interactions with Humic Substances at the Interface between Solid and Aqueous Phases

Solid phase surfaces in soil and sediment systems are often chemically highly reactive (Healy, 1980). Different surfaces may be positively or negatively charged or neutral and any charged surfaces attract oppositely charged counterions from solution to the interface. The relative proportions of ions attracted depends on the degree of acidity or alkalinity of the soil, on mineralogical composition and on the content of organic matter.

Many of the hydrophilic sites on humic macromolecules are dissociable acidic or basic sites and therefore possess a pH dependent charge which is often highly negative under environmental conditions (Tipping, 1988). Of the functional groups that are present in solid phase humic macromolecules, many will reside on the interface between the solid and aqueous phases (ie. partial dissolution of polar entities) and, in this manner, account for the reactivity of insoluble organic matter. In many respects, this behaviour is similar to that of macromolecules which make up cell walls in biological tissues which also bear a pH dependent negative charge due to the prevalence of acidic functional groups on their surfaces (Tipping, 1988; Tipping *et al.* 1988a; Wershaw, 1989).

#### 1.4.6.3 Effects of Functionality, Ligand Concentration and pH on Metal Interactions with Humic Substances in Natural Waters

Hydration in aqueous solution is achieved by the dissociation of ionic groups and the formation of hydrogen bonds between water molecules and hydrophilic sites on the humic macromolecule. The degree of hydration is related to the nature and number of hydrophilic sites on the macromolecule (Buffle, 1988). Up to 50% of the weight of a fully hydrated humic molecule can be attributed to bound water molecules. Dissolution in aqueous systems gives rise to the presence of internal water molecules bound directly to hydrophilic sites which subsequently influence the shape of the macromolecule by altering the attraction between strands.

Dissolved organic matter is generally present in low concentrations (0.1-8ppm in surface waters ; 50ppm in swamp waters; Leenher, 1974) in natural waters and although up to 80% may be humic compounds, the remainder comprises low molecular weight compounds which are likely to be of a similar type to humic precursor compounds (ie. they contain many of the types of dominant functional groups that are incorporated into the humic molecules). In terms of metal attracting capacity, components of humic substances have the greatest capacity of all components of the dissolved organic fraction in natural waters. In theory, the differing behaviour of low molecular weight molecules containing a single functional group (eg. those specified in Table 1.16) when compared with overall similar numbers of functional groups attached to a single high molecular weight molecule can be explained by considering the distance in solution between the functional groups as affected by solution concentration. The functional groups on individual low molecular weight molecules become increasingly separated with decreasing solution concentrations of these molecules. Large macromolecules such as humic substances have 'surface' functional groups which remain separated by a more or less fixed distance independent of concentration, unless degradation results in the splitting of the macromolecule. Otherwise, the groups remain closely associated and can influence one another in nearly all circumstances (Sposito, 1989).

### 1.4.6.4 Effect of Functionality on Metal Interactions with Different Components of Humic Substances in Soils, Sediments and Waters

Humic macromolecules potentially exhibit a range of solubilities relating to the inherent charge on the individual macromolecule and its degree of hydrophobicity. As mentioned in 1.4.6, solubility is also dependent on influences of charge-neutralising $H^+$ ions and of polyvalent cations which can render these polydisperse, polyelectrolytes insoluble in water (Hayes and Swift, 1978; Hayes, 1985). Binding of cations to humic molecules causes the release of solvation water molecules resulting in configurational changes which, in the extreme case, can cause precipitation of an aggregate. Components of humic substances with differing proportions of functional groups or hydrophobic/hydrophilic units will be affected to differing degrees by these influencing factors. In the simplest case, fulvic acid as defined by the separation procedure is stable in solution over the entire pH range (assuming other conditions remain constant). In the environment, partitioning of humic components between solid and aqueous phases is infinitely more complex. Powell and Town (1992) reported that the solubility of humic substances is dependent on pH, ionic strength and the nature of the electrolyte ions in solution and suggested that a different molecular size fraction of humic substances will be present in solution at each observed combination of pH and ionic strength, with clear implications for metal complexation. Anthropogenic influences may also be important and provide an external variable. For example, variations in soil conditions due to pollution (eg. acid rain) will influence the partitioning of humic substances between the solid phase and soil solution (Tipping, 1988; Tipping and Hurley, 1988; Allard, 1986; Cronan *et al.*, 1985; Davis and Gloor, 1981; Oliver *et al.*, 1983; Vance and David, 1989; Giesy *et al.*, 1986). The magnitude of this effect at any locality will be controlled by factors affecting the deposition of such pollutants.

Additionally, as a consequence of the co-existence of hydrophobic and hydrophilic components in the same macromolecule, humic substances form micelle type structures in aqueous solution, with hydrophobic units oriented inwards to minimise contact with polar entities, and exposing a surface covered with hydrophilic complexing sites. The functional groups or ligands oriented outwards are therefore particularly accessible to metal ions. Alteration of the electric field exhibited by the macromolecule will result in conformational change on approach/attraction of a metal ion. The reduction in local charge allows the humic chains to exist more closely and the conformational changes will be influenced both by the flexibility of the surrounding chain and by the steric bulk of ligands. Chin and Gschwend (1991) also noted that the results of fractionation of humic substances using gel filtration chromatography at different ionic strengths illustrated the ability of humic colloids to contract and expand as a function of matrix ionic strength. Behaviour of this kind can affect the propensity of the humic macromolecules to bind particle reactive substances in different sedimentary environments.

Marine humic macromolecules are therefore, in general, more tightly coiled than their freshwater counterparts, so that the configuration of the humic colloid may influence the sedimentary geochemistry of associated radionuclides, other metals and hydrophobic anthropogenic organic compounds (Chin and Gschwend, 1991). The behaviour of metal-humic species in the marine and terrigenous environments is directly relevant to the present work. The sampling locations chosen in this study vary in their degree of marine influence. In particular, the sampling location on Southwick Merse on the coast of SW Scotland is exposed to water of alternately low and high salinity.

### 1.4.7 Metal Complexation by Natural Humic Compounds

The present study is primarily concerned with the interactions of radionuclides released into a marine system and subsequently deposited in coastal soils and sediments. Therefore it is necessary to consider the following:

i) general interactions of metals with humic compounds in solid and aqueous phases

ii) chemical behaviour of specific radionuclides which determines their distribution between solid and aqueous phases and subsequent behaviour

Interactions of metal ions with humic compounds have been described as ion-exchange, surface adsorption, chelation and coagulation. On a physical basis, the initial attraction may be accounted for in terms of colloidal properties of the macromolecule as a whole (Hayes *et al.*, 1989; Healy, 1980) since soluble humic macromolecules are considered as being of colloidal proportions. Metal ions can be sorbed on to humic colloids by monodentate or multidentate complexation (Kim, 1991) and so increase the concentration of metal ions in groundwater. It is generally considered that the functional groups predominantly in the regions of hydrophilicity in humic macromolecules also provide the sites at which metal ions or other attracted species may subsequently be bound. However, complexation reactions involving polyfunctional humic substances and often numerous metal ions and therefore simultaneously present multi-metal, multi-ligand interactions which are poorly understood.

Of the soil organic pool, humic macromolecules are known to have the highest complexation capacity for metals. Unlike simple inorganic anions they form stable complexes with a range of both hard and soft metal ions. Their complexation capacity arises from the large number of potential sites and also from the variety of functional groups present, which potentially allows them to provide simultaneously both hard and soft ligands. In other words, humic substances, because of their functional group diversity and distribution provide suitable sites for many trace metals by enabling different metal species to interact in different ways and with different functional groups in a variety of chemical environments. In contrast, many other natural biological complexants offer a single type of site which is capable of binding very specifically a particular metal species or group of metals (eg. desferrioxamine and $M^{3+}$ ions, haemoglobin and $Fe^{3+}$, proteins, enzymes, pigments etc.).

In the environment, a large number of pollutant and natural metal ions co-exist, displaying widely varying properties and present at concentrations differing by many orders of magnitude. Humic substances have the capacity for numerous and diverse interactions with many of these metal ions (Andreyev and Chumachenko, 1964; Perdue, 1976; Choppin, 1988; Shanbhag and Choppin, 1981; Szalay, 1964; Tipping *et al.*, 1988a; Wolfrum and Bunzl, 1986; Weber, 1988). For example, Uranium and other metals are accumulated by peat humic acids, with geochemical enrichment factors of $10^4$ from the very low concentrations in natural waters (Szalay, 1969).

The selective uptake of certain trace metals, particularly those of high charge can be described theoretically by the formation of double layers (DLVO theory). Humic colloids, due to their ability to accumulate such metals with enrichment factors of up to $10^5$, are therefore of considerable importance in the study of actinide behaviour with respect to the disposal of intermediate and high level radioactive waste (Kim, 1991) and low level liquid wastes as this study demonstrates.

For the purposes of this study of actinide behaviour, further reference is made to previous studies of metal mobility following complexation by humic substances under varying environmental conditions. The effect of changing conditions in natural water bodies has been observed where a significant proportion of humic material was flocculated over the salinity gradient experienced as material moved from freshwater to salt water in estuaries (Sholkovitz, 1976, 1978; Eckert and Sholkovitz, 1976; Tipping and Ohnstad, 1984; Backes and Tipping, 1987). Importantly, not all humic material was flocculated under any particular set of conditions due to the range of solubilities displayed by humic macromolecules. Tipping and Ohnstad (1984) also demonstrated that precipitation with $Ca^{2+}$ and $Mg^{2+}$ was dependent on the nature of the humic substances. In other words, the component of humic substances that remained in solution on the addition of these metal ions differed in chemical composition from that which was effectively removed to the solid phase.

Sholkovitz demonstrated that 3-11% of humic material was removed in river estuaries in SW Scotland and large scale removal of dissolved Fe together with humic material is also more widely observed in estuaries (Boyle *et al.*, 1974, 1977; Sholkovitz, 1978; Murray and Gill, 1978; Mayer, 1991; Moore *et al.*, 1979). The perturbation of the system in this manner has consequences for mobility and bioavailability not only of Fe but also of associated important trace elements, nutrients and trace organics such as pesticides sorbed onto humic macromolecules (Mayer, 1991; Gamble, 1986; Gamble and Schnitzer, 1973; Khan and Schnitzer, 1972; Picard and Felbeck, 1978). Further apparently contradictory evidence of the importance of humic macromolecules as chelators was reported by Duursma and Sevenhysen (1966) who showed that the concentration of soluble Fe species in natural water bodies was in excess of that predicted on the basis of the solubility product of iron hydroxide. They concluded that certain iron humates are soluble under the conditions present in seawater and are resistant to hydrolysis to insoluble iron hydroxide. However, the flocculation of a large proportion of metal humates under estuarine conditions is proposed as an important mechanism for the scavenging of metal species from the water column to sediments.

Humic and fulvic acids are not only responsible for most of the metal immobility attributed to soil organic matter (Stevenson and Ardakani, 1972) but are also implicated in the formation of soluble complexes with metals, particularly in dilute solutions (Wildung and Garland, 1989). In the assessment of the long term behaviour of radionuclides in the terrigenous environment, the factors influencing their solubility and the formation of soluble species in the soil solution must be determined (Wildung and Garland,1989). The major factors governing bioavailability of actinide elements are their solubility in the soil solution and the stability of soluble species in the soil solution. In particular, the formation of stable complexes with organic matter can potentially enhance the solubility of actinides and hence their mobility (Choppin, 1988; Saar and Weber, 1982). The formation of soluble complexes can result in a reduction in their toxicity to plants since it is considered that complexed forms of a metal are less bioavailable than the free metal ion (Saar and Weber, 1982). Alternatively, the formation of insoluble complexes reduces the mobility of the species and results in a reduction in toxicity by complete removal to the solid phase.

A further area of study has been the bioavailability of radionuclides following complexation by natural organic compounds. The uptake of non-essential and pollutant metals, for example Pu, by plants has been observed and was significantly enhanced by fulvic acid complexation, relative to a citrate control (Livens, unpublished data, 1987) (Table 1.18).

| | Pu-fulvate | Pu-citrate |
|---|---|---|
| Soil to shoot (Bq $kg^{-1}$ shoot/soil) | $1.7 \times 10^{-4}$ | $9.0 \times 10^{-5}$ |
| Root to shoot (Bq $kg^{-1}$ shoot/root) | $1.9 \times 10^{-2}$ | $1.7 \times 10^{-3}$ |

**Table 1.18 Plant Transfer Data for Pu Complexed with Fulvic and Citric Acids (Livens, unpublished data, 1987)**

This complexation also resulted in increased root-to-shoot translocation of Pu within the plant (Livens, unpublished data, 1987). The mechanism of uptake by plants and, in particular, the nature of the radionuclide-organic complex within the plant is uncertain.

### 1.4.7.1 The Influence of Humic Substances on the Solution Speciation of Actinides

Before discussing the characteristics of actinide-humic complexes it is first necessary to restate some aspects of the inorganic chemical behaviour of metals and, in particular, the actinides. All metal cations are hydrated in aqueous solution and interactions with other species in solution involve exchange mechanisms in which preferred ligands displace the co-ordinated water molecules. Less commonly, other weaker ('outer sphere') complexes may be formed by the attachment of ligands via the water molecules of the hydration shell. Dissolved actinide ions, $M^{3+}$, $M^{4+}$, $MO_2^+$ and $MO_2^{2+}$, are extensively hydrated and, in the absence of complexing ligands, their behaviour is governed by conditions of $E_h$, pH, ionic strength and actinide ion concentration which may effect their hydrolysis and subsequent precipitation as insoluble polymeric species. The solvation of actinide species is a special case of their more general interaction with other species in solution. In aqueous solution, ensuing reactions are exchange reactions where the effectual complexation of the actinide ion follows the exchange of hydration water for the preferred ligand. The affinity of the metal species for any ligand is governed by the same rules as hydrolysis. Tendency of actinide ions to form cationic, anionic or neutral species in solution influences their subsequent interactions with reactive species. For example, formation of anionic species is more favourable for U than for Np (Katz *et al.*,1986) and so carbonate species of U are more stable than those of Np. Again size/charge effects are the dominant factors in determining the reactivity of the metal and its affinity for ligands.

All actinide species in solution are prone to complexation. The actinyl ion is linear (to a first approximation) and ligands are arranged in the equatorial plane. Where water is the ligand, there is a pentagonal arrangement of water molecules in the equatorial plane. A necessary condition for the formation of any complex is that the ligand has an affinity for the central actinide ion strong enough to compete with that of co-ordinated water. The actinides are hard metal cations, as defined by their ratio of charge/radius, and as such form their strongest bonds with oxygen containing ligands via the oxygen atom. Similarly a strong affinity for N is reflected by the formation and stability of certain complexes via N donors. Both O and N are hard ligands whereas S is a soft ligand and as such forms weak complexes with the actinide ions. As for hydrolysis the affinity of the metal for a given ligand increases in the order:

$$MO_2^{+} < M^{3+} \simeq MO_2^{2+} < M^{4+}$$

In aquatic systems there are a number of complexants with an affinity for actinide ions. The common inorganic anions, $CO_3^{2-}$, $SO_4^{2-}$, $NO_3^{-}$, and $PO_4^{3-}$ possess very different affinities for the actinide ions. The most stable complexes are formed with carbonate ligands and, due to its high concentration in sea water, and often in groundwater, the formation of such complexes is a key component in the solution behaviour of the actinides. Low concentrations induce hydrolysis followed by precipitation of polymeric species. At concentrations present in sea water, depolymerisation of actinide hydroxides occurs with the formation of stable, soluble carbonates. These species are by far the most readily formed with actinyl ions and under alkaline conditions in sea water $MO_2(CO_3)_3^{4-}$ will predominate. At the oxidation potential normally prevailing in sea water, U is present exclusively in the +6 state and this is likely to be the most stable complex of U and a strong possibility also for Pu in the absence of high concentration of stronger organic complexing agents. Conversely, the existence of these species indicates high solubility and hence high concentrations of U in sea water and it is the inability of Th to form soluble Th(IV) carbonate species under the prevailing conditions together with its high particle affinity that differentiates between solubility of U and Th in sea water.

Laboratory studies also confirm that the presence of carbonate ions increases the solubility of uranium by approximately two orders of magnitude. In distilled water the maximum concentration of added uranyl species in solution is 50 ppb whereas solution concentrations in a synthetic groundwater containing carbonate are of the order of 1ppm (Forsyth *et al.*, 1986). Organic substances are predicted to form strong complexes with $Pu^{3+}$, $Pu^{4+}$, $Am^{3+}$ and $Np^{4+}$ (Bondietti, 1982) and may also be able to reduce $Pu^{6+}$ to $Pu^{5+}$ or $Pu^{4+}$ (Cleveland, 1979). $Pu^{5+}$ and organically complexed $Pu^{4+}$ are predicted to be the mobile species of Pu under aerobic conditions (Bondietti, 1982). It is suggested (Choppin, 1983; Choppin *et al.*, 1986) that complexation by organic matter significantly enhances the solubility of Pu, in particular by solubilising $Pu^{4+}$. Organic complexants such as citrate and EDTA can, however, reverse the relative stabilities of the +3 and +4 oxidation states. The greater strength of complexation of $Pu^{4+}$ relative to $Pu^{3+}$ is illustrated by the partial oxidation of $Pu^{3+}$ to $Pu^{4+}$ in the presence of citrate or EDTA (Foreman and Smith, 1957 : in Choppin 1983). A strong correlation between $Pu^{4+}$ in solution and natural organic matter content has been reported in marine systems, in which resulting complexes would be stabilised by high alkalinity due to increasing ionisation of functional groups (Shnitzer and Khan, 1972).

Soluble Np complexes with Aldrich humic acid have been observed under conditions predicted to be prevalent in proposed German high level radioactive waste repository systems (Bidoglio *et al.*, 1988). On the basis of these studies, it was concluded that natural humic substances would be capable of transporting Np from the repository to the far field environment.

In natural systems, concentrations of the actinides in solution, in particular U (in the absence of suitable ligands) and Th, are often extremely low and, although the actinides are highly susceptible to hydrolysis, the concentration of the hydrolysed species is lower than their solubility in the water body.

The low concentration of Th in sea water also suggests that formation of polymerised species would be unlikely due to the low probability of a hydrolysed Th species encountering one or more other Th species. Subsequent adsorption, due to the high particle reactivity of Th species is responsible for the almost complete removal of Th from the water column (McKee *et al.*, 1984). In a natural system, at pH>7, sorption of Th occurs almost completely on to clays and solid organic matter, resulting in significant depletion of Th relative to U in aquatic systems with adequate particulate loading. This is subject to seasonal effects (eg.

variations in primary productivity) and is also enhanced close to coastlines due to increased resuspension of particulates. Anomalies to this general case have, however, also been reported including the measurement by Nozaki and Horibe (1983) of enhanced $^{232}$Th concentrations in solution towards the ocean floor. It is suggested by Chin and Gschwend that sediment bed-to-overlying water releases of colloidal organic material appear to occur and may play an important role in the cycling of particle reactive chemical species (metals and anthropogenic organic compounds). Other studies have shown that there is a seasonal phenomenon which leads to high concentrations of Th and Pb associated with organic "fluff" on the ocean floor which is easily resuspended (unpublished data, McKave, 1995). Langmuir and Hermann (1980) have predicted that the concentration of Th in solution is greatly increased by the formation of soluble organic complexes and suggest that $Th^{4+}$ can therefore be compared with $Pu^{4+}$ with respect to organic complexation. Ligand concentration and characteristics must be considered in the assessment of the stability and solubility of actinide species in aqueous systems. It is well known that organic substances are important in complexing U and in ore formation. In acidic environments, soluble organic compounds may be important in $U^{6+}$ migration, whereas at higher pH, carbonate species are likely to dominate. Thus, maximum sorption of $U^{6+}$ on to most natural colloidal materials including humic substances occurs in the pH range 5.0-8.5. Th is believed to form stronger complexes with dissolved organics than U (Langmuir and Herman, 1980, Nash and Choppin, 1980). The high particle affinity of Th results in almost complete sorption of uncomplexed Th on to clays and solid organic matter at $pH > 7$. Marine organisms may also influence the geochemical behaviour of Th by the more efficient uptake of Th relative to U (Cherry and Shannon, 1974) and since they are potentially precursors of marine and estuarine sedimentary humic substances, they may therefore exert an influence on the relative amounts of U and Th associated with this component. Specifically, it would be expected that U/Th ratios in humic substances occurring in estuarine sediments may be lower than predicted from seawater ratios.

### 1.4.7.2 Studies of Actinide-Humic Complexation

Chelating resin chromatography in combination with laser fluorescence has been used to estimate conditional stability constants for $UO_2^{2+}$ binding by humic substances at environmentally relevant metal-ligand ratios and to determine the binding capacity of humic material for $UO_2^{2+}$ (Giesy *et al.*, 1986). Conclusions from this study show that at environmental concentrations, a significant proportion of U is humic-associated (25%) and, more importantly, is bound to natural humic substances by a number of sites with different binding affinities. The attraction of metal species towards negatively charged humic macromolecules will result in concentrations in the condensed layer adjacent to the surface being orders of magnitude greater than in the bulk solution. The metal ions exhibit a range of reactivities and hence surface affinities that depend on the predominant form of the metal ion. This affects the residence time of a metal ion in solution and its potential interaction with humic substances (Pentreath *et al.*, 1986; Honeyman and Santschi, 1988). The higher the reactivity, the more likely it is that the metal ion will become particle bound and the shorter will be its residence time in solution (Buffle, 1988). In the presence of carbonate, the uranyl species is present as the carbonato complex $[UO_2(CO_3)_3]^{4-}$ which is anionic and therefore electrostatically repelled by highly negatively charged humic macromolecules. Uranyl ion complexation responds to changing $E_h$ and pH conditions. For example, it is known that a reduction of pH in coastal areas causes increased complexation of U by humic macromolecules (Choppin and Mathur, 1991). The mechanistic details of the interaction of the uranyl species with humic substances are however uncertain (Choppin and Mathur, 1991; Choppin and Stout, 1989). Andreyev and Chumachenko (1964) suggest that U will be reduced by organic matter if the system contains partially oxidised compounds (eg. alcohols, aldehydes and acids) and that reduction involves insoluble, large organic macromolecules and therefore takes place within the U-organic molecule (ie. with U withdrawn from the bulk solution). The reduction of U is shown to be thermodynamically feasible at 25 °C and 1 atm. pressure whilst the degree of reduction depends on the precise nature of organic matter and the acidity of the medium.

### 1.4.7.3 Kinetic Studies of Actinide-Humic Interactions

A further dimension to the complexity of the binding properties of humic material is provided by the inclusion of kinetic factors. In decreasing order, the rates of reactions have been reported (Buffle, 1988) to be as follows:

i). exchange of hydrated water, exchange of protons, formation and dissociation of ion pairs.

ii). formation and dissociation of chelates.

iii). chemical reactions among several phases (solid formation, certain redox reactions, adsorption, organism assimilation)

iv). physical sedimentation and mixing processes.

Kinetic considerations have been invoked to provide insight into metal ion interaction with humic macromolecules in studies where $Th^{4+}$ and $UO_2^{2+}$ were added to solutions containing humic material and it was observed that complete binding of the nuclides was effected in less than one minute. Further studies showed that the kinetic expression for dissociation of these newly bound nuclides altered with time prior to their removal. Seven first order dissociation reactions were shown to contribute to the overall rate of dissociation (Choppin, 1988). With increasing time the importance of the slower processes also increased. It was proposed, therefore, that two stages are involved in binding; initially the metal ions are attracted to the charged surface and held in a condensed layer adjacent to the polyionic surface. Thereafter, some fraction migrates and becomes chemically bound to specific sites. It is proposed that this includes some sites within the molecular structure. This will also result in further modification of the overall humic structure. This discussion of kinetics brings together ideas regarding the colloidal nature of the material and the principles of site selectivity and coordination chemistry. A kinetic discussion of humic substances as a polydisperse ion-exchanger system (ion exchanger particles of different diameters) suggests that the rate of ion exchange of such a system is not simply predictable by comparison with a range of separate monodisperse systems (Bunzl, 1978; Bidoglio *et al.*, 1991; Burgess, 1992). The overall rate of attainment of equilibrium is slower than for a monodisperse system containing only the large diameter particles. Results show that the smaller particles in the polydisperse system always react much more rapidly and initially sorb beyond their equilibrium value. Following this, counterions are released and sorbed by the more slowly reacting larger particles. This can result in underestimation of the time taken for the system to reach equilibrium where this is estimated from the change in solution concentration of the free counterions. In the later stages, little change in solution concentration masks the continuing exchange of counterions from smaller to larger molecules (Bunzl, 1978).

More recently (Rao *et al.*, 1994), cation exchange has been used to study the kinetics of the interactions between U and humic materials. The initial binding of the free metal added in a buffered dilute perchlorate solution was effectively complete within 1-2 minutes. Two subsequent cation exchange experiments showed that the ion exchange characteristics of the humic material altered with time and that the retention of metal by the cation exchange resin decreased with increasing time during the second stage of ion exchange. They concluded that a portion of the added U was only weakly bound to the humic material. The strongly bound U was redistributed within the humic material with increasing time and, due to the decreasing retention of U by the resin, it was suggested that redistribution of U resulted in conformational changes and the subsequent relocation of U within the humic molecules (ie. unavailable to further ion exchange). Attempts to relate this to potentially different stereochemical associations of U when compared with $Eu^{3+}$ data could not be substantiated conclusively. Theoretically it was proposed that i) equatorial co-ordination of ligands around the linear $UO_2^{2+}$ ion where the binding would be stronger due to the higher effective charge associated with U (+3.3) ii) chelation of a 'condensed $UO_2^{2+}$' species would be less strong due to the lower effective charge (+2.2) (Rao *et al.*, 1994).

The influence of humic substances on the geochemical behaviour of actinide elements in this manner has important implications for the assessment of high activity waste storage proposals and therefore modelling of humic characteristics and prediction of long term mobility of humic-actinide species is an important area of study (eg. Higgo et al, 1992; Falck, 1989). Versions of modelling codes (PHREEQE) based on the effects of variations in Eh and pH on the inorganic speciation of metals have recently been modified in order to improve their predictive capacity where organic polyelectrolytes, humic substances, are present (Falck, 1989).

### 1.4.8 Modelling of Metal-Humic Complexes

A major problem in modelling metal interactions with humic substances is the need to describe adequately the heterogeneity in the distribution of binding sites. The conclusions drawn by Kim (1991) suggest that the interaction of metal ions with humic and fulvic acids can be adequately treated by the use of a single binding constant. A single ligand approach is adopted by Higgo (1992) leading to the attainment of only a single intrinsic stability constant for metal complexation for incorporation into models of humic behaviour. Intrinsic stablitiy constants are obtained from conditional constants over a wide range of experimental conditions. Conditional rather than true stability constants are measured since the latter are not strictly applicable to variations in humic binding capacities under differing environmental conditions.

Where a more complex treatment of humic-metal interactions has been attempted, further modelling problems have arisen due to the difficulty in incorporating a multi-ligand approach or additionally, the competitive binding of a range of metals. A number of authors have concluded that U is first adsorbed by organic matter as uranyl complexes, and with increasing time, reduction occurs and uraninite is formed (Breger, 1974; Meunier, 1990) and is subsequently held in the solid phase. In order to model this interaction, thermodynamic data are required and in particular a single conditional stability constant for the formation of the organo-uranyl complex. This information cannot be obtained due to the number of different complexes that are potentially formed between humic substances and

$U^{6+}$, so present modelling codes are unable to cope satisfactorily with this mechanism of interaction (Jamet *et al.*, 1993). The direct reduction of uranyl complexes to form $U^{4+}$ minerals is thought to be important in strongly reducing environments (Boyle, 1984) and can be more straightforwardly incorporated into present codes.

Additional consideration of the behaviour of actinides in their interaction with humic substances shows that the concentration of added ions associated with humic material varies with time and also that the nature of associations change with time. The necessary time for equilibration of actinide speciation must be determined and incorporated into laboratory experiments used in the determination of modelling parameters.

## 1.5 Humic Substances and Their Role in Soils, Sediments and Waters

Considering the soil system as a whole, the particle-water interface will play a major role in regulating the concentration of most reactive metal ions in natural waters (Chin and Schwend, 1991; Ure, 1991, Baskaran and Santschi, 1992; Allard, 1986; Choppin, 1983; Tipping and Hurley, 1988; Tipping and Woof, 1991). It is suggested that humic substances can regulate the free ion concentration in solution and in turn determine the physiological conditions for the growth of algae and other microorganisms. Their effect on the free ion concentration in solution is also of importance for plant uptake of essential trace elements and nutrients (Dissanyake, 1989). Additionally, the mobilisation of otherwise unavailable metal ions is of the utmost importance in the study of anthropogenic nuclides and other trace organic compounds (including anthropogenic organic compounds eg. pesticides, industrial organics) in the environment (Livingston and Bowen, 1982; Cherry and Shannon, 1974; Fisher, 1986; Gamble, 1986, Khan and Schnitzer, 1972; Wershaw, 1989).

### 1.5.1 Alternative Complexants in the Organic Fraction

A further potential interaction, considered by Pickering and Batts (1992), follows the partial degradation of the humic fraction. They contend that the alteration of the organic fraction releases small ($C_1$-$C_5$) highly soluble carboxylic acid groups with a capacity to chelate metals in the soil solution and so enhance the movement and consequently the toxicity of pollutants (Pickering and Batts, 1992; Kieber et al, 1990). In areas of industrial activity where processing of waste may induce the degradation of humic material the solution phase transport of discharged metals is forseen via such organic acids. Berry and Bond (1990) indicate the importance of the degradation of cellulosic materials and other organic waste products arising from the alkaline chemistry of concrete pore water and microbial action. Such degradation products can have a marked effect on the sorption of Pu and U onto geological materials and results suggest that complexation of Pu and U by such degradation products enhances their solubility under these conditions. In the absence of such complexants the reactivity of Pu leads to hydrolysis and the formation of possibly polymeric $Pu(OH)_x$ species which are insoluble as a consequence of high particle reactivity.

### 1.5.2 Importance of Humic Substances

Overall, it must be considered that humic substances are not the only potential organic complexants of metals in the environment. However, i) they are the most widely distributed natural organic complexant, ii) they comprise a major portion of the organic fraction in most soils, sediments and waters, iii) they make up the most resistant portion of the organic fraction with respect to degradation and as such provide a long term potential for influencing the mobility of actinides in the environment. Therefore, their characterisation, both structurally and in terms of actinide complexation, is the focus of this study.

# CHAPTER 2

# METHODOLOGY

## 2.1 INTRODUCTION

The objective of this study was to investigate the associations of Th, U, Pu, Np and Am with natural humic substances extracted from soil and sediment samples. An integral part of the study was the characterisation of humic material from soils with contrasting environmental conditions. In particular, three study sites were selected which experienced varying degrees of marine influence. Samples from different depths at these locations were also collected to allow comparison of the characteristics of humic material present at surface and depth in soils and sediments. Importantly, humic materials from different depths in soils and sediments are of varying ages with those present at depth having been subject greater diagenetic alteration.

The study only involved the characterisation of humic material extracted from environmental soil and sediment samples from these study areas. Commercially prepared humic material (eg. Aldrich Humic Acid) was not used for the characterisation of actinide interactions with humic substances since an extrapolation back to environmental interactions is impossible on the basis of the results of such a study.

## 2.2 Sample Collection and Preparation

Soil and sediment samples were collected from several locations around the Irish Sea. A limiting factor was the large number of subsamples which had to be analysed in order to characterise the humic material present, both with respect to chemical composition and interactions with the actinides. In order to provide sufficient material and to allow examination of variations in the composition of humic substances and their interactions with actinides as a function of depth, samples of approximately 5kg were collected from two or three depths at each site. All of the sampling sites were known from previous studies (MacKenzie et al., 1987; Scott et al., 1991; Livens, 1985; Hursthouse, 1990, Allan, 1993) to receive inputs of Sellafield waste radionuclides via deposition of contaminated marine particulate material. The various sampling sites, and the sampling procedures used, are described below.

### 2.2.1 River Esk

A set of samples was collected from the River Esk in W Cumbria from the positions shown in Figure 2.1. The sampling sites are illustrated in Plate 2.

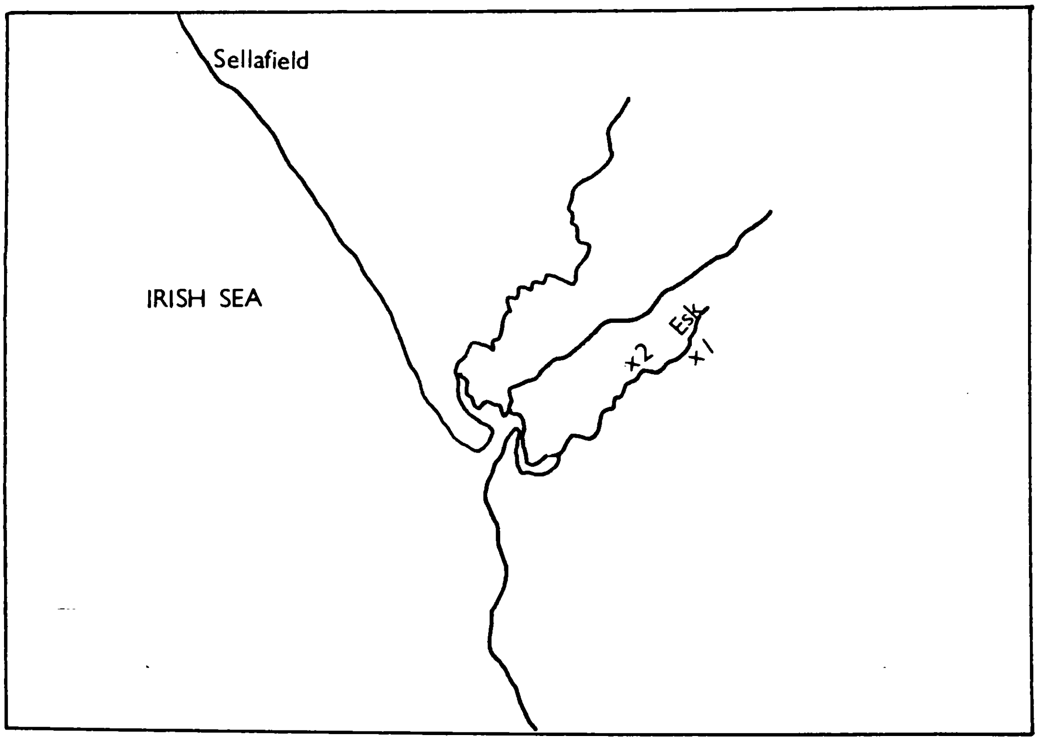


**Figure 2.1 Map of W. Cumbria showing the River Esk Sampling Sites**

Bulk soil samples were collected from Site 1, situated near the bank of the River Esk at a position close to, but below, the upper tidal limit. The location is exposed to marine conditions and deposition of marine sediments only during very high tides. A spade was used to cut a section of the soil and a portion representing the depth interval 0-5cm was carefully removed. The 5-10cm depth interval was then removed and discarded, and after cleaning the spade, the 10-15cm depth interval was sampled.

Bulk soil samples were also collected from Site 2 which was situated on the floodplain region between the River Esk and Muncaster Castle. This location is inundated with marine material only infrequently on occasions of spring tides etc. but is prone to waterlogging and the soil is therefore subject to predominantly reducing conditions. A small pit (30cm depth) was dug, one face was selected and carefully cleaned before the samples were removed. Again 0-5cm and 10-15cm samples were carefully cut and removed.

### 2.2.2 Southwick Merse

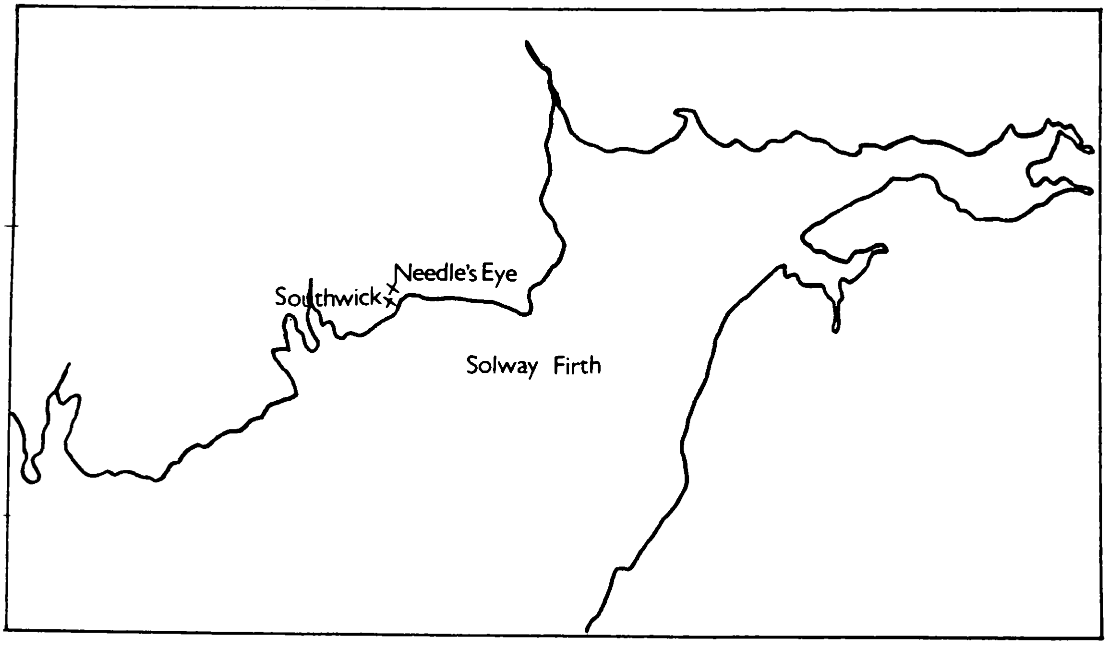


**Figure 2.2 Map of SW Scotland showing Southwick Water and Needle's Eye**

Bulk sediment samples were collected from the edge of the Merse (the local term for saltmarsh areas) forming the bank of the Southwick Water at a location known to be subject to marine inundation and accumulation. Sedimentation rates of 3-5cm per year have been estimated (MacKenzie et al., 1993) which are considerably in excess of the accumulation rates at either of the two locations

studied in W Cumbria (Livens, 1990; estimation on basis of radionuclide depth profiles; 3mm per year). The samples were collected as before, but at the depths 0-5cm, 40-45cm and 65-70cm. These depth intervals were chosen in order to provide suitable time intervals for the investigation of diagenetic changes affecting Sellafield waste radionuclides. This location was also selected on the basis of previous studies to allow comparison with existing data (Ben Shaban, 1989; Scott et al. 1991; Allan, 1991).

All samples were collected during May 1991. The individual samples were placed in marked polythene bags and sealed following their removal from the sampling location. The samples were frozen until use. Each soil or sediment sample was dried at 40°-50°C, the dried grass removed, and the dry weight recorded. Finally, each sample was sieved (2mm) and homogenised before storage in labelled polythene bottles.

## 2.3 Extraction of Humic Substances from Soils and Sediments

### 2.3.1 Alkaline Extraction

The objective of this part of the study was to extract samples of the bulk humic substances from each soil or sediment. As discussed earlier (section 1.4.5), an ideal extraction of humic substances should allow the isolation of organic matter which is representative of the entire molecular weight range of the humic material. Ideally, the solvent used should effect complete dissolution of the humic fraction of soils and sediments by disrupting existing hydrogen bonds and providing alternative groups to form humic-solvent hydrogen bonds. In aqueous solvents, the dissociation of functional groups including carboxyl and phenolic hydroxyl results in the partial dissolution of humic macromolecules. The solvation of less hydrophilic macromolecules requires the formation of hydrogen bonds with solvent molecules.

As discussed in section 1.4.5.1, aqueous solutions of NaOH have predominantly been used as the solvent for this purpose since they extract the greatest amount of humic material from soils (in total, about two-thirds of the total soil organic matter (Stevenson, 1982)). Alkaline solutions, however, do extract some non-humic organic material and it is estimated that this comprises about 5% of the total extract from some surface soils (Stevenson, 1982). 0.1M NaOH has been suggested by some workers (Levesque and Schnitzer, 1966) to extract the highest amounts of carbon and nitrogen although they claim that humic and fulvic acids prepared following 0.5M NaOH extraction have a lower ash content. It has, however, been shown that alkaline extraction results in the oxidation and possibly the breakdown of the humic macromolecules in the presence of oxygen (Swift and Posner, 1972) and this effect is greater when the extractant is 0.5M NaOH. This effect is lessened by approximately one order of magnitude by the use of 0.5M $Na_2CO_3$ instead of 0.5M NaOH (Bremner, 1950) indicating that the uptake of oxygen is elevated at higher pH (Table 1.15). 0.5M $Na_2CO_3$ is markedly less efficient at extracting humic material from soils. Table 2 also suggests that uptake of oxygen may be increased at higher ionic strength. Prolonged exposure of humic materials to alkaline solutions, on the timescale of days, has also been shown to alter the composition of the humic fraction (Tipping, 1984). It is therefore essential that extraction can be completed over a short time period. (Further discussion of other extractants can be found in section 1.5.3).

To achieve the most efficient but least harmful extraction of humic substances, 0.1 M NaOH was used as the extractant in the initial phases of this study. Later 0.5 M $NH_4OH$, (pH 11.5) was found to be a preferable extractant due to:

i) the lower pH of extraction,

ii) efficient dissolution of humic material and associated actinides and

iii) reduced potential matrix interference in subsequent analyses

but was used only in the later stages of the study. [Its use as an extractant or eluent is indicated at appropriate stages in Chapter 3]. Extraction was achieved by the respective addition of 200ml or 2000ml aliquots of dilute alkali to 50g soil or 500g sediment (ie.1:4 g/ml) . These experiments were not carried out in a $N_2$ atmosphere since it has been shown that the uptake of alkali from solution in the presence of oxygen is not eliminated by its use and instead the use of lower ionic strength and lower pH values has been implemented to minimise uptake of oxygen by the humic material. The most important factor, given present knowledge, is the reduction of the time of exposure to the alkaline solution. The sample was dispersed in alkali by continuous mixing using a magnetic stirrer. At half hour intervals the aqueous phase was removed and a further aliquot of alkali was added. The solution containing the humic material was centrifuged for 40min. (4500rpm; 3637g) and the supernatant transferred to a second set of centrifuge bottles and centrifuged for a further 40min. in order to maximise the removal of suspended inorganic particulate material. The supernatant was then retained for dialysis. This procedure was repeated until the alkaline aliquots were colourless and exhaustive extraction of the humic fraction had, therefore, been achieved. The number of aliquots required to achieve complete extraction varied between samples and was, as would be expected, dependent on the organic content of the soil or sediment. All extractions were completed within an eight hour period and, following mixing of all extracts, the pH lowered to 7 by dialysis (tubing : M.Wt. cut off = 1000 daltons) against distilled water. Where possible the dialysis water was changed at half hour intervals (with the exception of the overnight period) and the total procedure, therefore, was completed in a period of 30 hours. Exposure to the highest pH values, however, need only extend over about ten hours.

### 2.3.2 Acid Precipitation of Humic Acid

As described in section 1.4.5.2, humic materials from soils and sediments are traditionally fractionated on the basis of their solubility in acid and alkali. Humin is the portion of the high molecular weight organic material which is totally insoluble in both acid and alkali. The humin fraction is less commonly analysed due to its intractability. Humic acid is designated the portion soluble only in alkali whilst fulvic acid is soluble in both acid and alkali.

Initially in this study, humic acid and fulvic acid were prepared from the alkaline extract (0.1M NaOH) by addition of 12M HCl until the pH dropped from 13 to 1, causing the precipitation of humic acid and leaving fulvic acid in solution.

Humic and fulvic acids were then separated by centrifugation (40 minutes, 4500rpm; 3637g) and the solid humic acid washed with 100ml distilled water and centrifuged for a second time. The supernatant water was discarded and the humic acid resuspended in distilled water prior to freeze drying.

At a later stage in the project, as discussed in detail below (section 3.5), it was concluded that the acid precipitation described above caused unacceptable alteration of the humic substances. It was therefore decided to discontinue acid precipitation and later work was carried out using humic substances. These were isolated by alkali extraction and centrifugation to remove suspended mineral matter (as described above) and then dialysed to lower the pH to approximately 7.

### 2.3.3 Dialysis of Fulvic Acid and Humic Substances

The combined extracts were transferred to dialysis tubing (Medicell size 10 dialysis tubing) and dialysed against distilled water until the pH of the solution inside the dialysis bag attained a value of 7. This ensures the loss of small, soluble, non-humic components which were co-extracted, the reduction in salt content and the removal of excess alkali required prior to freeze-drying, actinide analyses and spectroscopic investigations. Little colour was transferred to the dialysis water indicating minimal loss of humic material.

Whilst a humic/fulvic separation was being used, the fulvic acid remaining in solution after acidification was transferred to dialysis tubing and dialysed against distilled water (in order to reduce the $Cl^-$ ion content of the solution) until the pH of the solution inside the dialysis tubing reached approximately 5 [pH of available distilled water was approximately 5]. Under the same conditions as above (ie. same frequency of water changes), more colouration of the dialysis water was observed. Previous studies (Livens, 1989) included the analyses of the most coloured dialysis water and calculations show that a small proportion of the actinide species may be lost.

### 2.3.4 Freeze Drying of Humic and Fulvic Acids and Humic Substances

Freeze drying has several advantages over other preconcentration methods. [The use of ammonium hydroxide as the solvent is most effective in providing a final humic product with little inorganic residue and is therefore preferred both for extraction purposes and at later stages in the analysis.] However, freeze-drying results in the concentration of all non-volatile solutes so that dialysis of fulvic acids and humic substances is essential in order to avoid exposure to concentrated alkali. In addition, freeze drying of humic acid under acidic conditions is known to result in charring of the humic acid and to lead to chemically induced artefacts in subsequent analytical studies.

### 2.3.5 Ash Content of Humic Substances

The method for extraction of humic substances involving only alkaline dissolution followed by dialysis and freeze drying provides a potentially less altered humic material (see section 1.4.5). An indication of the contaminant content was obtained by determining the ash content of the bulk humic material. A weighed amount of humic material was ashed at 550°C overnight and the residue weighed. FTIR spectroscopy of the bulk humic material (see KBr disc preparation) was also used to detect the presence of silica associated with the humic sample.

## 2.4 Determination of Humic Content of Soils, Total Organic Content and Percentage Humic Association of Radionuclides

### 2.4.1 Organic Content of Soils and Sediments

Total organic content of the soil sample was estimated from mass loss on ignition. 1-5g soil was placed in a preweighed platinum crucible, and heated overnight at 550°C and then reweighed. This process was repeated until the sample was of constant weight.

### 2.4.2 Humic Content of Soils and Sediments

Total humic content was measured by exhaustive extraction of humic substances from 1g of soil, using the extraction methodology discussed earlier (section 2.3.1). This was achieved by repeated extraction using consecutive 20 ml aliquots of alkali until the supernatant after centrifugation was colourless. The residue was washed with distilled water, centrifugation repeated, the supernatant removed and the residue then air-dried and reweighed. The loss in mass was taken to represent the amount of humic substances present in the soil.

## 2.5 Gel Chromatographic Fractionation of Humic Substances

The objectives of this part of the study were initially to optimise the procedures used for gel chromatographic fractionation of humic materials and to provide fractions of humic substances which were more easily analysed and characterised than bulk humic substances. A single interaction of actinides with humic substances is an assumption implicit in many models which incorporate organic species. Therefore the aims were also to investigate actinide distribution within the humic fraction following the analysis of individual fractions and to chemically characterise analogous fractions using a range of spectroscopic techniques in order to ascertain the validity of the above assumption.

### 2.5.1 Theory of Gel Column Chromatography

Size exclusion chromatography can be subdivided into gel filtration and gel permeation chromatography where the former uses aqueous solvents and hydrophilic packings whilst the latter is based upon non-polar organic solvents and hydrophobic packings. Gel filtration is commonly applied to the separation of high molecular weight, natural product molecules from lower molecular weight species and from salts (group separations) but also for the fractionation of different high molecular weight species where size differs by greater than 10%.Separation of different solutes is achieved using a porous polysaccharide gel (eg. Sephadex G type from Pharmacia) packed in a chromatographic column. The chromatographic system is heterogeneous, with a porous solid phase (the gel) and a continuous liquid phase (the solvent) which fills the gel pores. Solute molecules passing through the column are, in principle, separated according to differences in size. In the simplest case, small molecules diffuse into the pores from the surrounding solution whereas larger molecules are unable to enter the pores. With the addition of eluent, the solute molecules move down through the column and large molecules move continuously down and elute first, whilst smaller molecules are delayed and elute later (Pharmacia, 1993, 1995). Molecules are thus eluted in a predictable way in order of decreasing molecular size.

### 2.5.2 Column Preparation

Sephadex G gels from Pharmacia were used in this study and a full description of their structure and physical and chemical properties is found in 'Gel Filtration' (Pharmacia, 1993,1995). The gel matrix is chosen primarily for its inertness and, in particular, lack of adsorptive interactions between the solute and the gel is an important factor. The Sephadex gel structure is produced by cross-linking of sugar chains to form a three dimensional network giving gel pores with a carefully controlled range of sizes and individual gel types have pore size ranges regulated by the degree of cross-linking of the gel. For example, gels with a high matrix content ie. highly cross-linked such as G25 Sephadex have smaller pore sizes. Sephadex gels are bead-formed gels consisting of dextran chains crosslinked with epichlorohydrin molecules. The choice of gel is determined by the size of the solute molecules but also by the chemical characteristics of the solute where interactions with the gel are likely. The more highly crosslinked Sephadex gels, G10, G15, G25 and G50 are most frequently used to separate peptides and other small biomolecules, whereas the less stable, less crosslinked gels, G75, G100, G150 and G200, are more commonly applied to the fractionation of proteins and other macromolecules. Where more than one gel type offers a suitable pore size range, an influencing factor is the time taken for elution of the solute. Less crosslinked gels have lower maximum flow rates and consequently elution of the solute is much slower. Contrarily, gels with a higher matrix content are less suitable for solutes where strong solute-gel interactions are expected. However, this apparent disadvantage may be favourable in the separation of chemically different species of similar molecular size. The retardation of aromatic species by G10, G15 and G25 gels allows the separation of aromatic peptides from other non-aromatic substances.

Optimisation of gel fractionation is therefore frequently hindered by conflicting factors. Criteria such as maximum recovery, minimum sample dilution and short elution time have to be balanced with maximum run-to-run reproducibility for analytical purposes. Band separation can be improved by lengthening the column but this results in longer elution times and greater diffusion. The effect of the latter can be reduced by using a smaller initial sample volume.

The Sephadex material is commercially available in powdered form and swells to form a gel in aqueous solution (Sephadex LH20 is specifically for use with organic solvents eg. DMF) (Pharmacia, 1993, 1995). These gels display good chemical stability and can withstand exposure to 1M NaOH or 0.1M HCl. A summary of some important gel characteristics is provided in Table 2.1 below.

| **GEL medium** | **pH Stability** (short term) | **Bed Volume** (ml/g dry gel) | **MW fractionation range** | | **Approximate max. flow rate** (ml/min.) (2.6 x 30cm column with distilled water at room temperature) |
|---|---|---|---|---|---|
| | | | Dextrans | Globular Proteins | |
| G25 | 2-13 | 4-6 | 100-5000 | 1000-5000 | - |
| G50 | 2-13 | 9-11 | 500-10,000 | 1500-30,000 | - |
| G100 | 2-13 | 15-20 | 1000-100,000 | 4000-150,000 | 4.2 |
| G200 | 2-13 | 30-40 | 1000-200,000 | 5000-600,000 | 1 |

**Table 2.1 Technical Information (Pharmacia 1995)**

The eluent chosen for the experiment is frequently used to prepare the gel column and hence to reduce equilibration time prior to fractionation of the sample.

Maximum flow rate is determined by the grade of the gel with medium and coarse grades being essential for preparative purposes where high flow rates and low operating pressures are required.

A 3.5cm diameter x 50cm length glass gel column fitted with a fine sintered glass bed support was used in this study. A coarse bed support is more easily contaminated and consequently is a source of experimental artifacts. The column and tap were constructed to ensure a small dead volume at the outlet. This is essential to prevent remixing of the separated fractions.

The gel was prepared according to the manufacturers recommendations. A slurry of the gel was prepared using the chosen eluent, 0.1M NaOH or 0.5M $NH_4OH$, and allowed to swell for 6-24h, dependent on the gel type (with the more cross-linked gels requiring the shorter time periods), ensuring that the slurry was not so thick that air bubbles were retained. When settled, the gel comprised approximately 75% of the total volume of gel and solvent together. Where the gel suspension is too thin, uneven packing of the gel column may result, leading to band broadening and a loss in resolution. The gel must not be excessively stirred as this may result in breaking of the beads (eg. use of magnetic or mechanical stirrers).

The gel column was manually packed by tilting the column and pouring the well-mixed gel suspension in a single operation down the inside wall. The column was immediately adjusted to the vertical position and the flow through the column commenced soon after filling for the best packing effect. Where the eluent has been used to prepare the gel, the column should be already equilibrated and the passage of 2-3 column volumes of eluent is undertaken only to stabilise the gel bed. It is essential, particularly for the less mechanically stable gels (G100, G150, G200), that a slow flow rate is maintained since exceeding the maximum rate readily leads to compression of the gel bed and can result in the cessation of flow through the column. The packing of the column was checked both visually for heterogeneities and air bubbles and by measurement of the void volume using 2mg $ml^{-1}$ Dextran 2000, which is characteristic of the gel type and column length/diameter. Assuming no mixing or diffusion, the void volume, $V_o$ represents the theoretical volume of solvent required to transport through the column organic molecules which are too large to enter the gel. However, due to mixing and diffusion, non-retained components appear in a Gaussian shaped band with concentration maximum at $V_o$. For components small enough to enter into all the pores, a band maximum will appear at the permeation volume of the gel, $V_i$. The total column volume is defined as the sum of the volume outside and inside the pores together with the volume of the gel, $V_g$ ie. total volume $V_t = V_g + V_o + V_i$. Gel filtration is theoretically a non-interactive technique and so all solute molecules which are not excluded due to size, should elute between the void volume and the permeation volume ie. within well-defined limits.

In order to achieve maximum run-to run reproducibility constant column conditions must be maintained with respect to column length, gel type, volume, eluent composition, and flow rate. Following this procedure rigorously enables the collection of reproducible fractions and the maximum recovery of sample. To summarise, gel filtration optimally offers :

i) short and well-defined separation times

ii) narrow bands leading to good sensitivity

iii) no sample loss ie. no interactions with stationary phase

iv) no column contamination due to interactions of the solute with the packing.

However, a few limitations are :

i) relatively short elution times (in comparison with HPLC, GC) mean that the number of bands which can be separated is small

ii) inapplicability to the separation of solutes of similar size eg. isomers.

### 2.5.3 Eluent and Sample Preparation

In many gel chromatographic experiments there are no limitations on the choice of eluent and the most suitable eluent for the overall experiment may be selected. Restrictions on the eluent composition are however required under certain conditions. Theoretically, eluent composition is unimportant for the separation mechanism where the solute molecules are separated on the basis of size. For example, uncharged molecules may be eluted using distilled water, with the limiting factor being solubility in this solvent. However, the properties of the solute molecules and to some extent also the gel matrix do result in interactions between the solute and the gel matrix. Highly acidic or basic solutes, highly charged molecules and aromatic groups interact to varying degrees with the gel at low ionic strength. Unwanted ionic interactions can be eliminated by the use of an eluent of high ionic strength (0.1M) but interactions between aromatic units and ether linkages of the gel are less easily eradicated. Again, solubility is a further limiting factor since precipitation of the solute, which can occur on the top of the gel bed or during elution, is unacceptable.

The sample volume and concentration also influence the required column length and the separation achievable. Deviations from ideal separation are also inevitable where the macromolecular shape of the solute is significantly different from the spherical structures used to calibrate the column commercially. [Further discussion of the use of gel chromatography as a technique to determine molecular weight is found in section 1.4.4.7]

In this study, 100mg of humic material was dissolved in 10ml of 0.1M NaOH or 0.5M $NH_4OH$ prior to being placed on the gel. 0.5M $NH_4OH$ was preferable for the total experiment due to the reduced residual content of fractions prepared for mass spectrometric analysis. The humic material was also more readily soluble in this eluent and no sample precipitation on the top of the gel column was evident. Considerable care was taken to avoid disturbing the surface of the gel when placing the sample on the gel bed. An uneven surface results in uneven separated bands and hence a loss of resolution. The eluent was then allowed to drain through the column until the sample moved onto the top of the gel. A small amount of eluent was then passed down the sides of the glass column above the top of the gel bed to wash any remaining solute onto the gel. The eluent was again allowed to drain before the addition of a larger amount of eluent.

## 2.6 FTIR Spectroscopy

### 2.6.1 Theory

Molecular vibrational spectra are obtained following the absorption of IR radiation. Fundamental vibrational transitions (IR absorption) relating to major functional groups occur in far to mid-IR regions (10-4000cm$^{-1}$).

Assuming anharmonicity of vibrations, absorption of energy from an IR source produces an increase in the amplitude of vibrations occurring within bonds at ambient temperature. The amplitude rapidly decreases as molecules collide with their less active neighbours. A system is never saturated with energy. There are many more inactive than active molecules. This places limitations on the technique where the sample being analysed is highly heterogeneous. The increase (and re-equilibration) is dependent on the nature of the bond and its surroundings. However, bonds of similar strength and containing similar elements can result in broadly similar spectra which are indistinguishable without additional structural information. This places bounds on the usefulness of vibrational spectroscopy.

Limitations are also imposed by the mechanism by which absorption occurs. A molecule or functional grouping must have a momentarily induced or permanent dipole for absorption. Such molecules or functional units satisfy the conditions for absorption of quanta of electromagnetic radiation.

There are two types of vibration which can occur within a bond

i) stretching and ii) bending (deforming). Bending requires less energy therefore bending modes occur at lower frequencies than corresponding stretching modes. Polyatomic molecules contain many bonds and so numerous vibrational opportunities exist.

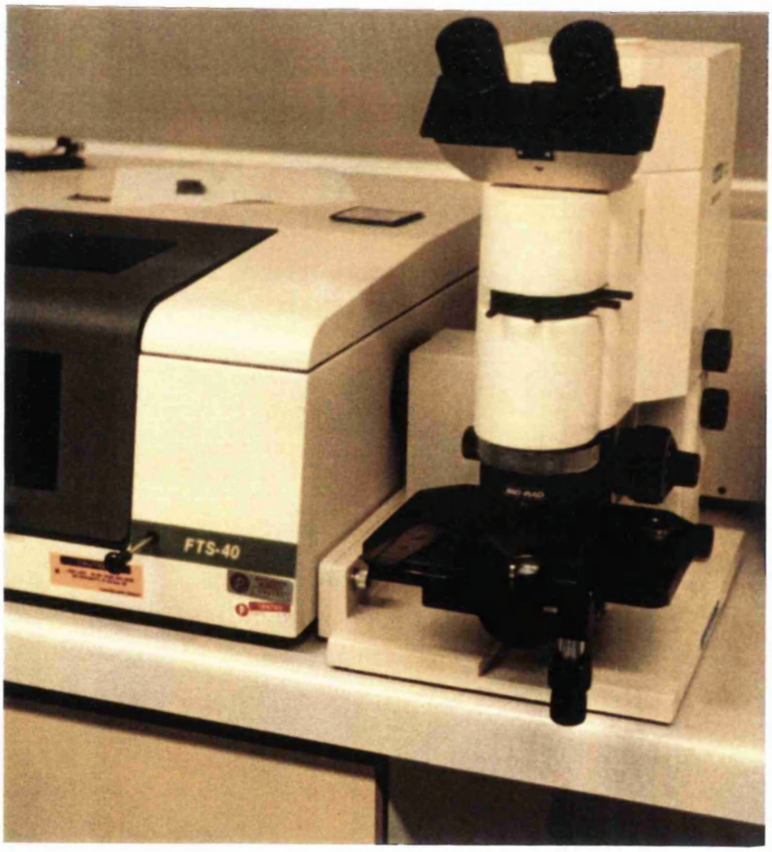
When the vibrational energies of two adjacent bands are very similar they may interact mechanically and couple. If coupling is strong enough, the original fundamental bands may be lost and new bands produced. Occasionally, overtones at $2\nu$ and $3\nu$ appear as additional bands in the spectrum. Coupling can also occur between overtones and suitable fundamental vibrations. This phenomenon is described as Fermi resonance.

FTIR spectroscopy is an appropriate tool in the structural characterisation of both pure organic chemicals and mixtures of natural or synthetic organic compounds since it requires little sample preparation and offers non-destruction of the sample prior to analysis, short analysis time and high sensitivity. Modern high-power infrared spectrometers use a single broad beam source. The output contains all the frequencies of interest. Previously, several monochromatic sources were used to scan over a range of frequencies giving the output at any one time related only to the specific scanning frequency. The most recent FTIR spectrometers incorporate an advanced scanning principle which allows the collection of double-sided interferograms in both the forward and backward scanning directions. This results in a high efficiency of data collection. A water cooled silicon carbide glowbar source and high optical throughput design produce large signal levels and therefore high sensitivity. The detector which contains a low noise amplifier allows good signal to noise ratios to be achieved.

The detected signal is digitised by an analogue to digital converter. The information in the single beam is processed using an interference effect. It is known that interference alters the amplitude of the total radiation reaching the detector as a function of time. Absorption components can be distinguished on this basis. Data acquisition and Fourier Transform is performed using an Acquisition Processor (AQP) card within the PC (connected to the spectrometer). Fourier Transform is the mathematical manipulation which converts the record of amplitudes into desired spectra. Fourier Transform is sometimes referred to as Fourier Inversion, the reason for this being the inverse relationship between time and frequency.

The technique of FTIR spectroscopy is advantageous over traditional IR techniques in that high speed multiple scans of the spectrum are made electronically. Data obtained from many individual runs are accumulated within the computer. The background can also be subtracted before the spectrum is printed. A high degree of sample throughput can be achieved since the multiprocessing capability of the PC enables the processing of previously acquired spectra whilst the AQP is collecting and processing new data. FT-IR spectroscopy is a relatively expensive technique but it can produce high definition spectra quickly and typically requires less than 1mg samples. It is particularly useful for the analysis of compounds being eluted in rapid succession from chromatographic columns. On-line use of FTIR spectrometers in association with chromatographic systems may minimise the potential for the creation of artifacts and has been used in studies of humic substances involving HPLC fractionation. In this study, off-line FTIR spectrometry was used in combination with gel chromatography which is more time consuming since the procedure requires the preparation of KBr discs following solvent removal prior to spectroscopic analysis. Additionally, these provided more structural information than solution phase spectra, where fine structure was obscured by the presence of broad water peaks.

In addition to FTIR spectroscopy, samples were also characterised using FTIR microscopy in reflection mode. The external coupling of a microscope to the main spectrometer promotes the analysis of extremely small samples. A spectrum can now be attained from sample sizes down to the diffraction limit of the probing radiation. The IR measurement is performed in the same manner as conventional optical microscopy. The optic mode was used for visual inspection of the sample. The sample was placed on a gold coated glass microscope slide and was observed as a bright image through the binocular objectives. Focusing is achieved in the optic mode using the coarse and fine adjustment of the microscope stage. The sample can be moved in the x and y directions using appropriate adjusters. The IR beam from the spectrometer optics follows the identical path to the light and the microscope was manually switched between optic and IR measurement mode. In the IR mode the beam passes through a small area (<100 micron) of the sample and is then directed onto a high sensitivity liquid nitrogen cooled detector. Conventional objectives display limited transparency to IR radiation, so all-reflecting optical devices are used in the IR microscope. One specific application of FTIR microscopy is the examination of small areas in larger samples. This is suitable for the determination of impurities on sample surfaces but is also applicable to the investigation of heterogeneous solids such as humic substances.


FTS-40
BIO-RAD

### 2.6.2 KBr Disc Preparation

For this study, carefully dried spectroscopic grade KBr was used for disc preparation. The preparation of KBr pellets is frequently used for FTIR spectroscopic studies of solid samples and KBr in pressed disc form offers a wide spectral range (4,000-385cm$^{-1}$) but is hygroscopic and requires careful storage. The discs are also brittle, although they display higher resistance to mechanical shock than other matrices used in disc preparation. Disc preparation is, however, time consuming and it is essential to exclude moisture at every stage from the KBr due to interference at important frequencies.

Spectroscopic grade KBr was initially ground to form a uniform powder and stored in a drying oven (60°C). The sample of humic material (bulk or fraction) was freeze dried and stored in a dessicator prior to use. A weighed amount of the sample (approximately 0.7mg) was ground to form a fine powder and was uniformly dispersed in approximately 200mg powdered, dried KBr using an agate pestle and mortar. The mixture was placed inside an evacuable die and was pressed between a highly polished anvil and plunger to produce pellets of highly uniform quality. The die was placed in a manual hydraulic press (10 ton) and a vacuum hose is attached to the die. The chamber is evacuated while the die is in the press and a 13mm clear disc is formed after approximately 2-3min. (Evacuation of the chamber is not essential but it is reported that this results in the formation of higher quality, longer lasting discs.)

### 2.6.3 Au Slide Preparation

A 0.5M $NH_4OH$ solution of humic substances (bulk or fractions) was used to provide a dry sample with minimal non-humic residue. 0.1M NaOH is an unsuitable solvent due to the formation on drying of residual salts, in particular carbonates. A capillary tube containing the solution was used to place a small drop of the sample on the Au plated slide. The droplet was air dried. A sample can be built up in layers by the careful addition of further droplets if a greater sample mass is required. The method is at present non-quantitative since the mass of sample in a droplet is unknown.

### 2.6.4 Operating Conditions

A Bio-Rad 2000 FTIR Spectrometer was used under clean room conditions. The sample chamber was flushed with a scrubbed air supply until the spectrum stabilised prior to switching the spectrometer to scan mode. 16 scans of the sample were combined and a background subtracted to give the final stored spectrum. The FTIR microscope is also flushed with a scrubbed air supply, although the sample is not held internally. Clean room conditions are therefore essential for the operation of the microscope.

## 2.7 UV/Visible Spectroscopy

### 2.7.1 Theory

The visible and UV spectra of organic compounds result from transitions between electronic energy levels induced by the absorption of UV or visible light of the appropriate energy. Transitions generally occur between bonding or lone pair orbitals and an unfilled or anti-bonding orbital and the wavelength of absorption therefore is a measure of the separation of the energy levels of these orbitals. Absorptions at >200nm are the result of excitation of electrons from p and d orbitals and $\pi$-orbitals and particularly $\pi$-conjugated systems. These transitions are most readily measured and most informative with respect to structural elucidation and comprise the dominant absorptions resulting from the irradiation of humic substances with a UV/visible source.

The spectrometer contains two sources : one of white visible light and the other of white UV light. During the scan, a change over from the white light source to the white UV source occurs in order to give the complete absorption spectrum over the range 900-200nm. An absorption line is not observed; the spectrum is generally broad as a result of vibrational and rotational fine structure and is further smoothed due to interactions of the solute with solvent molecules.

The cells used in UV spectroscopy are constructed so that the beam of light passes through a 1cm thickness of solution. Beer's Law states that absorption is directly proportional to the number of absorbing molecules.

$$\text{Absorption} = \log_{10}(I_0/I) = \epsilon cl$$

and where path length, l = 1cm

$$\text{Absorption} = \epsilon c$$

This is important with respect to the comparison of humic substances, humic acids and fulvic acids since the absorptions of the component humic acid and fulvic acid would be expected to be additive to give the absorption spectrum of humic substances. The relationship between absorption and concentration for UV absorption is not a property of IR absorption and therefore UV measurements within the context of this study are particularly valuable in providing information complementary to that obtained from FTIR spectra of the same materials.

### 2.7.2 Sample Preparation

It is essential that all samples are prepared using identical solvent conditions since the position of the absorption maximum UV/visible spectrum varies with pH and ionic strength. Suitable solvents contain no chromophores absorbing in the region important for humic macromolecules, 400-700nm. 0.5M $NH_4OH$ was used throughout since it satisfies the above criterion and was already the sample medium following gel chromatographic fractionation. The fractions were prepared directly following elution from the gel column. The undiluted samples were placed in UV cells and the absorption intensity at 465nm and 665nm measured. A matched cell containing pure solvent is also placed in the appropriate place within the sample chamber adjacent to the sample cell. The simultaneous measurement of the absorption spectra from each is followed by the subtraction of the solvent spectrum from that of the sample. It is essential that the sample is not exposed to the UV source for prolonged time periods since the energy associated with the UV source is sufficient to induce chemical reactions within the sample ($E(kJ/mol) = 1.19 \times 10^5/\lambda(nm)$ ; $\lambda = 297nm \Rightarrow 400kJ$). Samples exposed to UV/visible radiation in this manner were not used for further analyses.

## 2.8 Fluorescence Spectroscopy

### 2.8.1 Theory

The presence of certain chromophores in organic molecules enable these compounds to fluoresce. The functional groups which are responsible for the fluorescence properties of the molecules also absorb UV radiation. Where uncertainty arises in the interpretation of the UV spectrum of humic substances, complementary information can be obtained from their fluorescence spectra. The incident light is first passed through a monochromator before entering the sample. Excitation of the sample molecules at the chosen wavelength is followed by re-emission at longer wavelengths in all directions. The measurement of the emitted light is made at an angle, generally 90°, not in line with the source-sample axis. Fluorescence emission intensities may increase linearly with concentration up to a factor of approximately $10^5$. However, self absorption at higher concentrations is a problem for certain samples and is related to the structure of the molecules. This results in the emission of less light at higher concentrations than expected by extrapolation of results from lower concentrations but can be overcome by dilution of the sample. In the context of this study, the more highly condensed the humic macromolecular structure, the more highly quenched the emitted signal and therefore this technique can be used to validate interpretations based on $E_4/E_6$ ratios. In other words a low $E_4/E_6$ should be accompanied by a highly quenched fluorescence spectrum. This can be investigated by decreasing the concentration of humic substances in the solution prepared for fluorescence. Similarly, aromatic subunits result in the increase of the fluorescence intensity and this information is extremely valuable in combination with other spectroscopic techniques.

### 2.8.2 Sample Preparation

A tenfold dilution of the humic substance fractions was carried out prior to measurement of their fluorescence spectrum. For humic acids a hundred fold dilution was required in order to reduce the extensive quenching observed after only a tenfold dilution. The humic fractions were placed in cells identical to those used in UV/visible spectrometry and oriented correctly inside the sample chamber.

### 2.8.3 Operating Conditions

The excitation of the macromolecules was carried out at 230nm and an emission spectrum obtained. A filter at 700nm were used to eliminate emission from the solvent.

## 2.9 CHN Analysis

### 2.9.1 Theory

The total %C, H and N contents of freeze-dried humic fractions were determined by CHN analysis (Perkin Elmer 2400 CHN Elemental Analyser) using the facility at the University of Manchester. The technique involves the complete combustion of typically 1-2 mg quantities of sample in the presence of an excess of oxygen and combusting agents. The combustion products, $CO_2$, $H_2O$ and $N_2$ gases (and $SO_2$), are homogensised in the gas control zone before being fractionated using frontal chromatography and their stepwise detection using a thermal conductivity detector. Oxygen content was determined by difference.

### 2.9.2 Sample Preparation

Humic fractions obtained using gel chromatography were dialysed and freeze dried. 2 mg samples were placed in polythene vials and sent for CHN analysis.

## 2.10 Amino Acid Analysis

### 2.10.1 Theory

Amino acids and their derivatives can be separated and identified using GC/MS which has been calibrated using available standards. A DB$^{TM}$-5ms column (0.25mm i.d.; 0.25μm film thickness; 30m length) was used in this study. This is a capillary column which is composed of three parts : i) fused silica tubing ii) external polyimide coating iii) internal chemically bonded stationary phase. The stationary phase in this column type is a silicone polymer where the siloxane backbone has phenyl groups bonded to 2.5%, by number, of the silicon atoms; the remaining 97.5% of the silicon atoms have methyl groups bonded to them. This is a widely used column type and is applicable to the separation of a wide range of samples including amino acids.

### 2.10.2 Sample Preparation

Humic fractions obtained using gel chromatography were freeze dried prior to amino acid analysis. 0.5M $NH_4OH$ is a suitable solvent for samples being prepared for amino acid analysis due to its volatility and consequently little non-humic residue remains on freeze-drying. The method of preparation of humic substances for amino acid analysis is outlined below.

Following the removal of alkaline solution the samples are hydrolysed using 6M HCl and the residues of amino acids separated from the remainder of the sample by solvent extraction. The amino acids were converted to tert.-butyldimethylsilyl derivatives before analysis.

## 2.11 Radioanalytical Procedures

### 2.11.1 α-Spectrometry

Silicon surface barrier detectors were used in the α-spectrometric determination of U, Th, Am and Pu in samples of bulk soils and humic extracts. The appropriate alpha energies are listed in Table 2.2.

| Nuclide | α-Particle Energy (MeV) | Nuclide | α-Particle Energy (MeV) |
|---|---|---|---|
| Th-228 | 5.424 | ($^{237}$Np | 4.872) |
| Th-230 | 4.684 | $^{238}$Pu | 5.499 |
| Th-232 | 3.994 | $^{239}$Pu | 5.157 |
| U-232 | 5.32 | $^{240}$Pu | 5.168 |
| U-234 | 4.773 | $^{242}$Pu | 4.903 |
| U-235 | 4.597 | $^{241}$Am | 5.545 |
| U-238 | 4.195 | $^{243}$Am | 5.35 |

**Table 2.2 Alpha Particle Energies of Selected Actinide Elements**

Each detector consists of a thin wafer of Si containing junctions of n and p-type material across which a reverse electrical potential is applied. A thin layer of gold is evaporated onto one side and provides the positive electrical contact whilst aluminium deposited on the reverse side provides the negative contact. Radiochemical separations followed by electrodeposition onto metal planchettes provided suitable thin sources for α-spectrometry. The planchettes were placed close to the detector and the chamber pressure reduced to < 100 mbar before collection of the $\alpha$-spectrum. Background spectra were obtained in a similar manner by placing a blank planchette in front of the detector. A standard source ($^{244}$Cm, $^{241}$Am and $^{239}$Pu) was used to produce a three point energy calibration for each detector. The resolution of the detectors was approximately 20keV (FWHM) but peak widths are generally greater than this depending upon the source thickness.

### 2.11.2 ICP-MS and ETV-ICP-MS

A VG Plasmaquad PQ1 instrument was used to determine the masses of $^{238}$U and $^{232}$Th in humic extracts from soils.

The attachment of a quadrupole mass spectrometer to an inductively coupled plasma source is a relatively new analytical technique for quantitative and semiquantitative trace and ultra-trace multielement analysis. Plasma sources are commonly used in emission spectroscopic techniques with the plasma being formed when argon gas is ionised to give argon ions and free electrons in the gaseous phase. The excitation and acceleration of the free electrons results in the production of heat following the increased probability and rate of collisions. Temperatures in the hottest part of the plasma are calculated to be as high as 10,000K. The energy input is from a radiofrequency source at the base of the plasma plume and, since not directly connected, the plasma is described as inductively coupled. The plasma is suspended away from the silica glass walls of the torch chamber through a combination of the coolant gas flow and the shape of the radiofrequency electromagnetic field. Figure 2.3 is a diagrammatic representation of the instrumentation. The plasma itself is hollow and the analyte is sprayed into this hollow region in the form of an aerosol. The introduction of a sample into the plasma results in the formation of predominantly singly charged ions in the gas phase. The analyte ions are sampled using a skimmer cone and focussed using a lens stack before entering the quadrupole magnetic field. The ions reaching the spectrometer are predominantly detected at their mass number. The instrument is constructed of the inductively coupled plasma and a mass spectrometer, the latter operating at 3.7x10$^{-6}$ mbar whilst the ICP operates at atmospheric pressure. The pressure is reduced across the ion sampling interface which consists of a sharp angled skimmer cone located behind a shallow, water-cooled sampling cone. The skimmer cone has an aperture of 0.75mm whilst the sampling cone has a 1mm aperture and both are made from highly durable titanium nitride-coated nickel. The sampling cone is located at a distance of 10mm from the plasma. The pressure behind the sampling cone (2.7mbar) is maintained by a rotary vacuum pump and the intermediate pressure behind the skimmer cone is further reduced by a vapour pump (10$^{-4}$mbar). The rotary pump removes most of the gas associated with the sample and only 10$^{-4}$% passes through the skimmer cone. The expansion of the gas in the zone between the sampling cone and the skimmer ensures that no reactions occur between the plasma species due to their increased mean path, therefore maintaining the composition of the sampled gas.

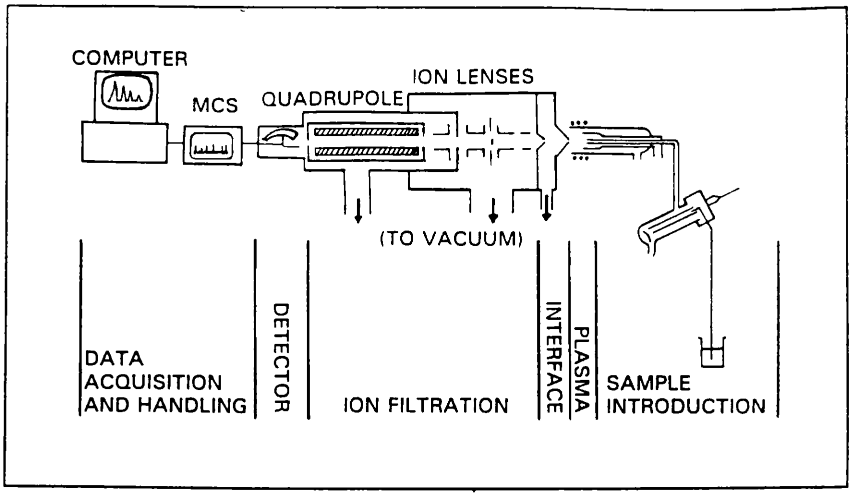


Figure 2.3 Schematic Diagram of an ICP-MS Instrument (Ross, 1993)

### 2.11.3 U and Th Analysis

#### 2.11.3.1 Preparation of Soils

5g soil samples were accurately weighed and placed in a muffle furnace at 550°C overnight. The soil was then transferred to a platinum crucible containing sodium carbonate and sodium peroxide and heated constantly for 10 minutes to form a flux which was allowed to cool before immersion of the crucible together with the sample in a 250ml beaker containing 50ml of distilled water to which 50ml of 12M HCl was added. $^{232}U/^{228}Th$ spike was added to the solution which was then refluxed for 2-3 hrs and subsequently allowed to cool before removal of the platinum crucible. The crucible was washed with 9M of HCl and the washings added to the solution which was then filtered using GF/C filters. The residue on the filter paper was washed with 12M HCl and the washings and solution collected in a 500ml flask. The solution was transferred to a 250ml beaker, reduced to near dryness and redissolved in a minimum volume of distilled water. 100ml of 12M HCl was added and the solution allowed to stand for 2-3 hours before refiltering using Whatman 541 ashless filter paper to remove precipitated salts. The filtrate was washed with 12M HCl and the combined solutions transferred to a clean 250ml beaker and reduced to near dryness. The sample was redissolved in 9M HCl. A DIPE extraction of Fe (40ml aliquots of acid conditioned di-isopropyl ether) was repeated until the remaining solution was pale. The solution was initially gently heated and 2ml of $H_2O_2$ added before heating to near dryness and redissolution in 9M HCl.

#### 2.11.3.2 Preparation of Fractions of Humic Substances

Fractions of humic substances obtained by gel chromatographic separation were placed in 100ml beakers and appropriate spike solutions added. 10ml of 16M $HNO_3$ was added and the solutions taken carefully to dryness. This process was repeated until a small white precipitate remained. The sample was then redissolved in 9M HCl for radioanalytical separations and α-spectrometry or dissolved in 0.3M $HNO_3$ with the addition of $^{236}U$ for analysis by ICP-MS.

### 2.11.3.3 U and Th analysis

2ml of $H_2O_2$ was added to the 9M HCl solution which was heated gently before being applied to a conditioned anion exchange column and a further 40ml of 9M HCl passed through the column. The combined effluent comprises the Th fraction and was retained for further chemical treatment. U was eluted with 75ml of 1M HCl and the solution evaporated almost to dryness. The sample was redissolved in 50ml of 9M HCl and passed through a second anion exchange column. U was again eluted with 75ml of 1M HCl and, following the addition of 2ml 5% m/v $NaHSO_4$, the solution was evaporated to dryness. 2ml of 12M $HNO_3$ was added and the sample taken to dryness. This was repeated with 2ml of 12M HCl. The sample was then prepared for electrodeposition (see section 2.11.7).

### 2.11.4 Pu and Am Analysis

2ml of 16M $HNO_3$ was added to the prepared 9M HCl solution prior to application to a conditioned anion exchange column (described in Table 2.3) and a further 40ml of 9M HCl passed through the column. The combined effluent comprises the Am fraction and was retained for further treatment. The anion exchange column was then washed with 100ml of 8M $HNO_3$ and 50ml of 12 M HCl. Pu was eluted with 50ml of 0.1M HI/11.8M HCl. 2ml of 5% m/v $NaHSO_4$ was added before the solution was reduced carefully to dryness. 2ml of 16M $HNO_3$ was added and the solution again taken to dryness. This was repeated with alternately 2ml 12M HCl and 2ml 16M $HNO_3$ until a small white precipitate remained. The sample was then prepared for electroplating (see section 2.11.7). The retained Am solution volume was reduced to approximately 5ml and diluted to 50ml with distilled water and applied to a conditioned cation exchange column (described in Table 2.3). The column was washed with 80ml of 0.5M HCl and 80ml of 2M HCl before Am was eluted with 200ml of 12M HCl. The Am solution volume was reduced to 5ml and diluted to 40ml with distilled water. 1ml of 1mg ml$^{-1}$ iron chloride solution was added and the pH adjusted to 7 by the addition of ammonia solution. The precipitate was retained following centrifugation and washed with water. After centrifuging for a second time the precipitate was redissolved in 20ml of 93% $CH_3OH$/1M $HNO_3$ and applied to a conditioned anion exchange column (see Table 2.3). A further 80ml of 93% $CH_3OH$ was passed through the column followed by 80ml of 0.1M HCl/0.5M $NH_4SCN$/80% $CH_3OH$. Am was eluted with 80ml of 0.5M HCl. The solution volume was reduced to 20ml and four 5ml aliquots of 16M $HNO_3$ were added. The first aliquot was added dropwise. The solution volume was reduced to near dryness and the sample redissolved in 20ml of 8M $HNO_3$ before being passed through a second anion exchange column. A further 40ml of 8M $HNO_3$ was passed through the column and the combined effluent retained. 2ml of 5% (m/v) $NaHSO_4$ was added to the solution which was evaporated slowly to dryness and excess ammonia added before redrying and the addition of 2ml of 12M HCl. The solution was then taken to dryness and prepared for electrodeposition.

| **Column** | **Element** | **Solution** |
|---|---|---|
| Anion (AG1x8) 100-200mesh Chloride Form (10cm x 1cm diam.) | Uranium | 60ml of 1.2M HCl followed by 60ml of 9M HCl |
| Anion (AG1x8) 100-200mesh Chloride Form (10cm x 1cm diam.) | Thorium | 80ml of 8M $HNO_3$ |
| Anion (AG1x8) 100-200mesh Chloride Form (8cm x 1cm diam.) | Plutonium | 60ml of 9M HCl |
| Cation ( 50W) Hydrogen Ion Form (8cm x 1cm diam.) | Americium | 80ml of 0.5M HCl |
| Anion 1 (AG1x8) 100-200 mesh Chloride Form (8cm x 1cm diam.) | Americium | 80ml of 93% Methanol/1M $HNO_3$ |
| Anion 2 (AG1x8) 100-200 mesh Chloride Form (6cm x 1cm diam.) | Americium | 60ml of 8M $HNO_3$ |

**Table 2.3 Conditioning of Anion and Cation Exchange Columns**

### 2.11.5 Np Analysis

#### 2.11.5.1 Preparation of $^{239}$Np Spike for the Determination of Chemical Yield

An 8M solution containing approximately 800Bq of $^{243}$Am provided a suitable activity of $^{239}$Np for the determination of chemical yield. The ingrowth of $^{239}$Np from $^{243}$Am ($t_{½}$ = 7380 y) in accordance with the 2.35d half-life of the daughter enabled the repeated removal of approximately 1 Bq of $^{239}$Np after 7 days. Hydroxylamine hydrochloride to adjust the oxidation state of Np was added to the Am/Np solution before 10ml of TTA (1M TTA in toluene). The solution is shaken continuously for 15 minutes resulting in the extraction of >99% of Np into the organic layer. Back extraction of Np using 10ml 8M $HNO_3$ was achieved by continuous agitation of the layers for 1-2minutes. The activity of the $^{239}$Np in 10ml 8M $HNO_3$ was determined using a Ge-Li detector. The gamma spectrum of $^{239}$Np contains 6 main peaks. Interferences present at 106 keV mean that the peaks at 228 keV and 277 keV are the most suitable for calculating the activity of $^{239}$Np. The solution was divided into two weighed fractions before addition to humic samples.

#### 2.11.5.2 Isolation of Np from Fractions of Humic Substances

Digestion of the samples was carried out as described in section 2.11.3. The digested sample was redissolved in 9M HCl and the oxidation state adjusted using 2ml 16M $HNO_3$ before application to a conditioned anion exchange column (conditioned as described in section 2.11.4). The column was washed with 9M HCl and the combined effluent may be retained for the subsequent measurement of Am. The column is washed using 200ml of 8M $HNO_3$ in order to elute U prior to the addition of 50ml 12M HCl and in each case the washings were discarded.

Np is eluted using 50-100ml of 5M HCl (experiments showed that virtually all of Np is eluted with 50ml of 5M HCl). The use of 5M HCl results in minimal co-elution of the remaining U. The solution was carefully reduced to dryness and redissolved in 10ml of 8M $HNO_3$ before gamma counting in standard counting vials. The sample was then carefully redried before redissolution in 1ml of 0.32M $HNO_3$ as required for aspiration into the ICP-MS via an ETV inlet. The samples were analysed using the VG PQ2 ETV-ICP-MS facility at the Jeffrey Schofield Laboratory at Westlakes, W Cumbria. A standard solution of $^{237}$Np was analysed after every sample and used to ensure the stability of the mass spectrometer and ensuring the accuracy of the determination of $^{237}$Np in the samples.

### 2.11.6 Measurement of Mass Balance for Gel Chromatography followed by Analysis by ICP-MS

The $^{238}$U and $^{232}$Th content of duplicate 2mg samples of bulk humic extracts were analysed by ICP-MS to calculate the total mass of $^{238}$U and $^{232}$Th in 100mg humic samples prior to gel chromatographic fractionation. The total mass of $^{238}$U and $^{232}$Th in the fractions was then calculated and compared with the respective totals for the bulk to give a measure of the retention of humic associated $^{238}$U and $^{232}$Th on the gel column.

### 2.11.7 Electrodeposition

The electroplating equipment consisted of a plastic tube mounted on a copper base (detachable) which supported the polished steel planchette. The seal between the plastic tube and the base was maintained by a rubber O-ring. Samples were dissolved in 10ml of 1M $NH_4Cl$ and placed in the electroplating chamber. The sample container was washed with a further 20ml of the plating solution which was then added to the chamber. A platinum electrode mounted to a plastic lid was then lowered into the chamber. Electrodes attached to the power supply were connected negative terminal to the copper base and positive terminal to the platinum electrode. The plating current was maintained at 3A whilst the voltage was approximately 15V for the period of electrodeposition. Electroplating times for each element are listed in Table 2.4.

| **Element** | **Time** |
|---|---|
| Uranium | 2 hours |
| Thorium | 3 hours |
| Plutonium | 2 hours |
| Americium | 3 hours |

**Table 2.4 Length of Time Required for Electrodeposition of Actinide Elements**

## 2.12 Measurement of U and Th associated With Humic and Fulvic Acids with Variation in pH

10ml solutions (pH 10) of bulk humic substances were placed in glass beakers and the pH adjusted using 12M HCl to specific values over the range 0.4 to 3.2. The humic and fulvic fractions were separated after centrifugation and analysed separately for $^{238}U$ and $^{232}Th$ by ICP-MS. $^{236}U$ was added as an internal standard.

## 2.13 Reference Materials

There are no widely accepted reference materials for determining the accuracy of actinide measurements in a humic matrix. In order to minimise the potential for obtaining inaccurate results with respect to the elution patterns, certain elution patterns were duplicated using separate portions of humic material and a comparison was made between results obtained using ICP-MS and α-spectrometric techniques. Additionally, ICP-MS samples were run in a nonconsecutive order (relative to gel chromatographic elution).

The precision of the ICP-MS data was carefully monitored. Three measurements from each sample were made and the standard deviation on these results used to define the precision. Any data sets with standard deviations of greater than 3% were discarded ie. a total elution pattern was discarded if even one sample had a standard deviation of greater than 3%.

# CHAPTER 3

# RESULTS AND DISCUSSION

## 3.1 Introduction

As described in section 1.4.1, humic material from soils and sediments was traditionally separated on the basis of its solubility in acid/alkali to give two fractions, humic and fulvic acids. The higher average molecular weight of humic acid relative to fulvic acid (Stevenson, 1982) led, in many studies, to the supposition that humic and fulvic acids were representative of the solid and aqueous phase humic material respectively. Studies of associations of metals with these fractions were assumed to give an indication of the solubility and hence mobility of such metal-humic complexes in a natural terrigenous system.

In the early part of this study humic and fulvic acid, prepared as described in section 2.1, were fractionated using Sephadex gels, and the fractions obtained was analysed for their radionuclide content. A similarly prepared set of fractions were characterised using FTIR spectroscopy. Following an evaluation of traditional methodology, and the observation of significant chemical alteration of the humic fraction as a result of the acid precipitation of humic acid, alternative isolation procedures were implemented for the remainder of the study. Spectroscopic characterisation of fractions of humic substances involved the use of FTIR, UV and fluorescence spectroscopy. This chapter will take the form of a discussion of

i) the use of gel chromatography in the fractionation of humic and fulvic acids,

ii) the alteration of humic material following the use of traditional isolation procedures

and

iii) the characterisation of humic substances isolated using modified methodology.

## 3.2 Characterisation of Humic and Fulvic Acids Extracted from Soils and Sediments in W Cumbria and SW Scotland

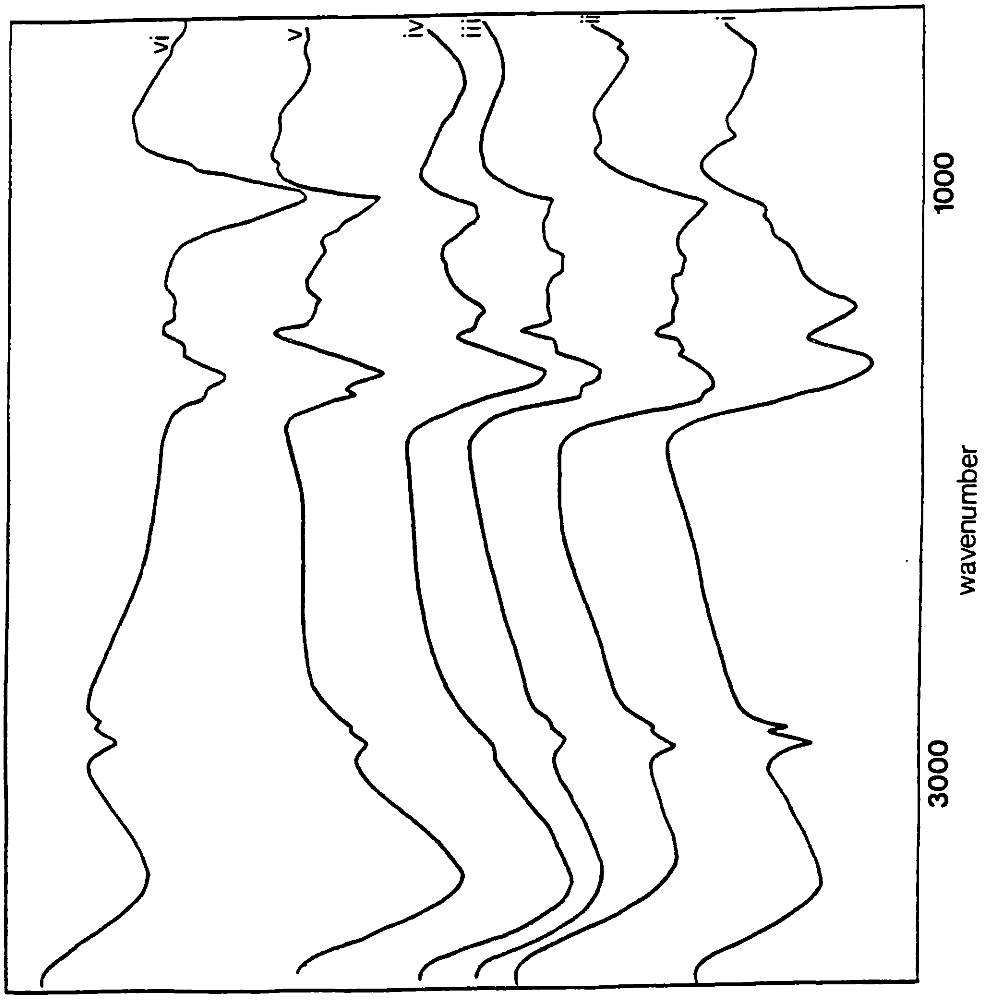
Humic acid was extracted using traditional methodology (ie. precipitation following the lowering of solution pH to a value of 1) from the following samples:
i) Esk 0-5cm
ii) Southwick 0-5cm
iii) Southwick 40-45cm
iv) Southwick 65-70cm.
Humic acid was also isolated from a highly organic soil at Needle's Eye, SW Scotland and for comparison Aldrich humic acid was included in the early part of this study.
Fulvic acid was extracted from Esk 0-5cm, Souwthwick 0-5cm and Southwick 40-45cm.
FTIR spectra of humic and fulvic acids from these soils and sediments and Aldrich humic acid are illustrated in Spectra 3.1 and 3.2.
The spectra are broad and the main absorption bands occur at 3400, 2950, 1720, 1650, 1590, 1400, 1250, 1050cm$^{-1}$ and are assigned to functional groups as described below :

3400cm$^{-1}$ band - broad absorption assigned to hydrogen bonded hydroxyl groups of carboxylic acids or aliphatic or aromatic alcohols.

2950-2850cm$^{-1}$ band - sharp but often weak band or bands assigned to aliphatic $CH_2$ or $CH_3$ groups.

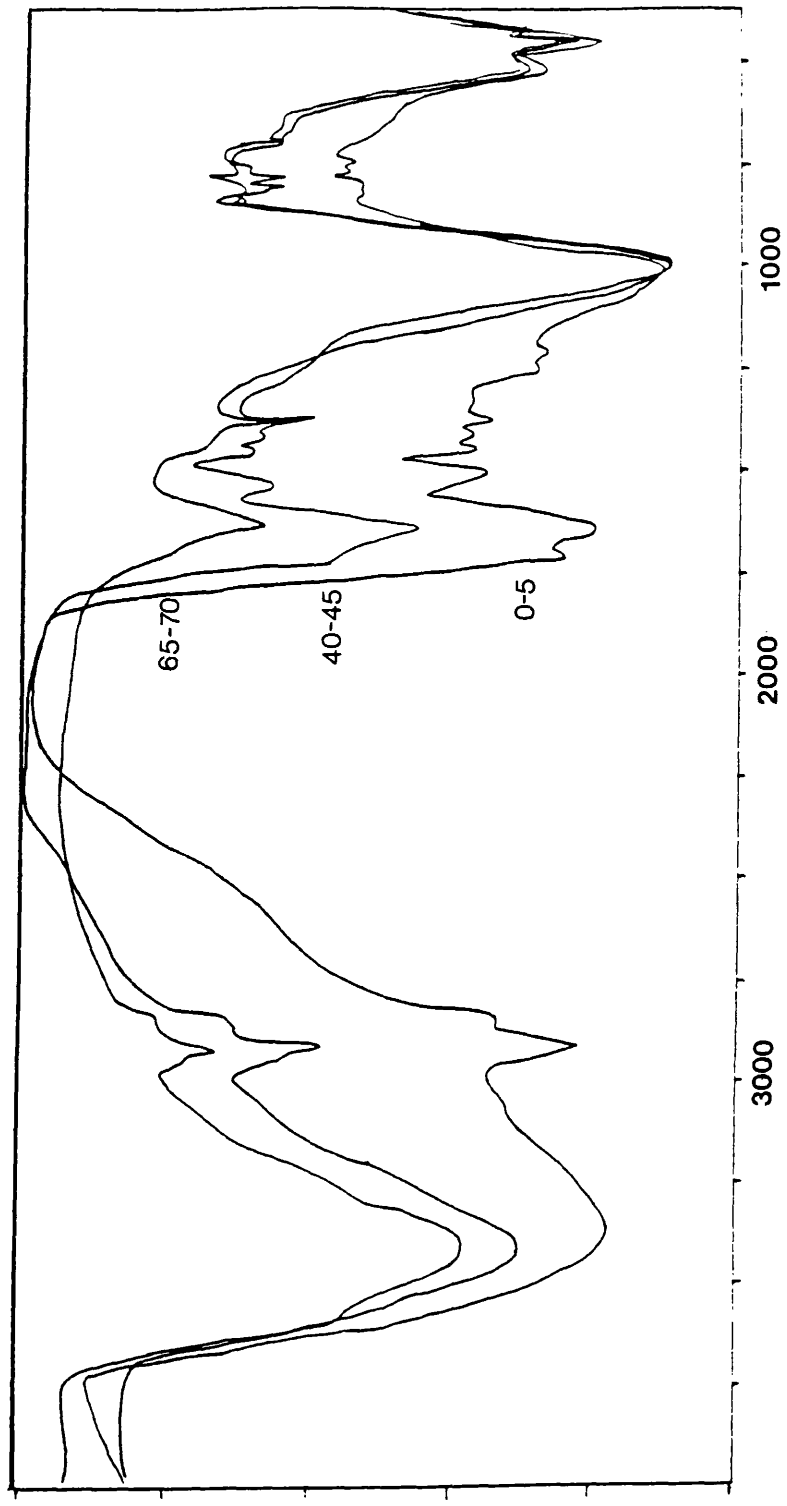
1720cm$^{-1}$ band - strong absorption assigned to the C=O unit of undissociated carboxylic acids.

1650cm$^{-1}$ band - strong absorption assigned to the C=O unit of other carbonyl compounds including aldehydes, ketones, ethers, esters, amino acids. Other



**Spectrum 3.1 Aldrich Humic Acid (Na-humate) and Humic and Fulvic Acids Isolated from Soils and Sediments**

| | |
|---|---|
| **i) Aldrich Humic Acid** | **ii) Esk Humic Acid** |
| **iii) Needle's Eye Humic Acid** | **iv) Esk Fulvic Acid** |
| **v) Southwick Humic Acid** | **vi) Southwick Fulvic Acid** |



Spectrum 3.2 Comparison of Humic Acids from Southwick Merse Isolated from 0-5cm, 40-45cm and 65-70cm Sediment Samples

interpretations include the contribution of aromatic C=C units to this band.

1590cm$^{-1}$ band - strong absorption assigned to the C=O unit of dissociated carboxylic acids ie. carboxylates

1400cm$^{-1}$ band - assigned to the C=O unit of undissociated carboxylic acids

1250cm$^{-1}$ band - assigned to the C=O unit of undissociated carboxylic acids

1050cm$^{-1}$ band - assigned to the C=O unit of dissociated carboxylic acids

These spectral features are characteristic of humic and fulvic acids and are consistent with the spectra obtained by Stevenson and Goh (1971), Schnitzer (1972), Kim (1990) and Choppin (1991) (section 1.4.4.3; Figure 1.8). Criticism of the use of FTIR spectroscopy to characterise humic and fulvic acids arises from the lack of structural information presented in spectra due to the overlap of absorptions occurring at similar wavenumbers. This study does not find significant differences between humic materials from diverse origins which is also consistent with observations of Stevenson (1982). However, a comparison of humic acids isolated from three sediment samples from increasing depths at the same location on Southwick Merse reveals that there is a progressive loss of oxygen containing functional groups (Spectrum 3.2). CHN data for 0-5cm and 40-45cm humic acids, presented in Table 3.1, confirms that there is a significant reduction in the percentage oxygen incorporated into 40-45cm humic acid. This is accompanied by a change in association of Pu from the organic to Fe/Mn oxyhydroxide phase with increasing depth in a similar sediment core (Allan, 1993) (Figure 3.1). Additional information is also obtained from C/H and C/N ratios (Table 3.1) which indicate that 0-5cm humic acid has a strong marine signature whereas 40-45cm humic acid has higher ratio values and therefore relatively less hydrogen and nitrogen. (Typical ratios for marine and terrigenous humic acids appeared in Table 1.11.)This is interpreted to be representative of the loss of the marine component

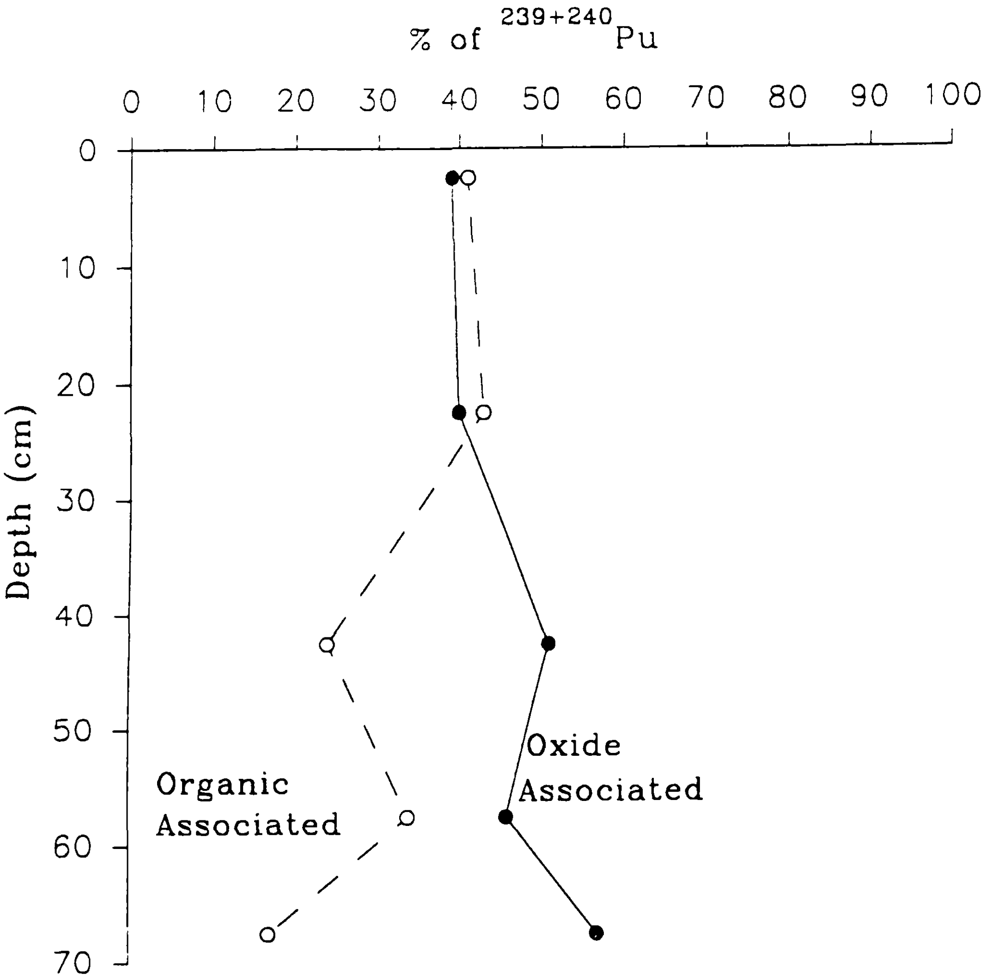


Figure 3.1 Plutonium Associations in Saltmarsh Sediments (Allan, 1993)

| Sample (Humic Acid) | %C | %H | %N | %O | C/H | C/N |
|---|---|---|---|---|---|---|
| 0-5cm Southwick Merse | 43.35 | 5.57 | 4.18 | 46.90 | 7.78 | 10.37 |
| 40-45cm Southwick Merse | 55.32 | 4.52 | 3.40 | 36.76 | 12.23 | 16.25 |

**Table 3.1 CHN Data for Humic Acid Samples from 0-5cm and 40-45cm Southwick Merse**

| Sephadex G Type | 15cm | 20cm | 25cm | 30cm | 35cm | 40cm | 50cm |
|---|---|---|---|---|---|---|---|
| G15 | | | NS | | | | NS |
| G25 | | | NS | | NS | | NS |
| G50 | | | NS | | NS | | poor |
| G75 | NS | | NS | | poor | medium | medium |
| G100 | NS | poor | poor | good | medium | medium | poor |
| G150 | poor | | medium | | medium | | |
| G200 | | medium | | | | | |

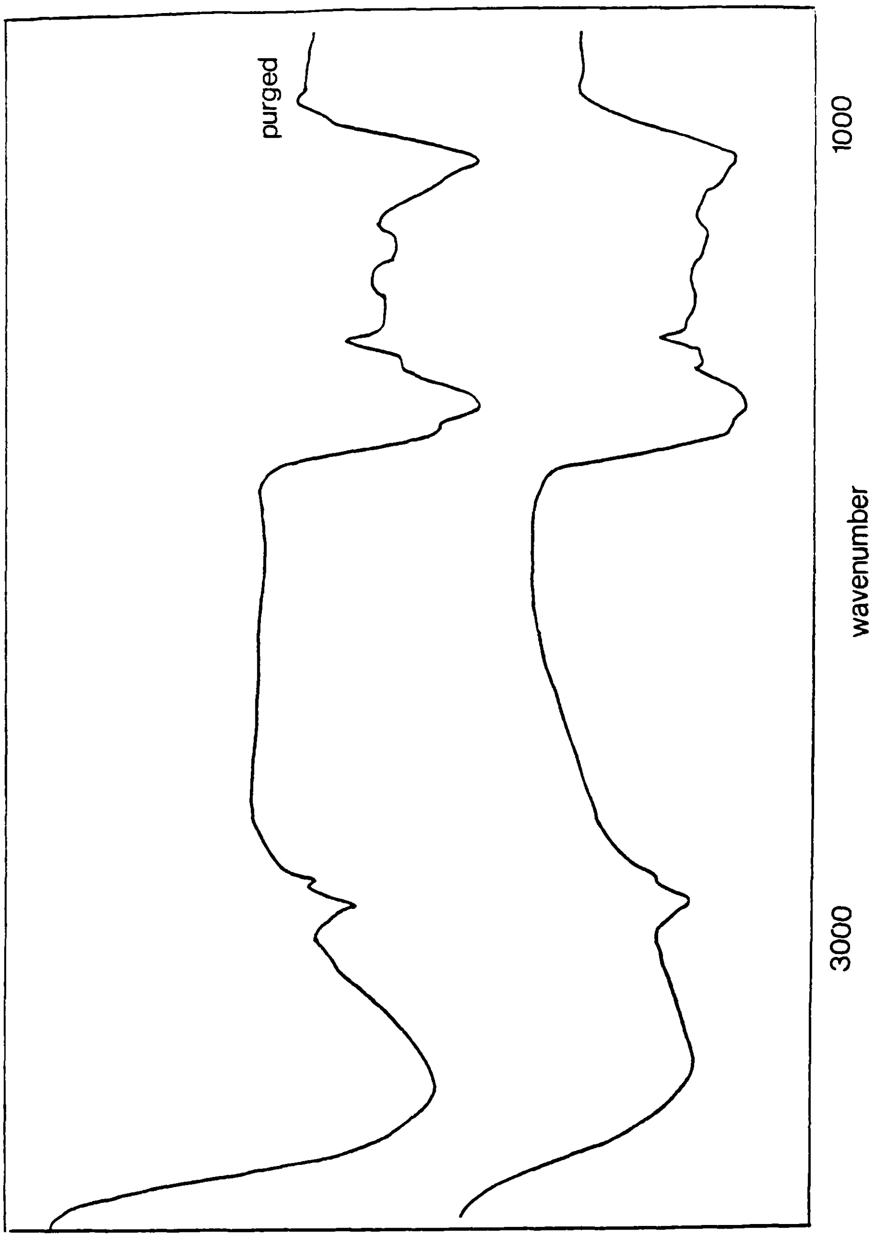
**Table 3.2 Optimisation of the Separation of Humic Acids (Esk 0-5cm and Aldrich Humic Acids) [NS : no band separation]**

due to preferential preservation of more stable terrigenous material and hence the increasing dominance of terrigenous material at depth. Moreover, this provides evidence of early diagenetic alterations of the humic fraction in such sediments.

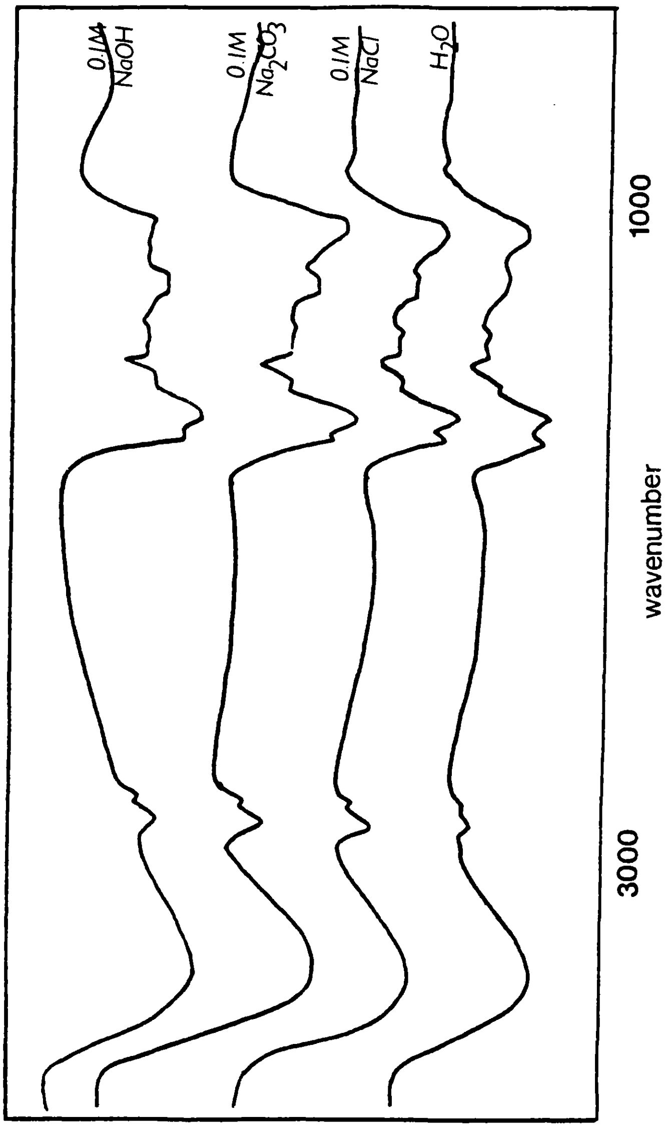
A development of the spectroscopic part of this work involved the comparison of spectra collected under atmospheric and moisture -free conditions. Spectrum 3.3 illustrates the improved resolution which can be obtained by the exclusion of moisture from the sample chamber of the FTIR spectrometer.

### 3.2.1 Characterisation of Humic Acid Isolated Following the Use of Various Extractants.

The potential alteration of the humic extract resulting from exposure to strong alkaline conditions was investigated by the comparison of four different extractants of varying alkalinity. These were water, 0.1M sodim chloride (pH7), 0.1M sodium hydrogen carbonate (pH 10) and 0.1M sodium hydroxide (pH13). The resulting humic acids were compared spectroscopically (Spectrum 3.4) and no chemical alteration was detectable in the FTIR spectra. Stronger absorptions at 1720, 1250 and 1050cm$^{-1}$ were observed for the water and 0.1M sodium chloride extracts and this is consistent with the extraction of a more highly hydrophilic portion of the humic acids in the soil as would be expected under these conditions. Most importantly an increase in these bands is not observed for the 0.1M NaOH extract demonstrating that significant oxidation of the humic acid has not occurred. Therefore, the use of 0.1M NaOH to extract all humic materials from the soils and sediments in this study without chemical observable alteration can be justified. This is important in the identification and elimination of other procedural artefacts as will be discussed in section 3.5.



Spectrum 3.3 Improvements in the Resolution of the FTIR Spectrum of a Humic Acid from an Esk Soil Resulting from the Purging of the Sample Chamber with a Scrubbed Air Supply



**Spectrum 3.4 Comparison of the Effect of Different Aqueous Extractants on the Composition of Humic Acid Isolated from an Organic Soil**

## 3.3 Chromatographic Fractionation of Humic and Fulvic Acids

Humic acids from the following samples were fractionated using G100 Sephadex gel :

i) Esk 0-5cm
ii) Southwick 0-5cm
iii) Southwick 40-45cm.
iv) Needle's Eye 0-5cm

Fulvic acids from Southwick Merse 0-5cm and 40-45cm were also fractionated using G100 Sephadex gel.

### 3.3.1 Optimisation of the Gel Chromatographic Fractionation of Humic and Fulvic Acids

The optimum gel chromatographic fractionation of humic acid and, likewise, fulvic acid was evaluated by :

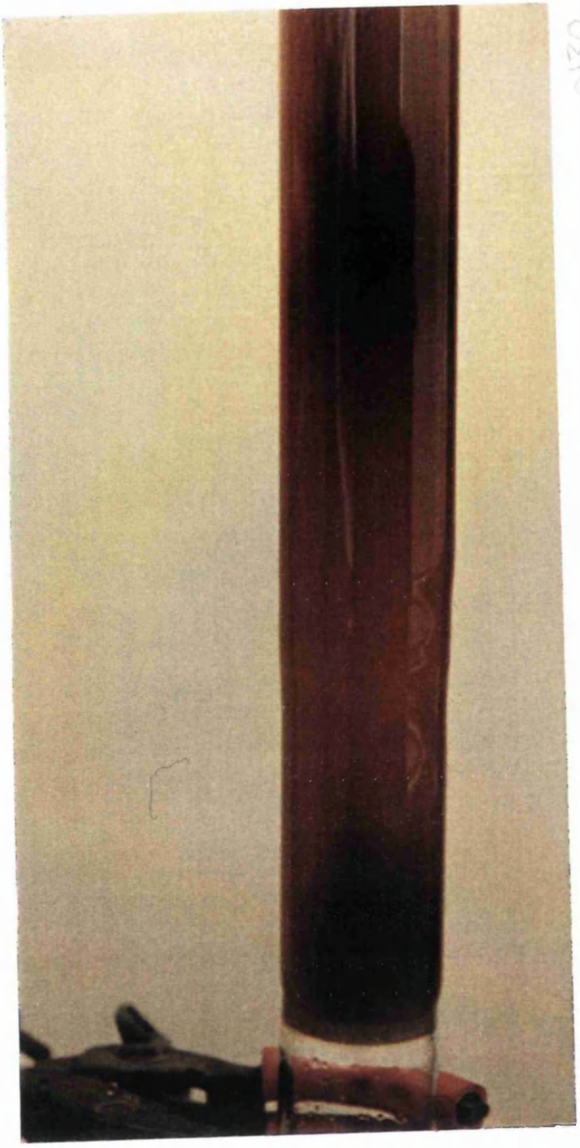
i) the observation of two bands during the passage of the humic material down the gel column. This is consistent with the work of Posner (1963), who monitored the fractionation of HA spectroscopically, and identified two maxima in the 260nm absorption in the UV/visible spectrum which coincided with the two bands observed on the gel column.

ii) the measurement of the maximum separation of the two bands without excessive smearing of the second band.

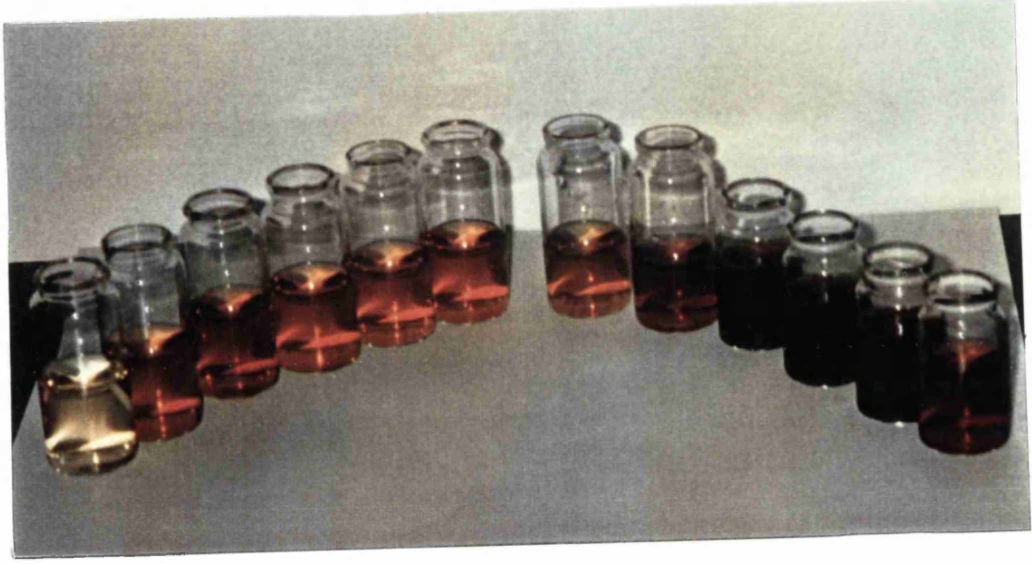
G15, G25, G50, G75, G100, G150 and G200 Sephadex gels were compared for 0-5cm humic acid from the Esk soil and Aldrich Humic Acid (Table 3.2). The optimum separation of the two bands was achieved using 30cm x 3cm diameter

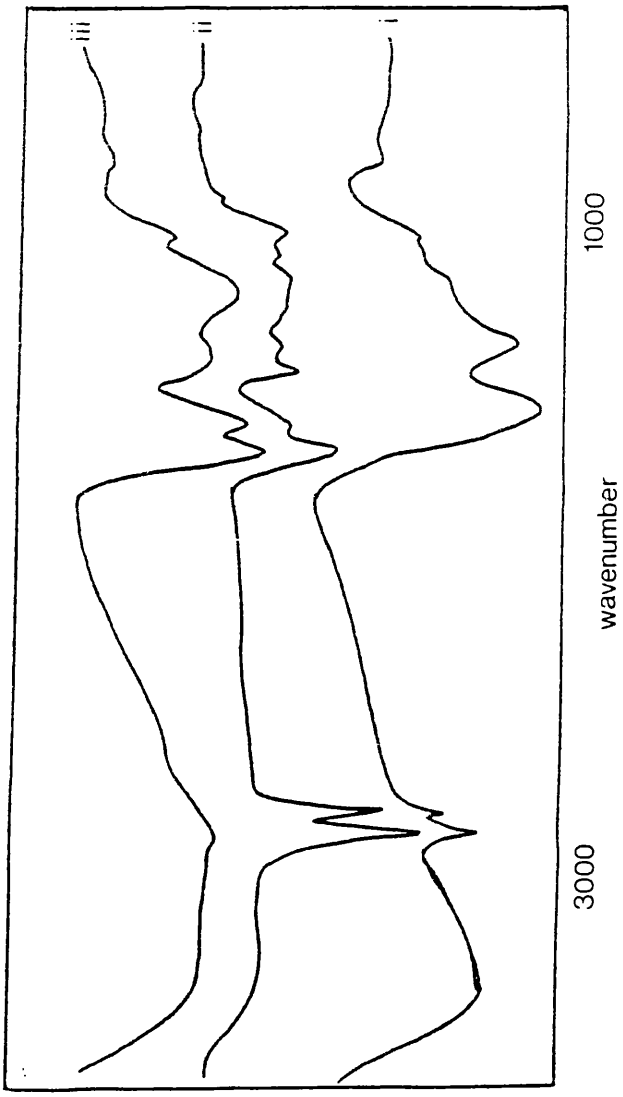
**a**

**b**

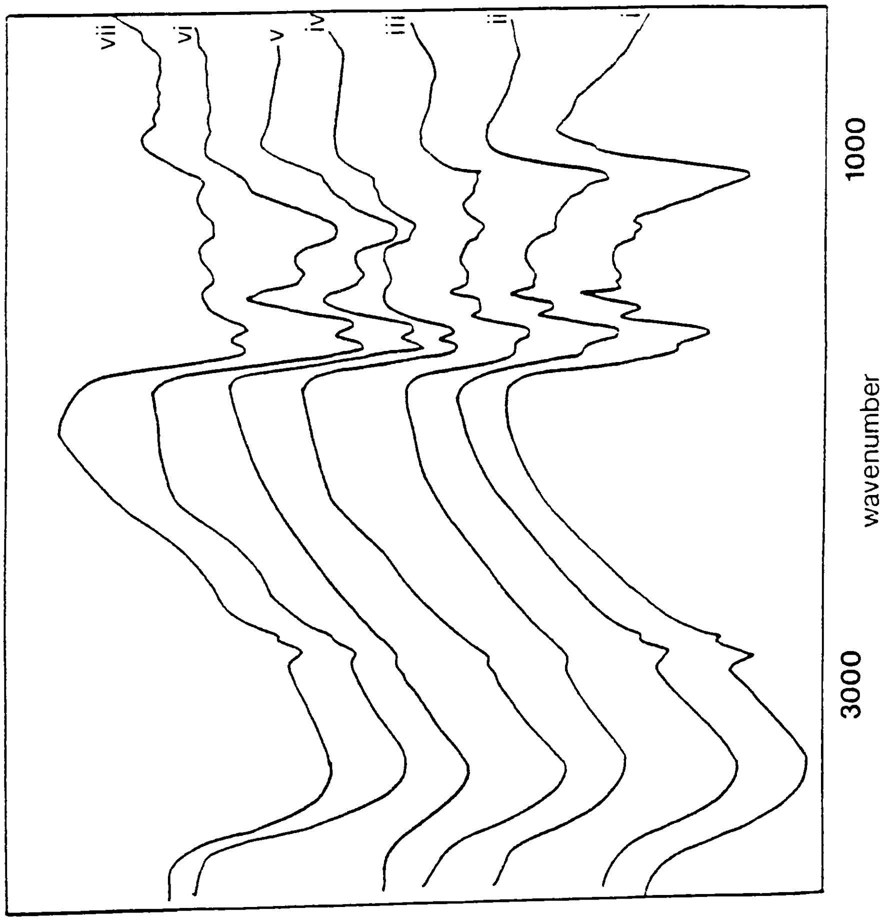


**c**

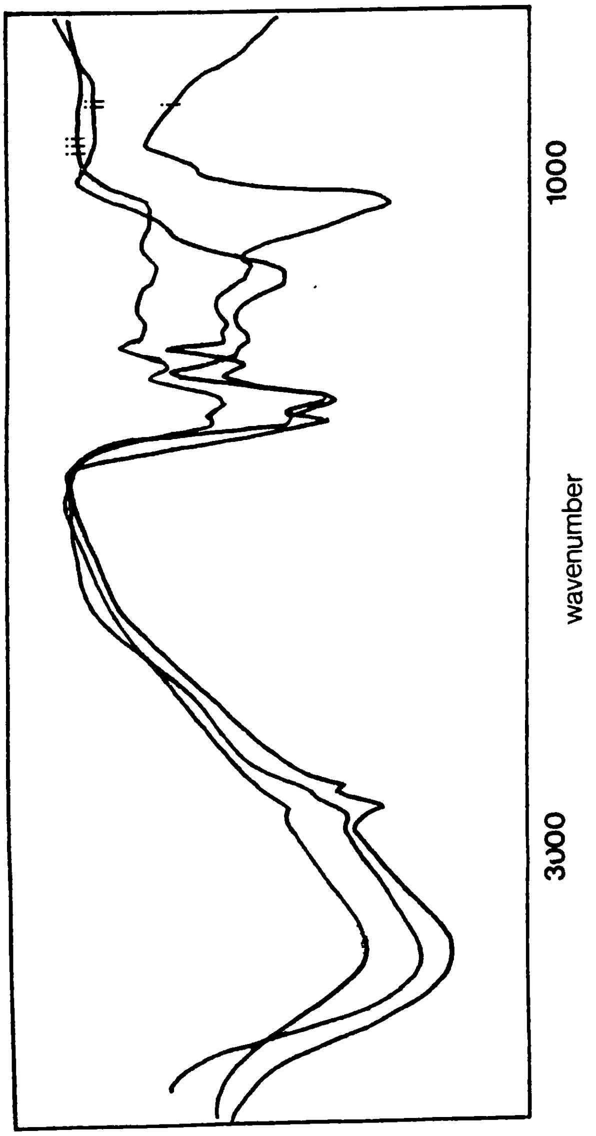




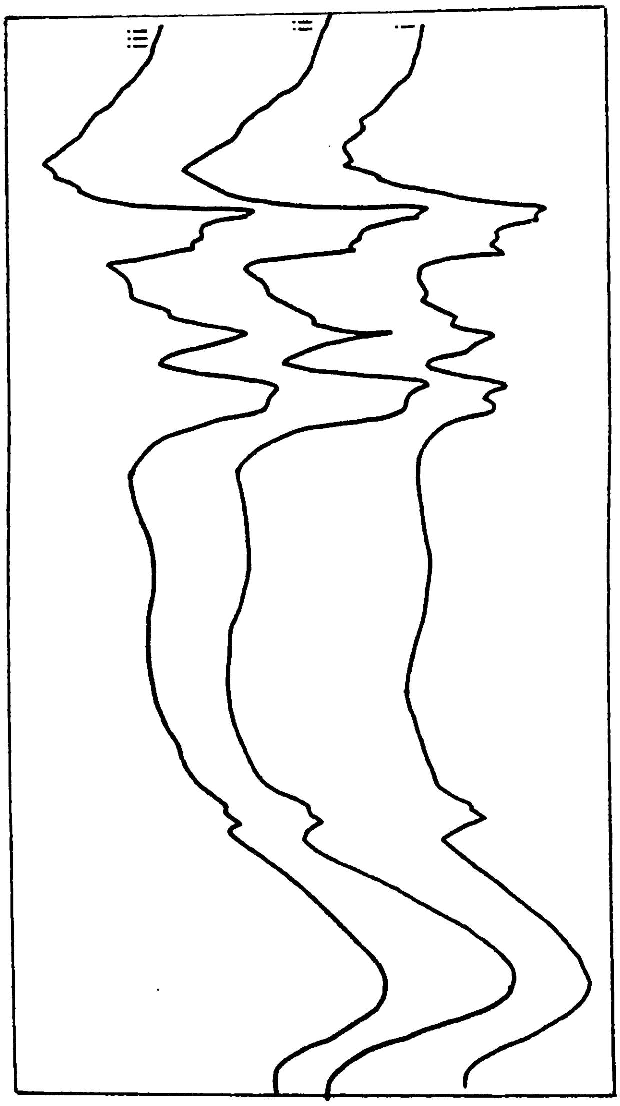
Spectrum 3.5 Gel Chromatographic Fractions of Aldrich Humic Acid
i) Aldrich Humic Acid ii) Band 1 iii) Band 2



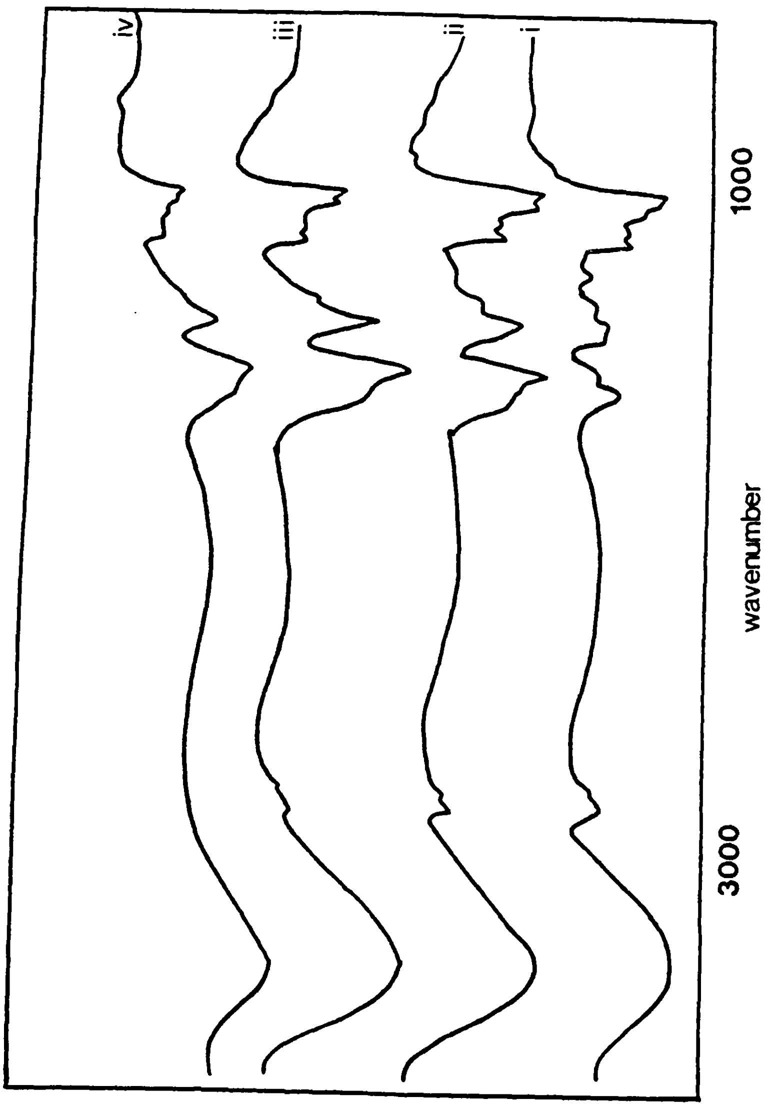
**Spectrum 3.6 Gel Chromatographic Fractions of Esk 0-5cm Humic Acid**
**i) - ii) Band 1 iii) - iv) Intermediate v) - vi) Band 2**
**vii) End Fraction**



**Spectrum 3.7 Comparison of Gel Chromatographic Fractions of Esk 0-5cm Humic Acid using Sephadex G100 Gel**
**i) Band 1 ii) Intermediate iii) Band 2**

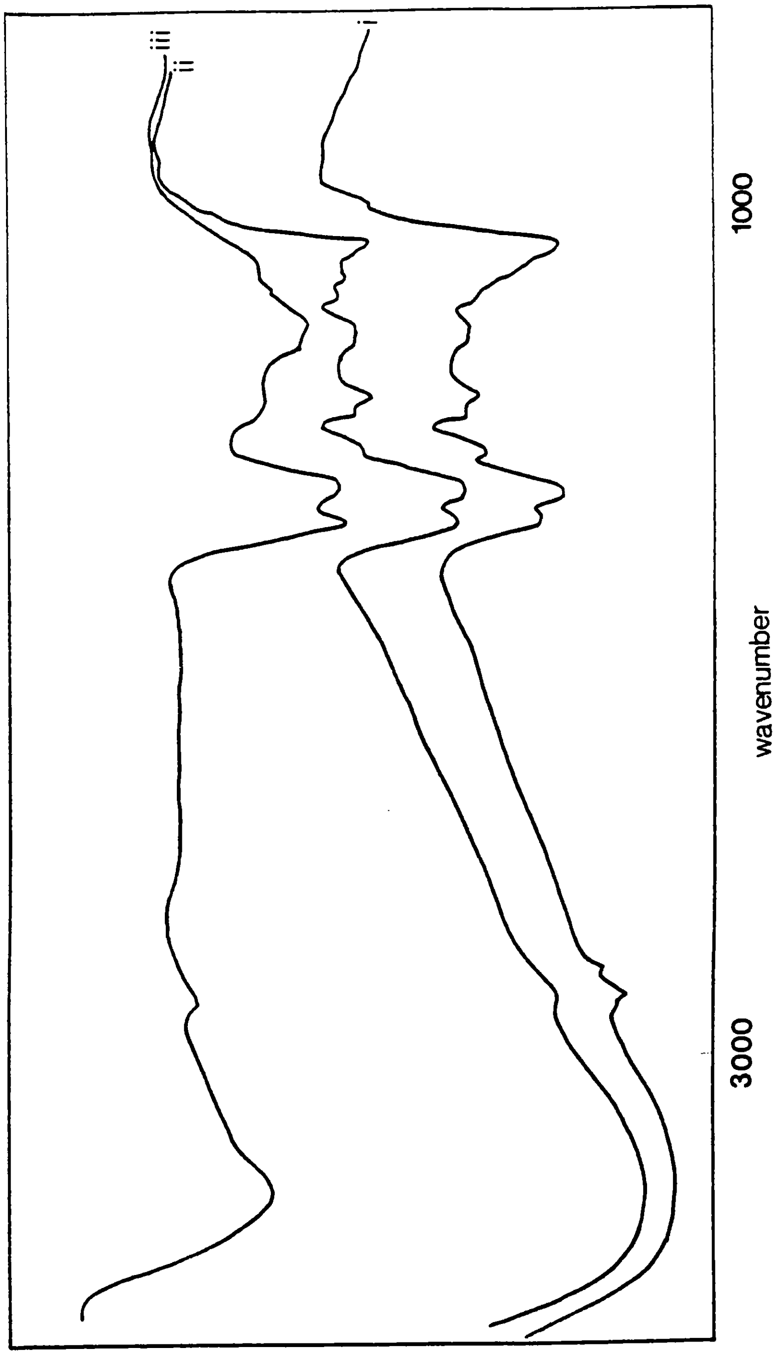


Spectrum 3.9 Gel Chromatographic Fractions of Esk 0-5cm Fulvic Acid using Sephadex G50 Gel
i) Band 1 ii) Intermediate iii) Band 2

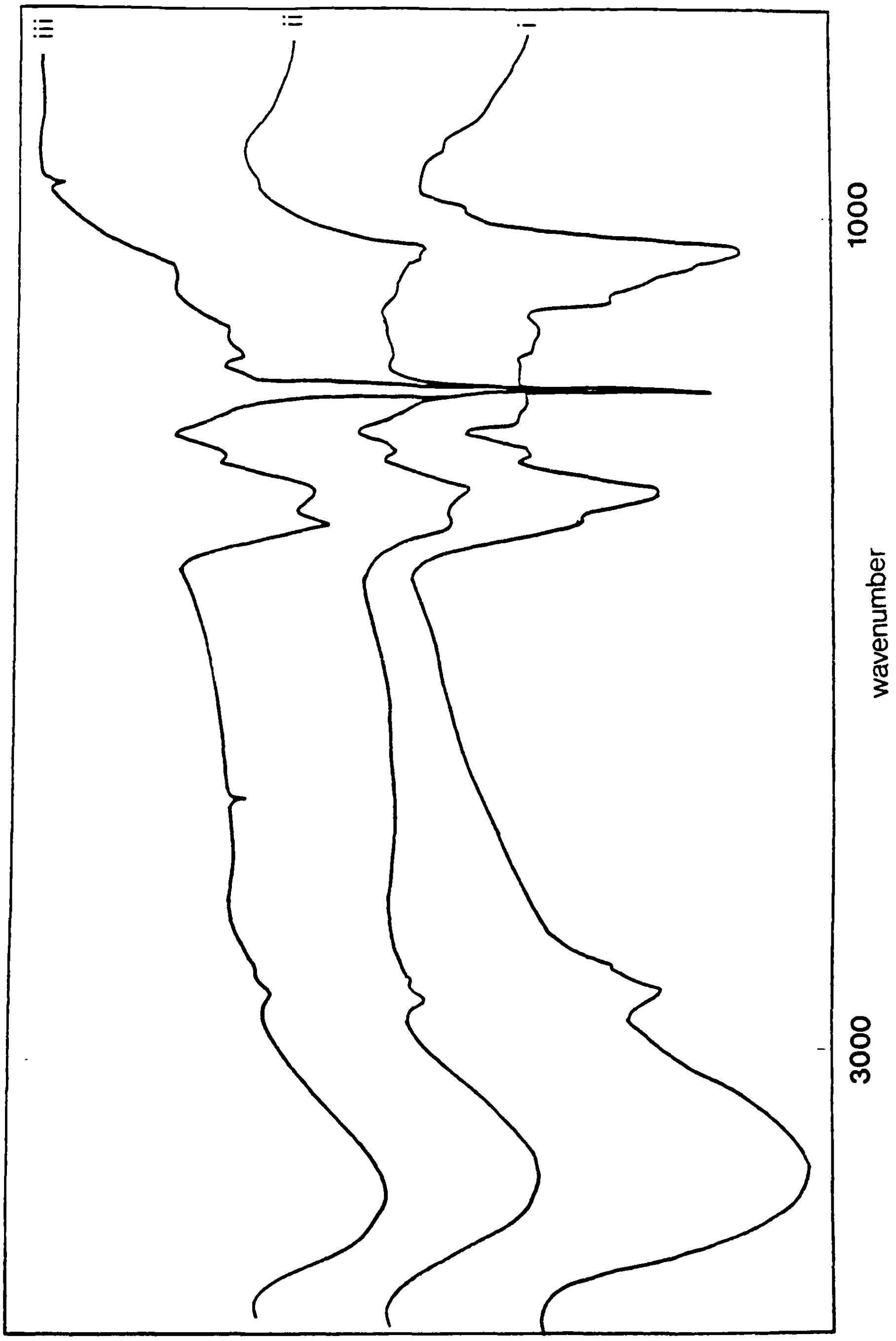


Spectrum 3.10 Gel Chromatographic Fractions of Esk 0-5cm Fulvic Acid using Sephadex G100 Gel

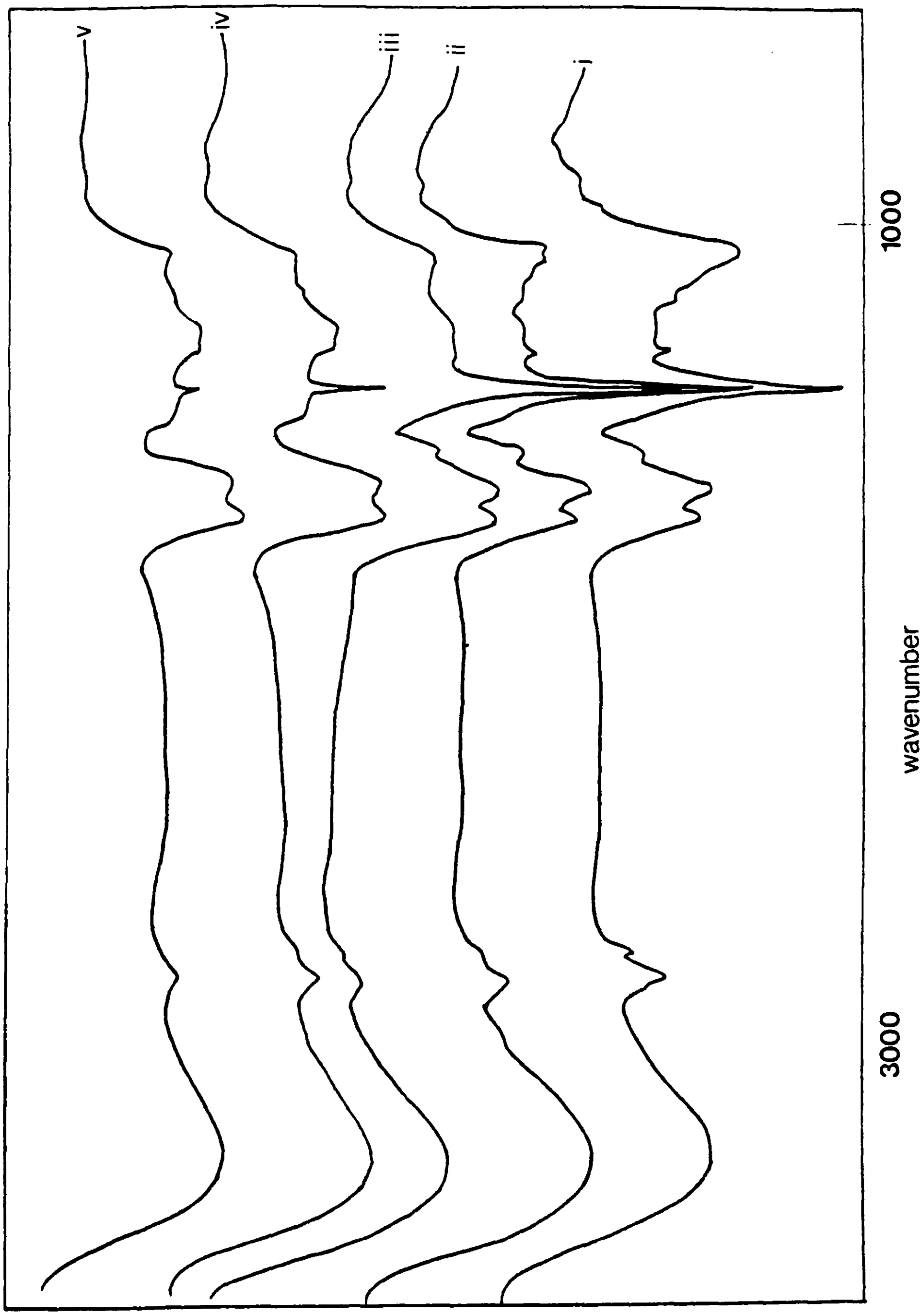
i) Band 1 ii) Intermediate iii) Band 2 iv) End Fraction



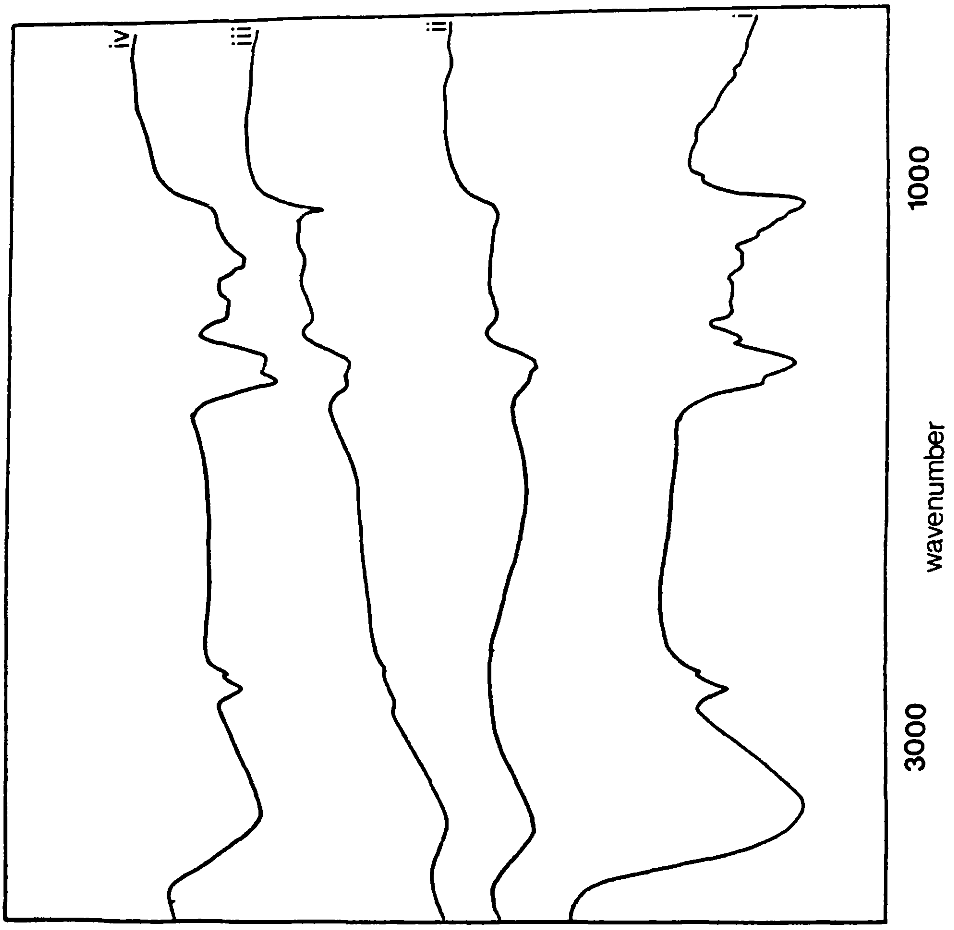
Spectrum 3.11a Gel Chromatographic Fractions of Esk 0-5cm Humic Acid using Sephadex G100 Gel and a 5% Methanol Eluent
i) Band 1 ii) Intermediate iii) Band 2



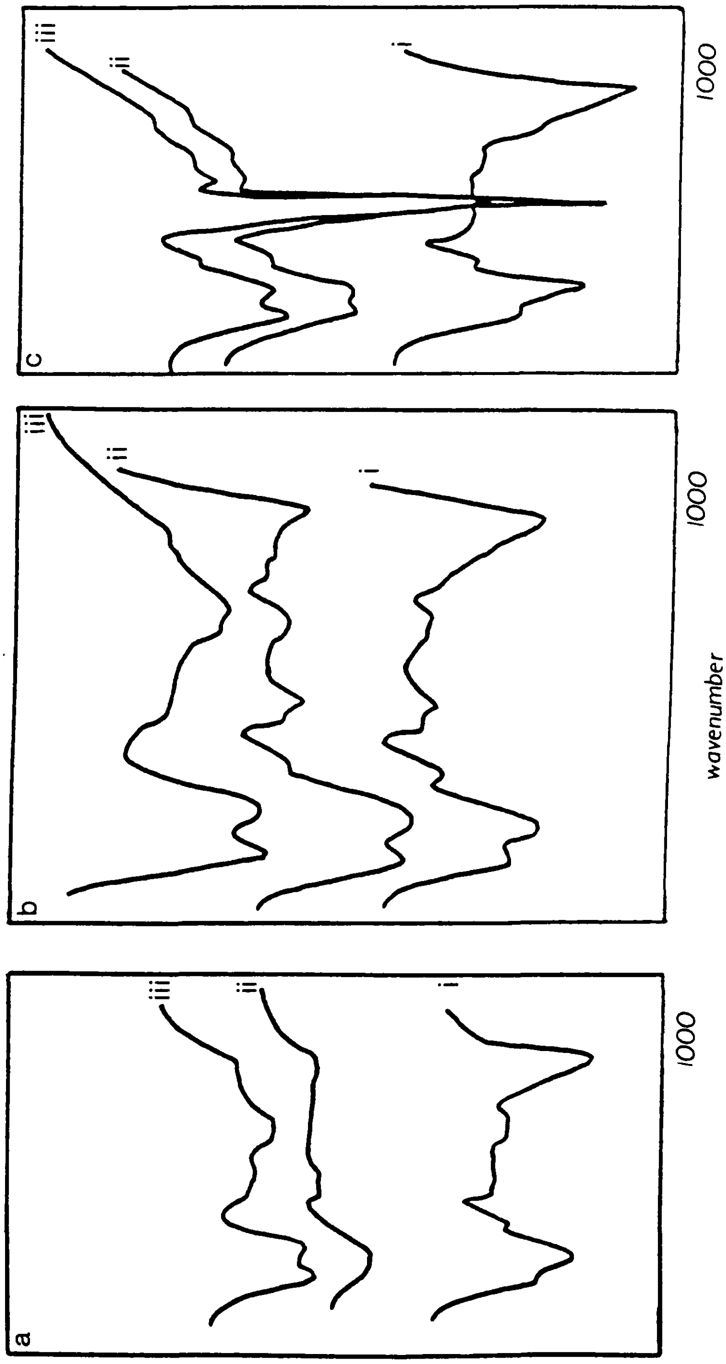
Spectrum 3.11b Gel Chromatographic Fractions of Esk 0-5cm Humic Acid using Sephadex G100 Gel and a 10% Methanol Eluent
i) Band 1 ii) Intermediate iii) Band 2



**Spectrum 3.11c Gel Chromatographic Fractions of Esk 0-5cm Humic Acid using Sephadex G100 Gel and a 10% Decan-1-ol Eluent**
**i) - ii) Band 1 iii) Intermediate iv) Band 2**

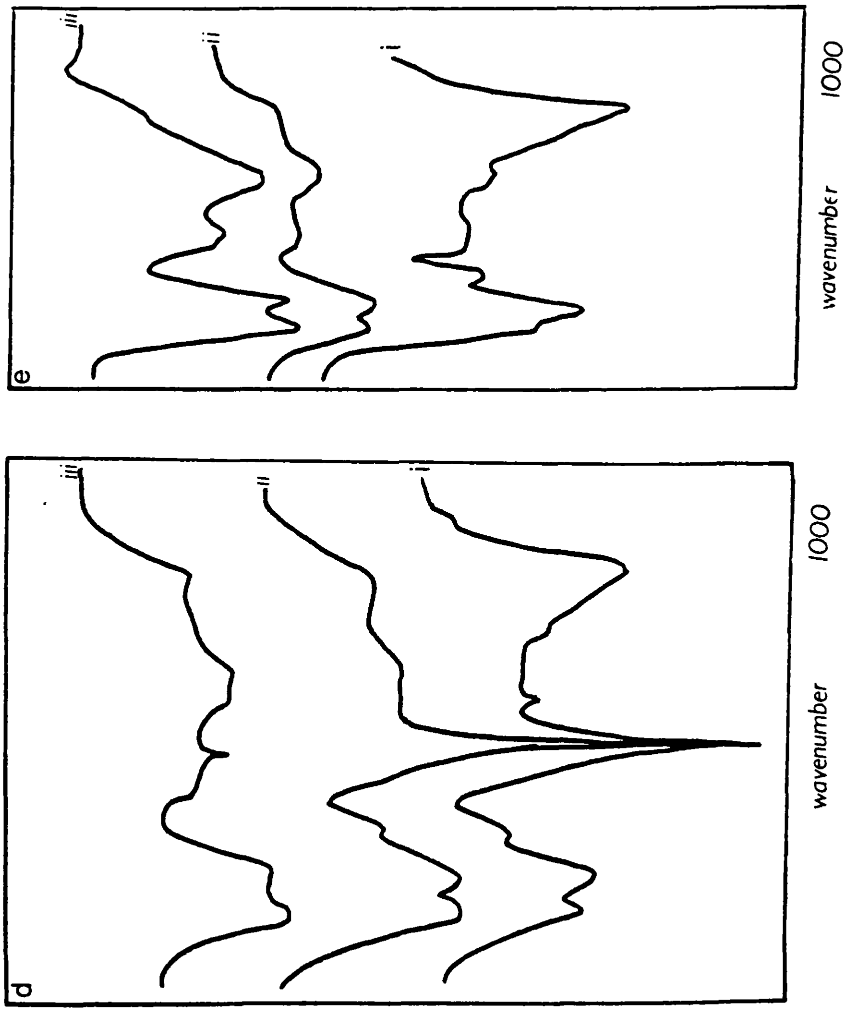


**Spectrum 3.11d Gel Chromatographic Fractions of Esk 0-5cm Humic Acid using Sephadex G100 Gel and a 5% ι-Amyl Alcohol Eluent**
**i) Band 1 ii) - iii) Intermediate iv) Band 2**



**Spectrum 3.12a b c Comparison of the Region 1700-1000cm$^{-1}$ of the Spectra of Gel Chromatographic Fractions of Esk 0-5cm Humic Acid Obtained using G100 Sephadex Gel and a Range of Eluents**

| | | |
|---|---|---|
| **a 0.1M NaOH** | **b 5% Methanol** | **c 10% Methanol** |
| **i) Band 1** | **ii) Intermediate** | **iii) Band 2** |



Spectrum 3.12d e Comparison of the Region 1700-1000cm$^{-1}$ of the Spectra of Gel Chromatographic Fractions of Esk 0-5cm Humic Acid Obtained using G100 Sephadex Gel and a Range of Eluents
d 10% Decan-1-ol e 5% t-Amyl Alcohol
i) Band 1 ii) Intermediate iii) Band 2

column containing G100 Sephadex gel and this is illustrated for 0-5cm humic acid from the Esk soil (Plate 3.1b).

Whereas G25 and G50 Sephadex gels, due to their smaller pore size, resulted in no significant separation, fractionation of humic and fulvic acids on Sephadex G100 gel is characterised by separation in to two distinct bands on the gel column. Fractions containing humic material from the first band are intensely coloured and cloudy (Plate 3.1c) whereas fractions containing material from the second band are somewhat less intensely coloured and clear. Intermediate fractions are paler and clear, indicating that good separation of the two bands has been effectively achieved. This is consistent with the results obtained by Livens et al. (1990) who also used Sephadex gels to fractionate humic materials of similar origins to those used in this study.

G150 and G200 gels using columns of greater than 20cm to effect band separation resulted in excessive smearing of the bands due to the longer elution times required.

The separation procedure was also monitored using FTIR spectroscopy and the results are shown in Spectra 3.5-3.12. These results will be discussed in terms of the optimisation of the gel chromatographic fractionation of humic and fulvic acid. The following section will further consider the use of FTIR spectroscopy in the characterisation of humic and fulvic acids.

Spectrum 3.5 illustrates the results of fractionation of Aldrich humic acid and highlights compositional differences between humic acid macromolecules contained in the two bands. A comparison with the spectrum of bulk Aldrich humic acid indicates that the spectroscopic features of the fractions are consistent with those in the unfractionated material. Spectra 3.7-3.8 show compositional differences between fractions of 0-5cm humic acid from the Esk soil. Band 1 macromolecules contain more carboxylate functional groups (1590, 1400 and 1050cm$^{-1}$) than band 2 macromolecules which contain more undissociated carboxyl groups (1720 and 1250cm$^{-1}$). This is consistent with the high proportion of trace metals associated with humic macromolecules in band 1 (Livens, 1989).

Macromolecules in intermediate fractions give rise to weaker absorptions than either band 1 or 2 humic material and since FTIR samples all contained identical amounts of humic material, this may indicate that these macromolecules have fewer functional groups (spectrum 3.8). Overall, the spectra of eluted fractions indicate that good separation has occurred and there are significant compositional differences between macromolecules from the different bands.

The spectroscopic data resulting from the gel chromatographic fractionation of humic acid isolated from the highly organic soil at Needle's Eye (Spectrum 3.8) can be compared with those obtained for the Esk humic acid. A trend of decreasing carboxylate and increasing carboxyl functionality with increasing fraction number is observed for these humic acids of diverse origin. The overall compositional differences are less marked for the Needle's Eye humic acid and may result from poorer degradation of humic precursors under the highly reducing conditions prevailing at this location.

Fulvic acid from the Esk soil was fractionated on both G50 and G100 Sephadex gels (Spectra 3.9 and 3.10). As discussed above poor separation was observed using G50 Sephadex gel and this is reflected in the uniform composition of the spectra of fractions of fulvic acid. Separation on G100 Sephadex did result in fractions with differing composition with the 1720 and 1400cm$^{-1}$ bands arising from carboxyl groups being most prominent in fractions corresponding to band 2. These results are similar to those of Schnitzer and Skinner (1968) and the trend is again identical to that identified for the fractionation of humic acid.

### 3.3.2 The Optimisation of Gel Chromatographic Fractionation of Humic Acids using Sephadex G100 with Different Eluents

Gel chromatography studies outlined by Shaw (pers. comm., 1993) suggested that the use of 5-10% (v/v) methanol/ 0.1M sodium hydroxide solutions as eluents could improve further the separation of the two bands prior to elution from the gel column. In this study methanol, decan-1-ol and t-amyl-alcohol were added to 0.1M sodium hydroxide solution to give the following eluents :

i) 5% (v/v) methanol
ii) 10% (v/v) methanol
iii) 10% (v/v) decan-1-ol
iv) 5% (v/v) t-amyl alcohol.

The results were again monitored spectroscopically and are shown in Spectra 3.11 and 3.12. However, no apparent improvement in the separation of the two bands is achieved since the same trend of changing functionality (1750-1500cm$^{-1}$ region) with increasing elution time is again observed and is also independent of the nature of the alcohol.

An additional feature of the spectra relating to the use of 10% methanol and 10% decan-1-ol is the appearance of a sharp band at 1390cm$^{-1}$. This suggests either that there has been some alteration of the humic material due to exposure to these solvents or, more probably, that a specific interaction has occurred between the alcohol and the humic material. The latter would be consistent with

i) the effect for methanol is increasingly apparent with the increasing proportion of methanol in the solvent

ii) the effect is more prominent in certain fractions than others for decan-1-ol indicating that the larger size of this alcohol results in a more specific interaction

iii) the effect is not observed for the sterically hindered t-amyl alcohol.

From these experiments, it can be concluded that there is no apparent improvement in the separation of components of humic acid resulting from the use of eluents containing proportions of these alcohols. Additionally, interactions of the alcohol with components of humic acids cannot be discounted and from this it is clear that the use of such eluents is, at best, inadvisable.

## 3.4 Characterisation of Gel Chromatographic Fractions of Humic and Fulvic Acids

### 3.4.1 Spectroscopic Characterisation of Gel Chromatographic Fractions of Humic and Fulvic Acids

In addition to monitoring the separation process occurring on the gel column it is also apparent that the spectra of fractions have greater resolution than those of bulk humic and fulvic acids and the well-characterisd trend of changing functionality with increasing elution time establishes FTIR spectroscopy as an extremely useful technique in the characterisation of humic and fulvic acids. In addition, an important observation is that the fractionation of humic and fulvic acids not only from the same soil but also from diverse origins produces the same pattern of compositional differences. This topic is discussed further in section 3.5.

### 3.4.2 Characterisation of Actinide Interactions with Gel Chromatographic Fractions of Humic and Fulvic Acids

In the early part of this study U and Th analyses, using $\alpha$-spectrometry and ICP-MS, were carried out for individual gel chromatographic fractions of humic acid from 0-5cm Esk soil and humic and fulvic acids from 0-5cm and 40-45cm Southwick Merse sediment. The elution patterns obtained are illustrated in Figures 3.2-3.4 and Tables 3.3-3.5.

75% of the $^{232}$Th and 45% of the $^{238}$U are associated with fractions 1-5 of the Esk humic acid (Figure 3.2). Moreover, the total number of atoms of $^{232}$Th is in excess of that of $^{238}$U and this is discussed in section 3.5. FTIR spectroscopy showed that early fractions contained higher proportions of carboxylate groups and it is known that early fractions generally have a high trace metal content (Livens, 1989), so it can tentatively be suggested that U and Th may be bound by carboxylate groups, although this could not be directly concluded from the spectroscopic evidence alone.

A similar pattern is evident for the 0-5cm Southwick humic acid and fulvic acid. 100% $^{238}$U is found in fractions 3-7 (Fig. 3.3). This again suggests a similarity in the behaviour of humic materials of diverse origin.

A different pattern is observed for the 40-45cm Southwick humic and fulvic acid (Fig. 3.4). Greater than 90% of $^{232}$Th and $^{238}$U is found in fractions 10-20. The

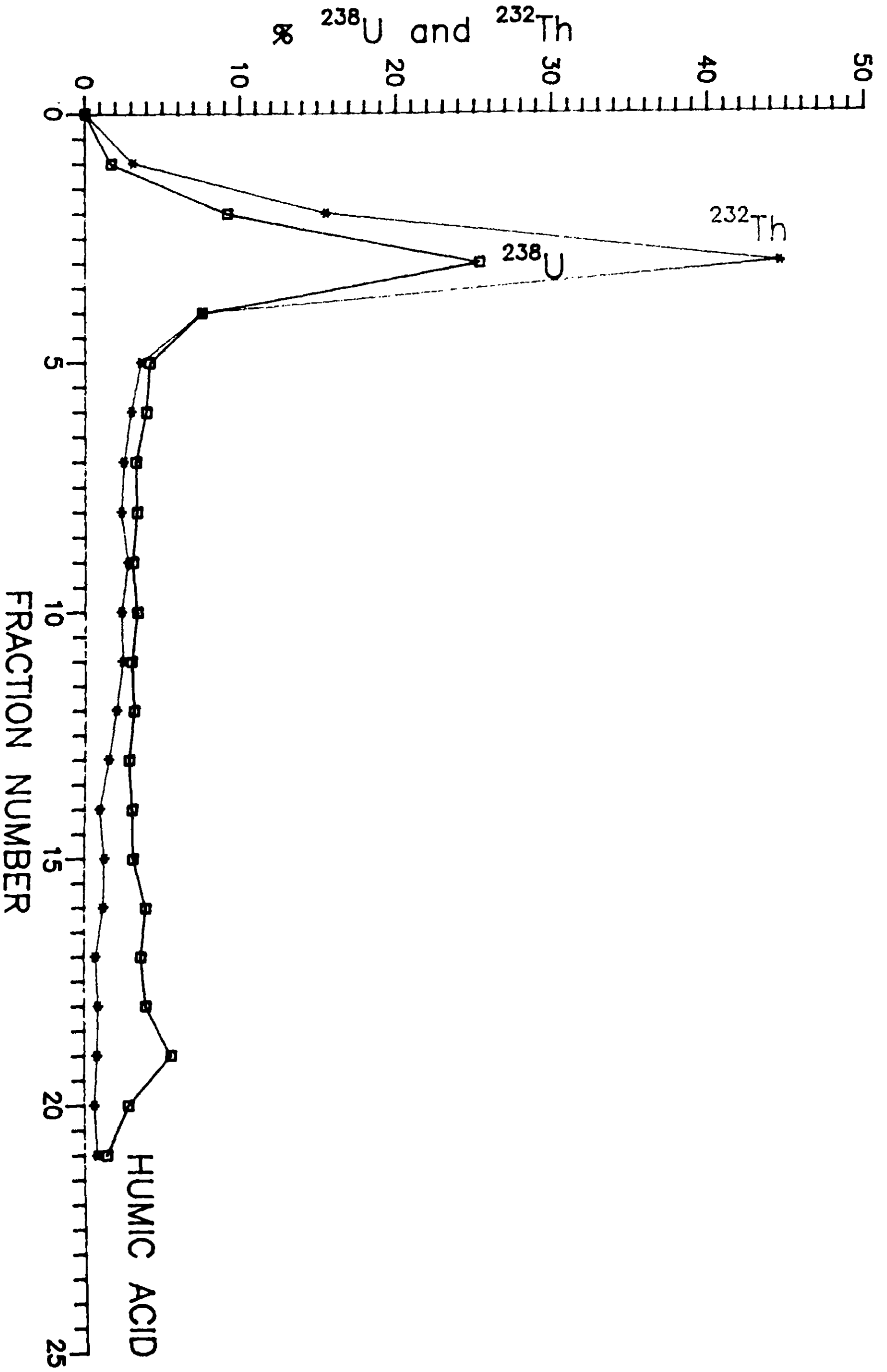


Figure 3.2 Esk 0-5cm Humic Acid : Percentage Distribution of $^{238}$U

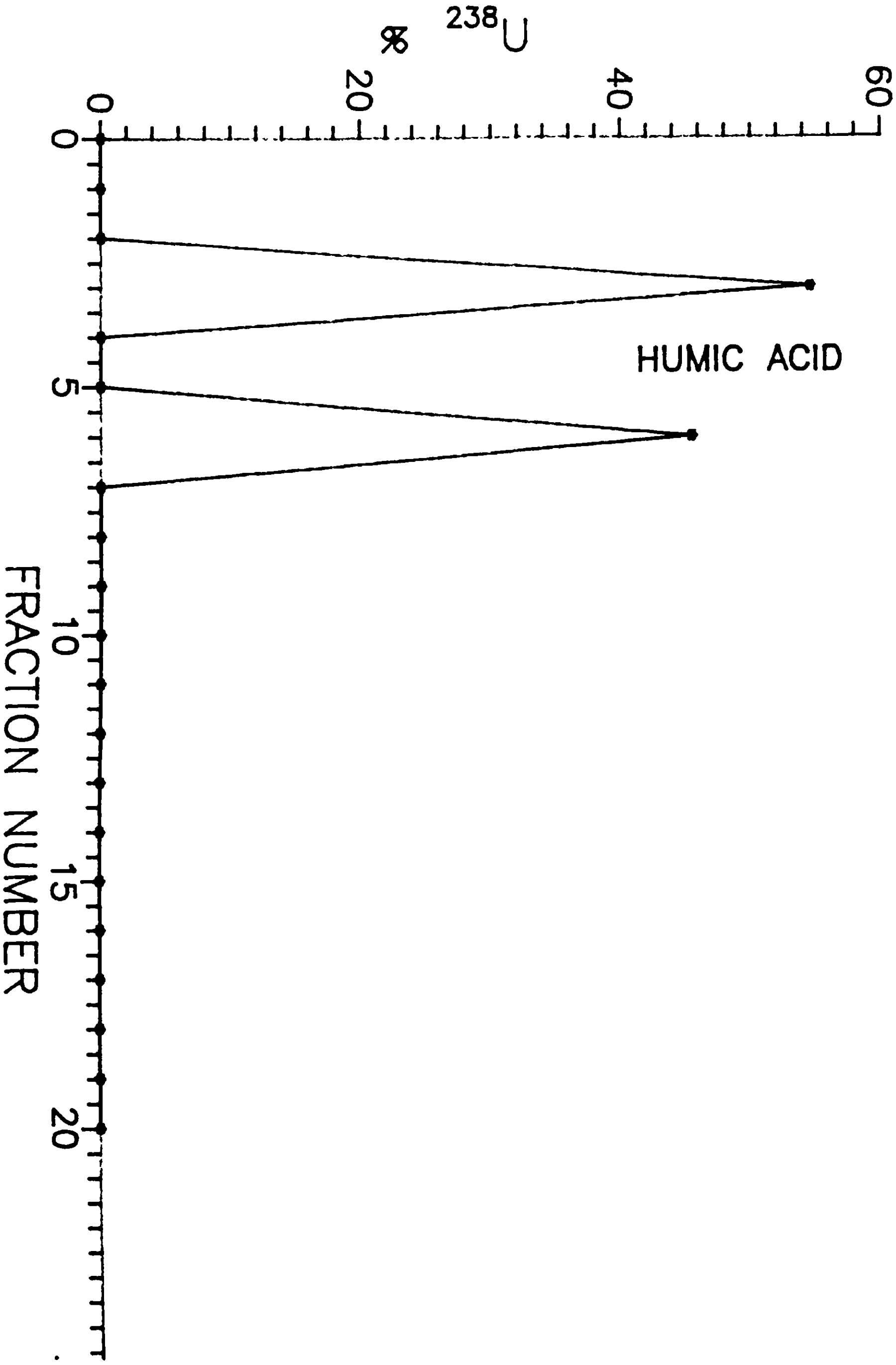


**Figure 3.3a Southwick Merse 0-5cm Humic Acid : Percentage Distribution of $^{238}$U**

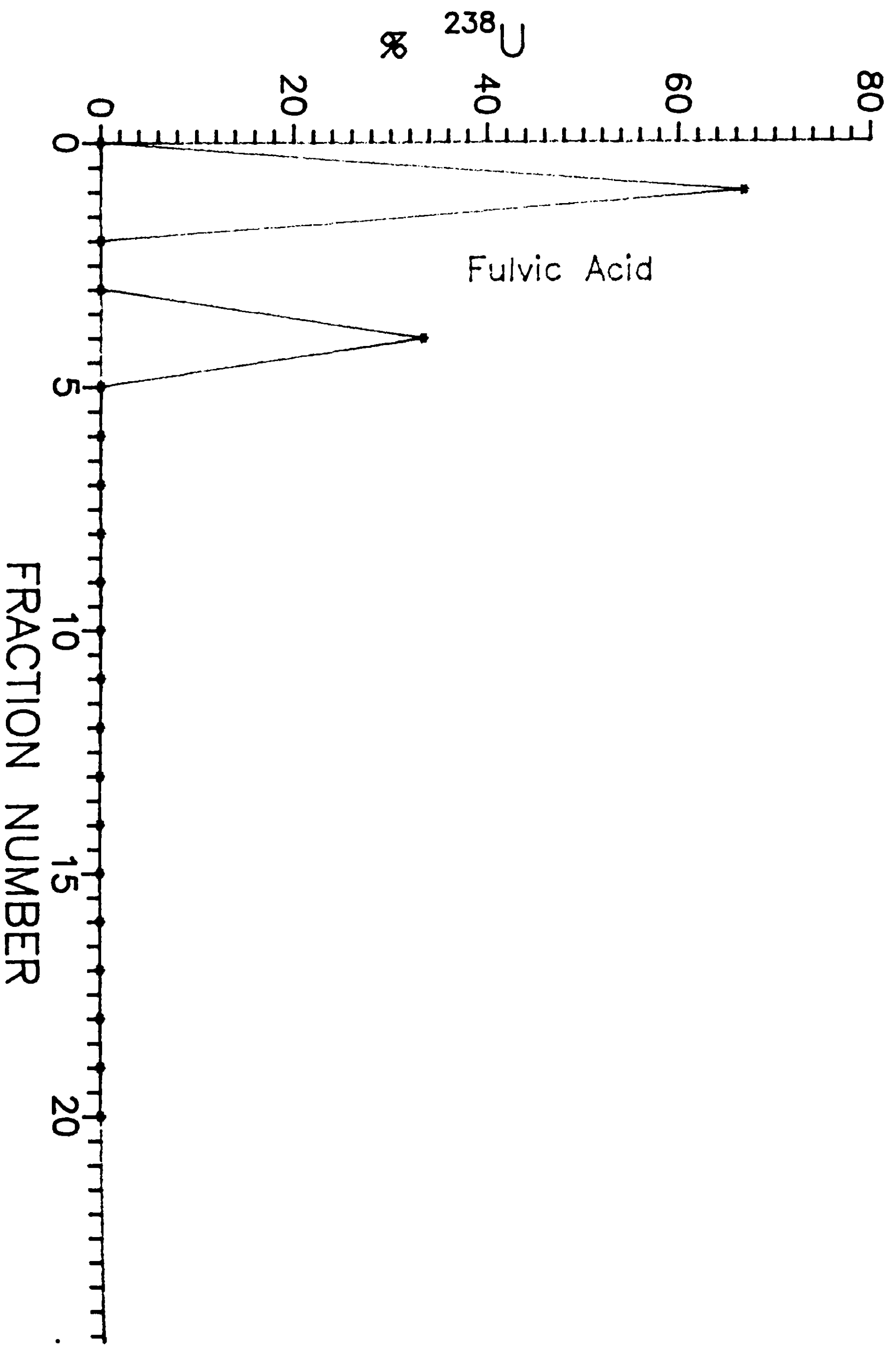


**Figure 3.3b Southwick Merse 0-5cm Fulvic Acid : Percentage Distribution of $^{238}$U**

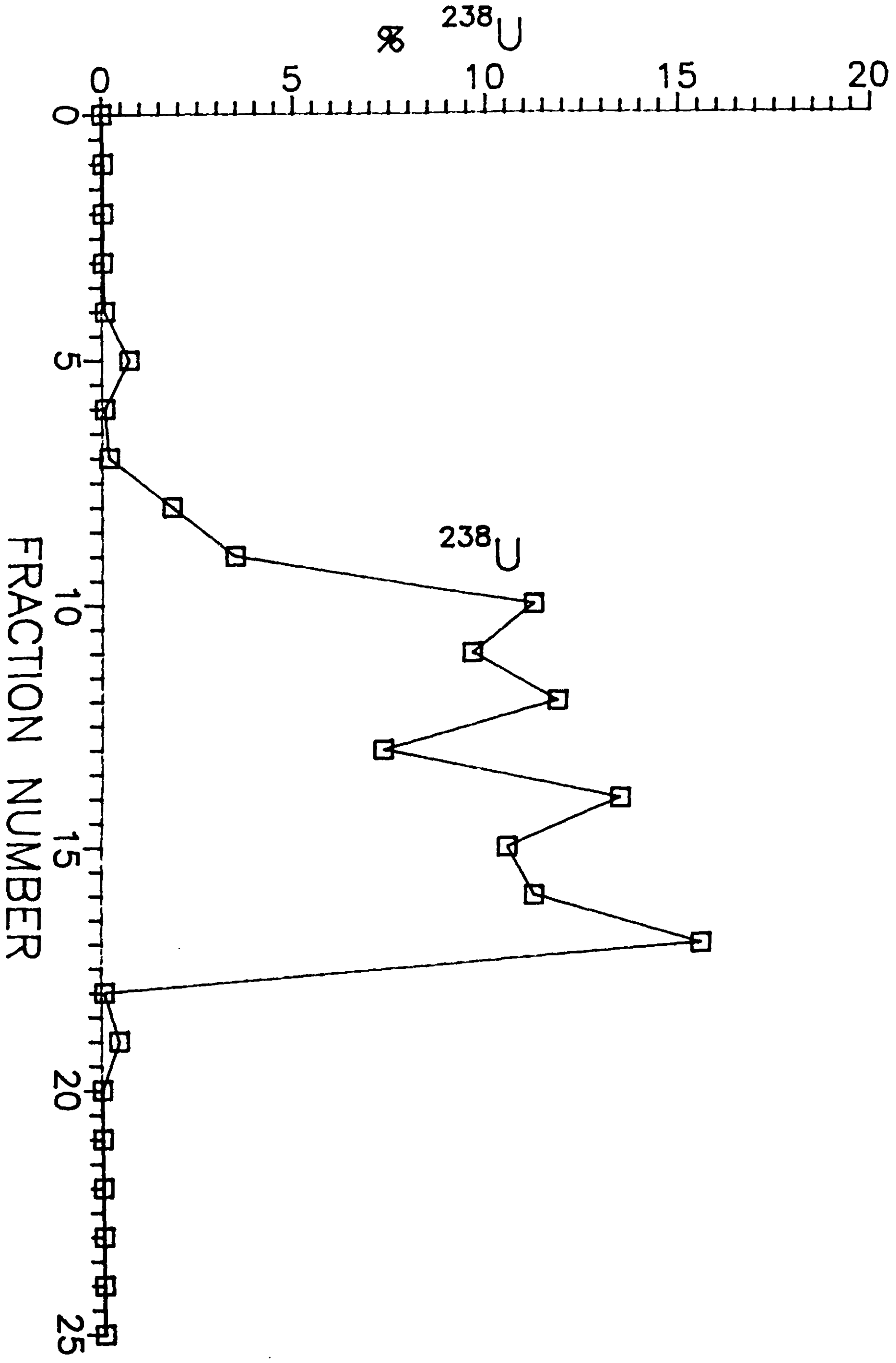


**Figure 3.4a Southwick Merse 40-45cm Humic Acid : Percentage Distribution of $^{238}$U**

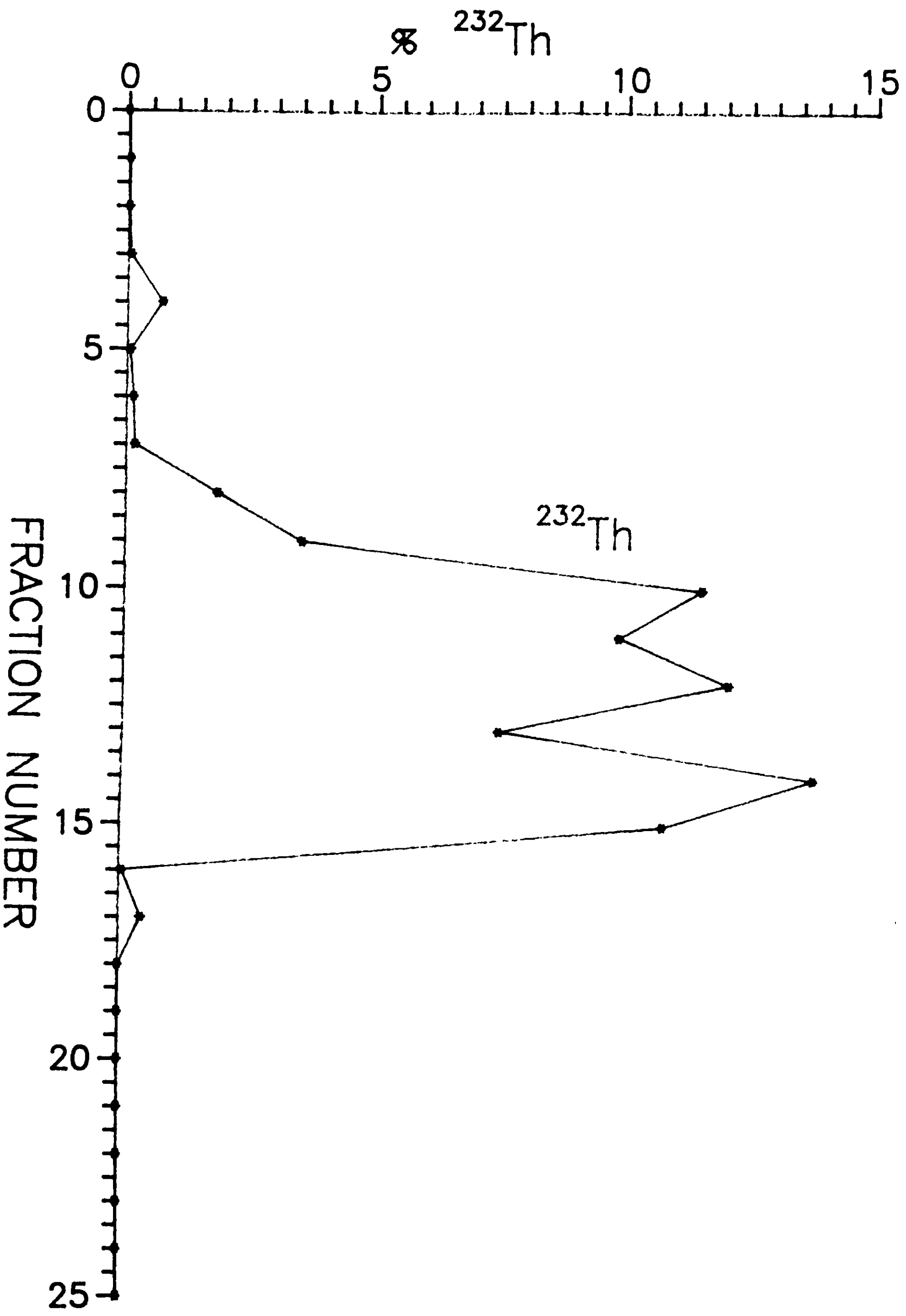


Figure 3.4b Southwick Merse 40-45cm Humic Acid : Percentage Distribution of $^{232}$Th

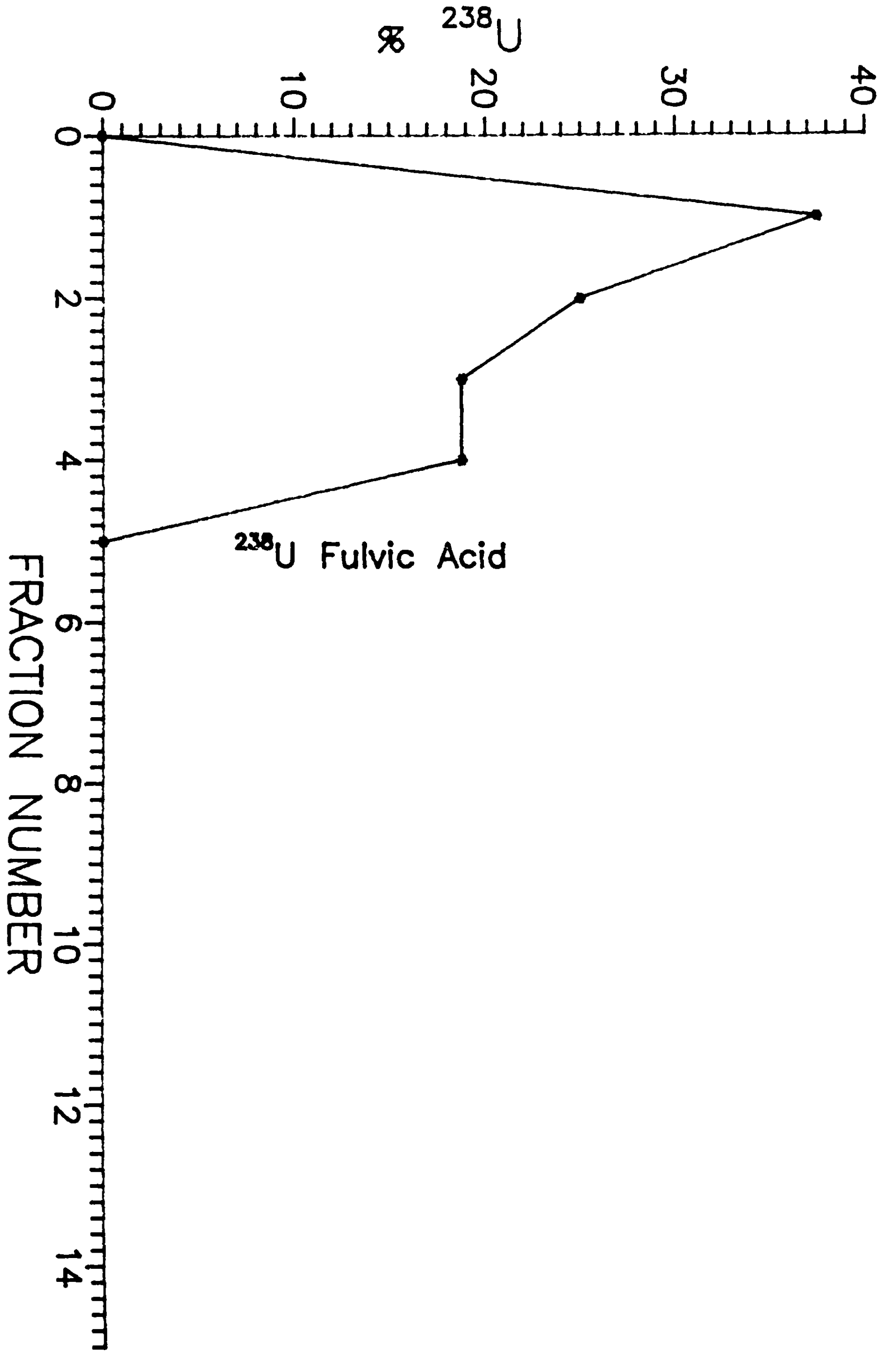


**Figure 3.4c Southwick Merse 40-45cm Fulvic Acid : Percentage Distribution of $^{238}$U**

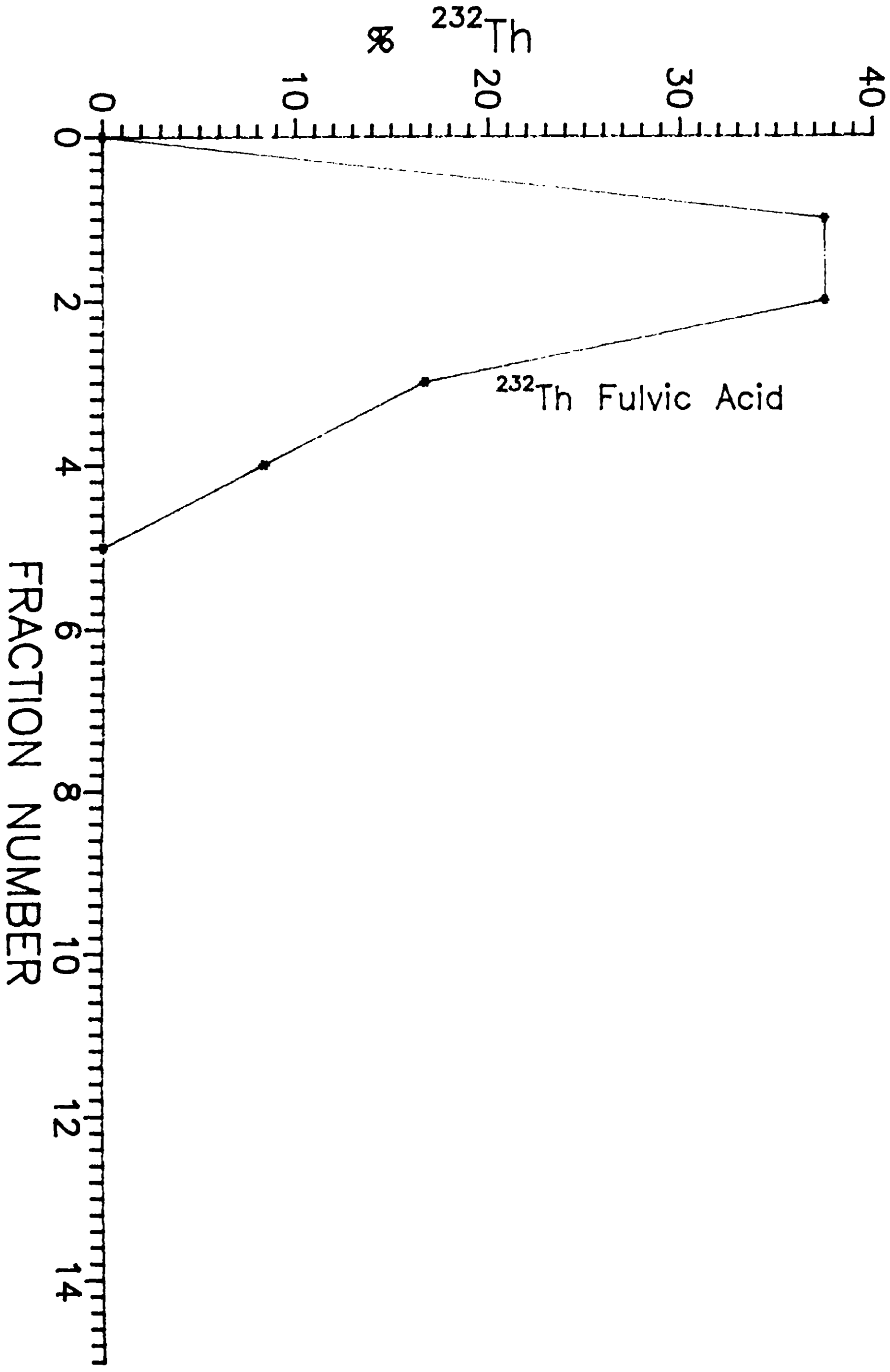


**Figure 3.4d Southwick Merse 40-45cm Fulvic Acid : Percentage Distribution of $^{232}$Th**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very high | 36.06 | 54.68 | 67.09 |
| 2 | medium | 18.62 | | |
| 3 | medium | 12.41 | 21.24 | |
| 4 | low | 8.83 | | 32.85 |
| 5 | medium | 10.52 | 24.02 | |
| 6 | medium | 13.5 | | |
| Activity | | Σ = 0.09 ± 0.01Bq (per 100mg) | | |

**Table 3.3a Esk 0-5cm Humic Acid Uranium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very high | 63.1 | 79.61 | 89.29 |
| 2 | medium | 16.51 | | |
| 3 | low | 9.68 | 14.11 | |
| 4 | very low | 4.43 | | 10.68 |
| 5 | very low | 3.17 | 6.25 | |
| 6 | very low | 3.08 | | |
| Activity | | 0.027 Bq (per 100mg) | | |

**Table 3.3b Esk 0-5cm Humic Acid Thorium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very high | 55 | 100 | 100 |
| 2 | very high | 45 | | |
| 3 | | - | - | |
| 4 | | - | | - |
| 5 | | - | - | |
| 6 | | - | | |
| Activity | | Σ = 0.19Bq (per 100mg) | | |

Table 3.4a Southwick Merse 0-5cm Humic Acid Uranium G100

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very high | 67 | 100 | 100 |
| 2 | high | 33 | | |
| 3 | - | - | - | |
| 4 | - | - | | - |
| 5 | - | - | - | |
| 6 | - | - | | |
| Activity | | Σ = 0.03Bq (per 100mg) | | |

Table 3.4b Southwick Merse 0-5cm Fulvic Acid Uranium G100

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very low | 0.3 | 1.07 | 4.38 |
| 2 | very low | 1.04 | | |
| 3 | very low | 3.31 | 44.81 | |
| 4 | very high | 41.50 | | 93.6 |
| 5 | very high | 51.40 | 52.1 | |
| 6 | very low | 0.7 | | |
| Activity | | $\Sigma = 0.169$Bq (per 100mg) | | |

**Table 3.5a Southwick Merse 40-45cm Humic Acid Uranium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very low | 0.08 | 0.91 | 6.32 |
| 2 | very low | 0.83 | | |
| 3 | low | 5.41 | 45.3 | |
| 4 | high | 39.90 | | 91.0 |
| 5 | high | 50.60 | 51.1 | |
| 6 | very low | 0.5 | | |
| Activity | | $\Sigma = 0.10$Bq (per 100mg) | | |

**Table 3.5b Southwick Merse 40-45cm Humic Acid Thorium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very high | 100 | 100 | 100 |
| 2 | - | - | | |
| 3 | - | - | - | |
| 4 | - | - | | - |
| 5 | - | - | - | |
| 6 | - | - | | |
| Activity | | $\Sigma$ = 0.08Bq (per 100mg) | | |

**Table 3.5c Southwick Merse 40-45cm Fulvic Acid Uranium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very high | 100 | 100 | 100 |
| 2 | - | - | | |
| 3 | - | - | - | |
| 4 | - | - | | - |
| 5 | - | - | - | |
| 6 | - | - | | |
| Activity | | $\Sigma$ = 0.009Bq (per 100mg) | | |

**Table 3.5d Southwick Merse 40-45cm Fulvic Acid Thorium G100**

broader radionuclide distribution is partially due to the longer time spent by these fractions on the gel prior to elution. The average molecular weight of these macromolecules could again be assumed to be lower than actinide-organic species from either of the 0-5cm samples.

Comparison of the actinide distribution patterns for 0-5cm and 40-45cm humic acid indicates that the distribution changes with depth and this is again consistent with the spectroscopic changes observed in this study (Spectrum 3.2) and the information obtained from sequential leaching studies by Allan (1993).

In every case, a simple distribution pattern is observed for U and Th. Elution patterns obtained by Livens (1989) showed that Pu and Am are also associated with humic and fulvic macromolecules eluting from the first band but additionally that there is a further association of both Pu and Am with macromolecules from the second band. Bulman (1990) also observed a bimodal distribution for Pu similar to that described by Livens (1989). From the experimental data obtained in this study :

i) it is apparent that the predominant interaction of U and Th in surface soils and sediments occurs with humic and fulvic molecules that elute in specific fractions at the exclusion volume.

ii) although U and Th in the remaining fractions may comprise a significant fraction of the total U and Th, comparison of the activity of these radionuclides relative to the concentration of humic material in each fraction suggests that the material in later fractions has a significantly lower binding capacity. This is the consistent with the FTIR spectra which indicate predominantly carboxyl as opposed to carboxylate groups and also with the observations of Rashid (1971) that not all acidic functional groups participate in metal binding.

iii) in comparison with literature data for Pu and Am (Bulman, 1990; Livens, 1989), the behaviour of U, Th, Pu and Am is characteristic of the individual element and can be generalised as :

Th > U, Pu > Am in band 1 and U > Th, Am > Pu in band 2.

## 3.5 Evaluation of Traditional Methodology Used in the Isolation of Humic and Fulvic Acids

A major concern noted by various authors (Stevenson, 1982; Schnitzer and Khan, 1972) has been the potential alteration of humic material following exposure to strong alkaline conditions. Stevenson (1972) and Farmer and Morrison (1960) concluded from FTIR spectroscopic observations that alteration of fulvic acid occurred following prolonged exposure to strongly acidic conditions (Section 1.4.5.3). In particular, the formation of a humic acid-like material upon exposure of fulvic acid to mineral acids was indicated by Stevenson (1972). The present study incorporated the investigation of both the effects of both strong alkali (section 3.2.1) and strong acid on the composition of the humic fraction of soils and sediments.

### 3.5.1 Characterisation of Humic Substances Extracted from Soils and Sediments in W Cumbria and SW Scotland

For the remainder of this study, only humic substances, as defined by the extraction procedure in section 2.3.1, have been characterised, due to the concerns expressed in the previous section but also following the results of this study for humic and fulvic acids. The characteristics of humic and fulvic acids from diverse origins are very similar (spectroscopically in terms of fractionated and unfractionated materials; also in terms of metal interactions)
If humic and fulvic acids are genuinely constituents of humic substances then the latter should exhibit properties that are a sum of those of the humic and fulvic acids. Although it could be argued that humic substances are so heterogeneous that such a comparison would be impossible, this hypothesis can be tested using gel chromatography to fractionate humic substances in a similar manner to that described above for humic and fulvic acids. This allows comparison of the

spectroscopic properties of and actinide interactions with fractions of these materials. At each stage in the following section (where appropriate) the hypothesis that the properties of humic substances are not the sum of the properties of humic and fulvic acids will be tested and the final conclusions summarised in section 3.5.1 and 3.5.2.

Humic substances were extracted from the following samples :
i) Esk 0-5cm and 10-15cm
ii) Muncaster 0-5cm and 10-15cm
iii) Southwick 0-5, 40-45 and 65-70cm.

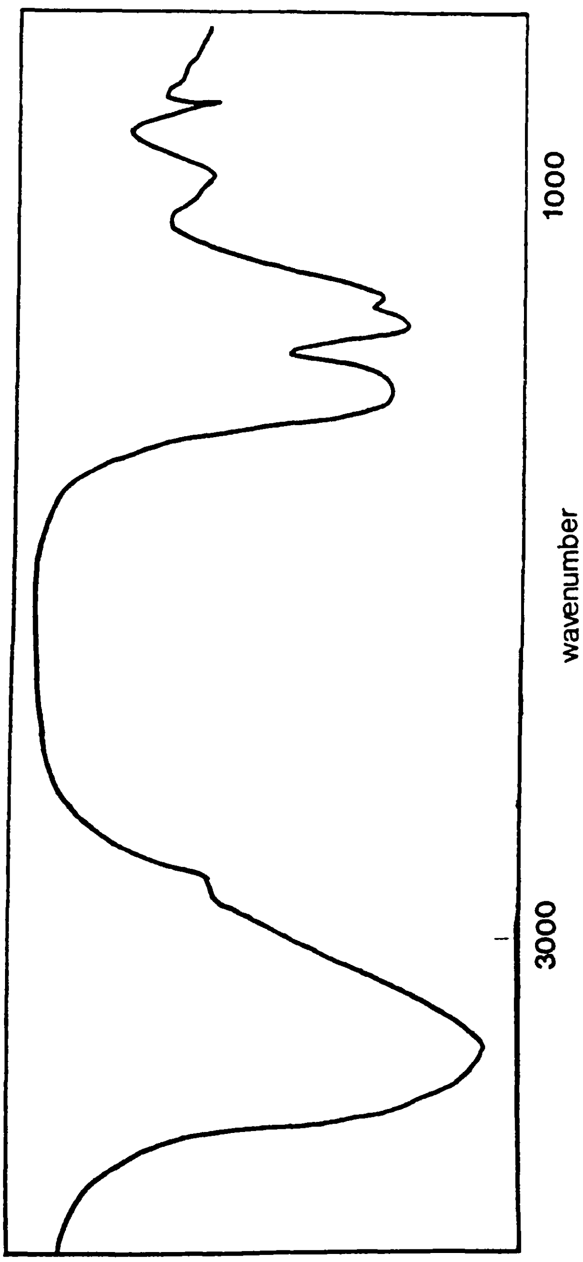
Typically humic substances had <2% mineral content following the incorporation of a second centrifugation (40min.; 3637g) of the alkaline extract (which did result in a visible residue although the dark humic extract had not previously appeared to be cloudy) so no purification steps were required. This is in strong contrast to the high mineral contents reported for humic acids which are of the order of 10 to >30% (Schnitzer, 1972; Stevenson, 1982). Fulvic acids have typically low ash contents and values quoted are often around 2% (eg. Schnitzer, 1972). A possible explanation of this is that mineral matter becomes associated with humic acid during the precipitation of humic acid under strongly acidic conditions. The presence of a measurable amount of fine non-organic particulate material suspended in solution has been observed by a second centrifugation of the alkaline extract. This is not commonly incorporated in to humic acid extraction techniques (with the exception possibly of Rao, 1994; Choppin, 1988 etc. who use redissolution, centrifugation and reprecipitation as a purification procedure) since the humic extract does not appear cloudy. Co-precipitation of the mineral material together with humic acid (ie. aggregate formation) can account for the significantly higher mineral content of humic acid relative to fulvic acid and does not necessarily indicate an association of humic acid with similar proportions of mineral material or with the same fraction of mineral material in the environment. The humic content of the samples studied detailed in Table 3.7 shows the

| **Sample** | **Loss on Ignition** |
|---|---|
| Esk 0-5cm | 12.0% |
| Esk 10-15cm | 6.6% |
| Muncaster 0-5cm | 8.0% |
| Muncaster 10-15cm | 4.9% |
| Southwick Merse 0-5cm | 5.1% |
| Southwick Merse 40-45cm | 3.9% |
| Southwick Merse 65-70cm | 4.0% |
| Offshore Sediment 0-5cm | 0.5% |

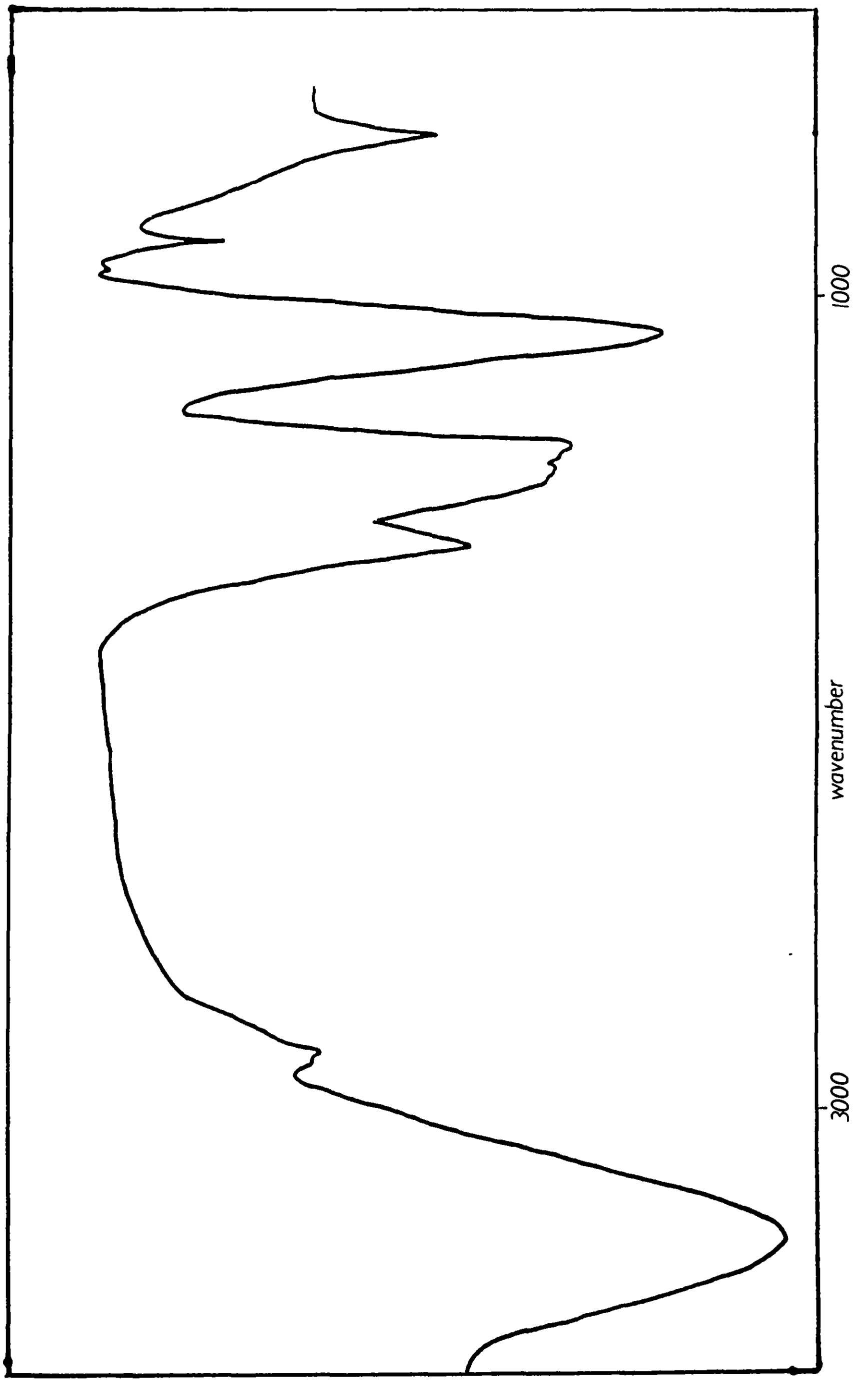
**Table 3.6 Percentage Loss on Ignition for Soils and Sediments**

| Sample | % Humic Substances | % of Total Organic Fraction |
|---|---|---|
| Esk 0-5cm | 8.0% | 67% |
| Esk 10-15cm | 4.7% | 71.2% |
| Muncaster 0-5cm | 5.5% | 54.5% |
| Muncaster 10-15cm | 3.4% | 69.4% |
| Southwick Merse 0-5cm | 1.8% | 35.3% |
| Southwick Merse 40-45cm | 2.7% | 69.2% |
| Southwick Merse 65-70cm | 2.9% | 72.5% |

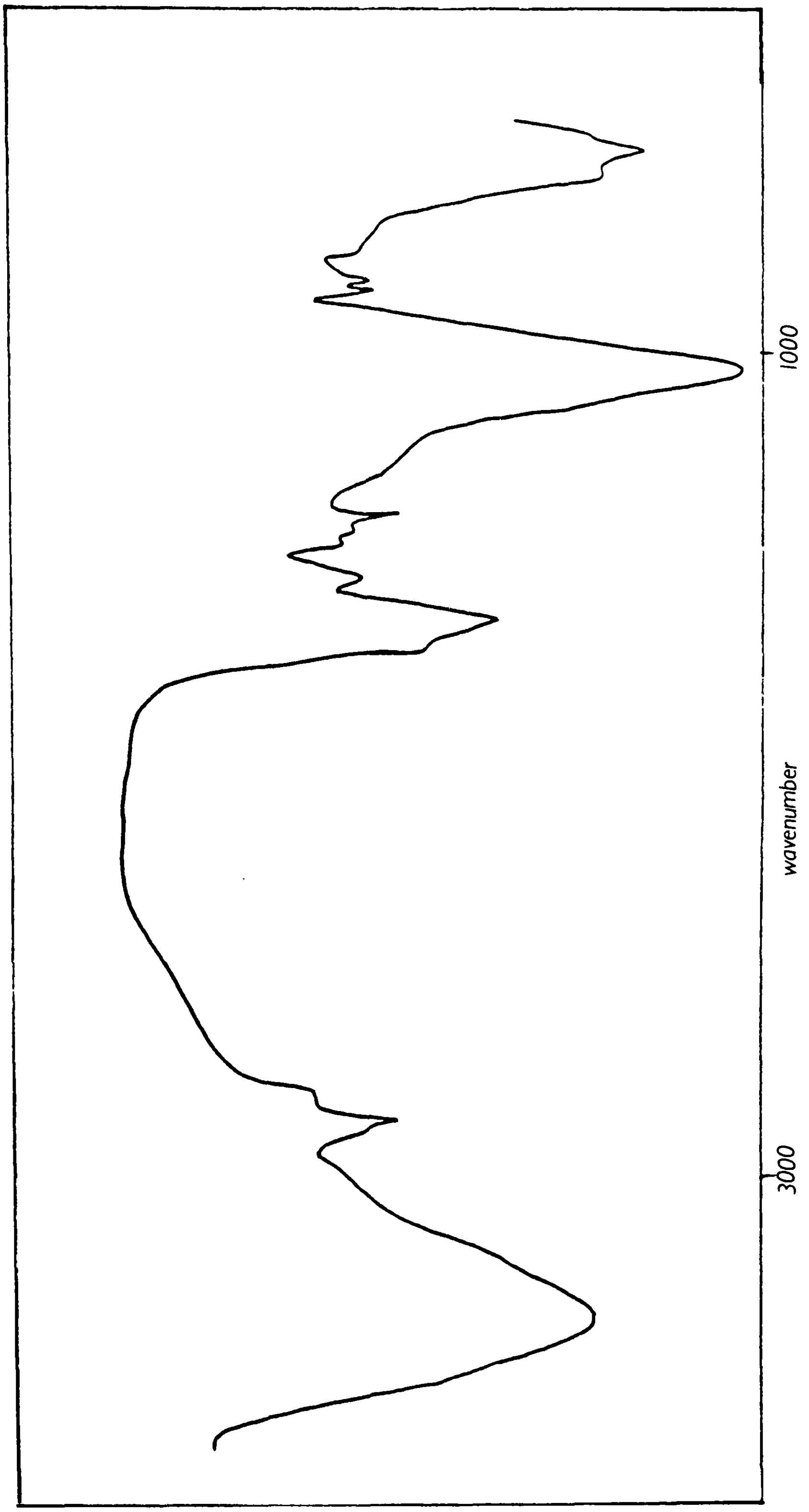
**Table 3.7 Percentage Humic Substances in Soils and Sediments**



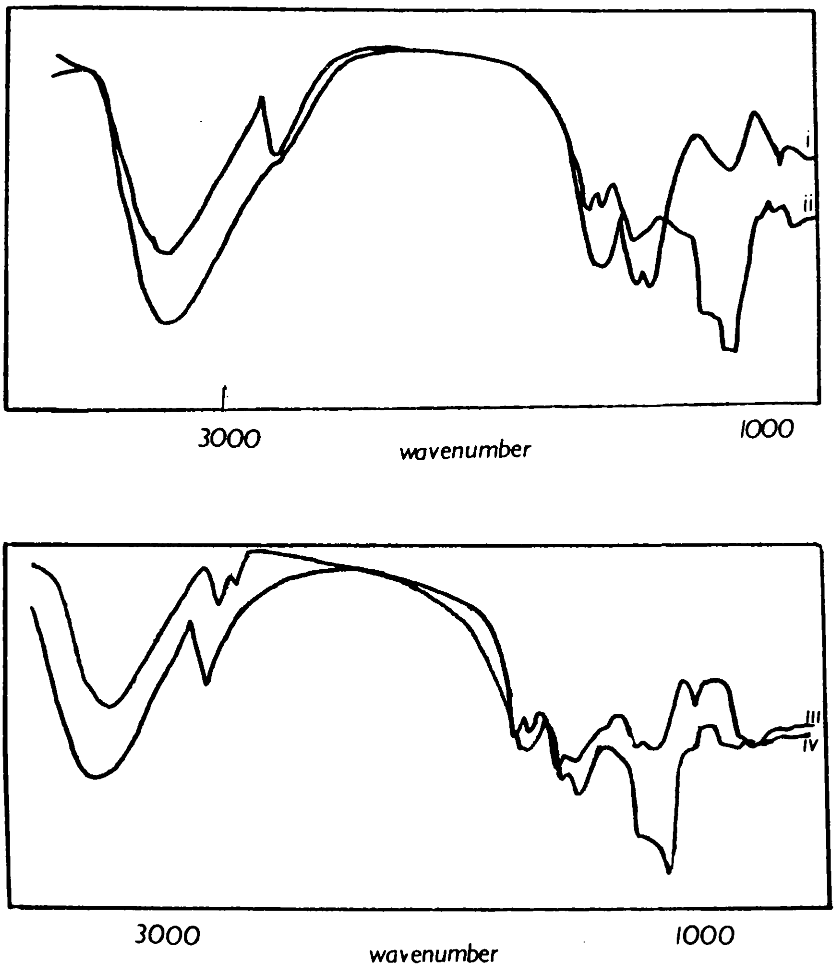
Spectrum 3.13 Humic Substances Isolated from Esk 0-5cm Soil



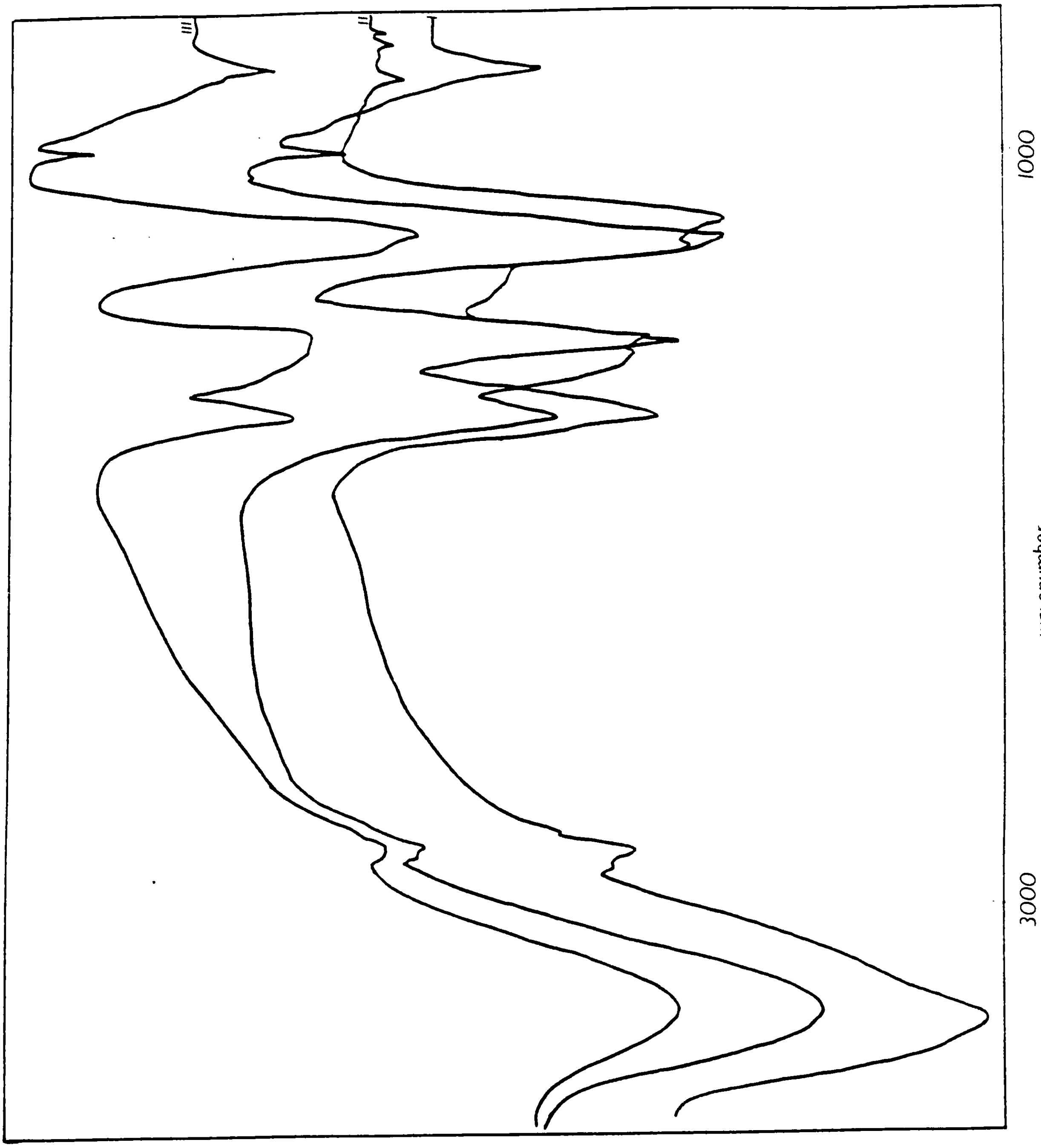
Spectrum 3.14 Humic Substances Isolated from Southwick Merse 40-45cm Sediment



Spectrum 3.15 Humic Acid Isolated from Southwick Merse 40-45cm Sediment



**Spectrum 3.16 Comparison of Humic Substances from Esk and Muncaster Isolated from 0-5 and 10-15cm Soil Samples**
**i) Esk 0-5cm ii) Esk 10-15cm**
**iii) Muncaster 0-5cm iv) Muncaster 10-15cm**



**Spectrum 3.17 Comparison of Humic Substances from Southwick Merse Isolated from 0-5cm, 40-45cm and 65-70cm Sediment Samples**
**i) 0-5cm          ii) 40-45cm     iii) 65-70cm**

following trend of decreasing content (Table 3.6 shows loss on ignition):

Esk 0-5cm > Muncaster 0-5cm > Southwick 0-5cm

A small decrease from 6% to 4% in the total organic carbon content of the Southwick Merse sediment was observed with increasing depth. Humic substances typically make up about 50-60% of the organic fraction in surface sediments. However, humic material comprised a lower proportion of the organic fraction in surface sediments than would be expected at this location but the proportion of the organic fraction comprising humic material increased with increasing depth (Table 3.6). This may have been due to the higher amount of halophytic grass material (including roots) present in the surface sediments (living biomass). In sample preparation, the removal of all vegetation was not achievable. A further observation from these results is that total amount of humic substances present at depth in comparison with the surface sediment did not vary greatly.

The extracted humic substances were characterised using a range of techniques including FTIR, UV and Fluorescence spectroscopy. FTIR spectra of humic substances from these soils and sediments are illustrated in Spectra 3.13-3.17. Broad bands are observed in all spectra of humic substances and the main absorptions are in the regions 3400-3000cm$^{-1}$, 1700-1400cm$^{-1}$ and 1200-1000cm$^{-1}$ and this general picture is similar to that of humic and fulvic acids. However, an additional absorption band at 865cm$^{-1}$ is apparent in the spectra of humic substances and can be related to the presence of substituted aromatic structures (Spectrum 3.13). No absorptions in the region <1000cm$^{-1}$ were observed for any humic or fulvic acid or any gel chromatographic fraction of these materials. This is particularly important because humic acid and fulvic acid are the only two constituents of humic substances and so this band should appear and potentially be stronger in humic and/or fulvic acid. This clearly indicates the loss or alteration of the structural features which account for this absorption in humic substances. The mechanism and justification proposed for this alteration is as follows :

i) the addition of hydrogen ions (pH 1 ; 0.1M) results in the reduction of inter and intra molecular repulsive forces enabling reactive units to approach more closely

ii) all humic substances contain a proportion of aromatic units (minimum 15% for marine humic materials) which are highly substituted. These types of compounds readily undergo coupling via O or C atoms and it is proposed that acid induced condensation is the chemical route to such products.

iii) the proximity of strands of the same molecule mean that this coupling may occur within and/or between humic molecules. The outcome is therefore likely to include a high proportion of molecules which are highly condensed (crosslinking) with a predominantly aromatic core and may have longer aliphatic side chains (with associated functional groups) predominantly on the exterior.

iv) since two bands are also observed for fulvic acid this process also occurs but to a lesser extent which can be explained in terms of the higher functionality of molecules which remain in the fulvic acid fraction (the inter and intra molecular distances will be larger). This would be consistent with the observation of the lower aromaticity of fulvic acid.

v) the effect on fulvic acid of prolonged exposure of acid is less readily explained in terms of colloidal principles although the precipitation of a humic acid like residue is supportive of a condensation type mechanism. Kinetic considerations might lead to the assumption that the replacement of cations with $H^+$ ions results, over prolonged periods, in a rearrangement of metal or $H^+$ ions within the humic material resulting in conformational change and subsequently condensation of the molecules in the fulvic acid fraction.

vi) it has been suggested that the first band eluted at the exclusion volume for humic acid contains molecules with a highly aromatic core in agreement with the results of Posner (1963). In order to account for this observation, Posner (1963) suggested that the presence of two bands on the gel column was indicative of two starting materials in the formation of humic substances : a high molecular weight, dark brown component derived from only partially degraded lignin and a low molecular weight, lighter brown component comprising easily decomposed plant material and degraded components of the high molecular weight molecules. This study suggests that the two bands are the result of a procedural artefact and this is discussed further in section 3.5.2.

Moreover, a comparison of humic acid and humic substances from Southwick Merse shows that absorptions occur at significantly different wavenumbers. This is partially due to the form of the humic material since the humic substances, although dialysed, are not in "acidified" form and so little absorption would be expected at 1720cm$^{-1}$. The remaining differences cannot be accounted for without invoking a chemical alteration of the humic material arising from the extraction procedure.

A comparison of humic material extracted from soils and sediments from different depths at the same location shows that there is a consistent loss of oxygen-containing functional groups with increasing depth and again indicates that the humic fraction is subject to degradation with increasing time after its formation (Spectra 3.16 and 3.17). A comparison of humic substances from the three locations also shows that there are characteristic differences between these humic materials . Although, it is not possible to directly relate these differences to specific chemical differences, it would appear that the main features occur at different wavenumbers, are present in differing amounts and in general represent more aliphatic structures for the marine influenced Merse humic material when compared with the more highly terrigenous Esk humic materials. Further differences are apparent when surface and depth humic materials are compared indicating loss of important oxygen containing functional groups and comparison between sampling locations shows that these differences are characteristic of the prevailing conditions. Therefore this study shows not only that humic materials from different origins are compositionally different but also that prevailing conditions give rise to highly characteristic degradation of the humic fraction with increasing time (Spectra 3.16 and 3.17). In summary of the results presented in this section :

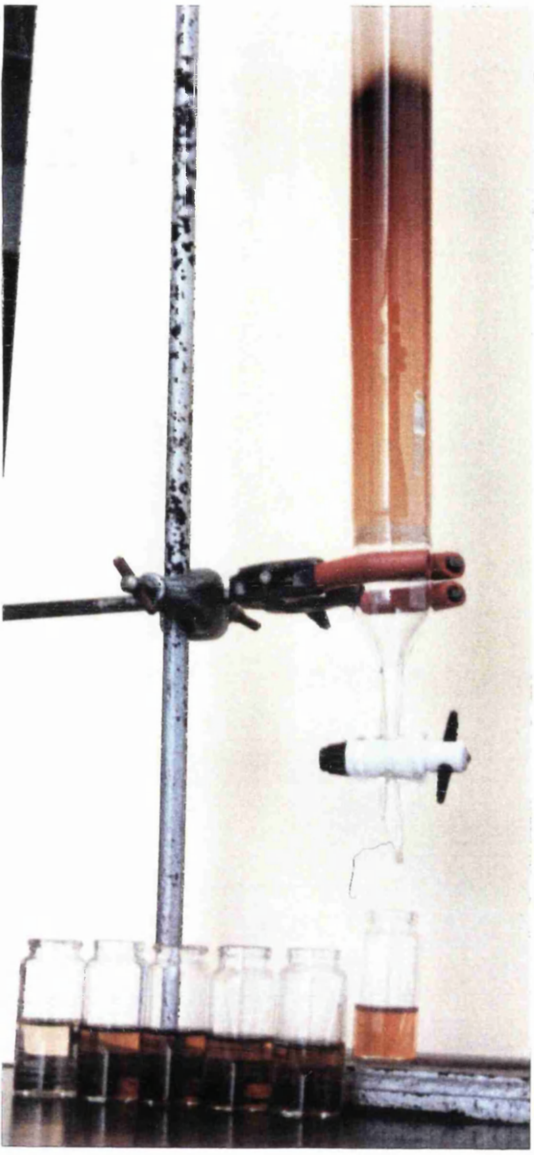
i) humic substances do not exhibit FTIR spectral characteristics that are a sum of humic and fulvic acids.
ii) humic substances from different environments exhibit different characteristics as would be expected from the diverse range of source materials (this will become more apparent in following sections). This contrasts with humic and fulvic acids which exhibit similar characteristics suggesting that the chemical diversity is lost by chemical alteration of humic materials.

iii) the results provide direct evidence of the alteration as a result of the chemical separation of humic and fulvic acids.

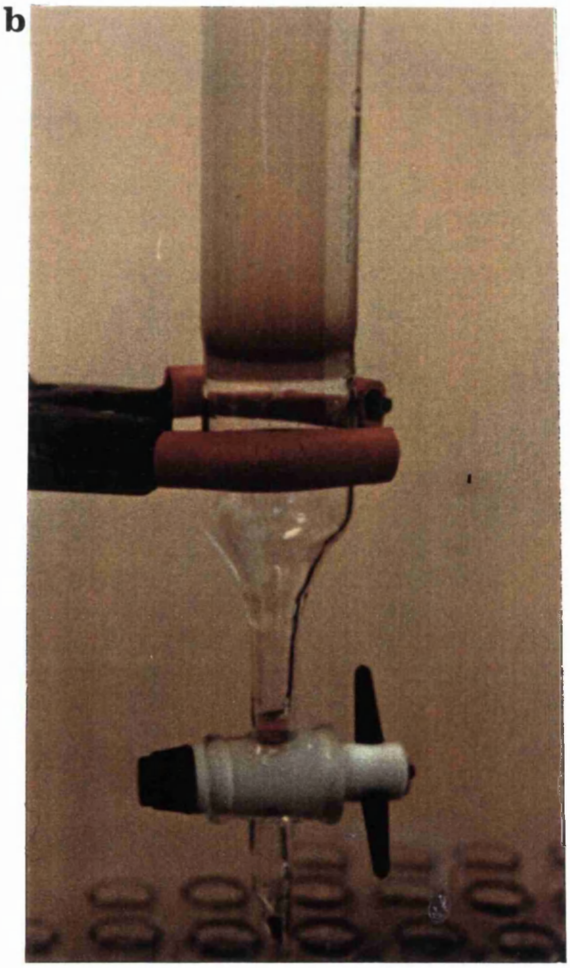
### 3.5.2 Charactacterisation of Gel Chromatographic Fractions of Humic Substances using FTIR Spectroscopy

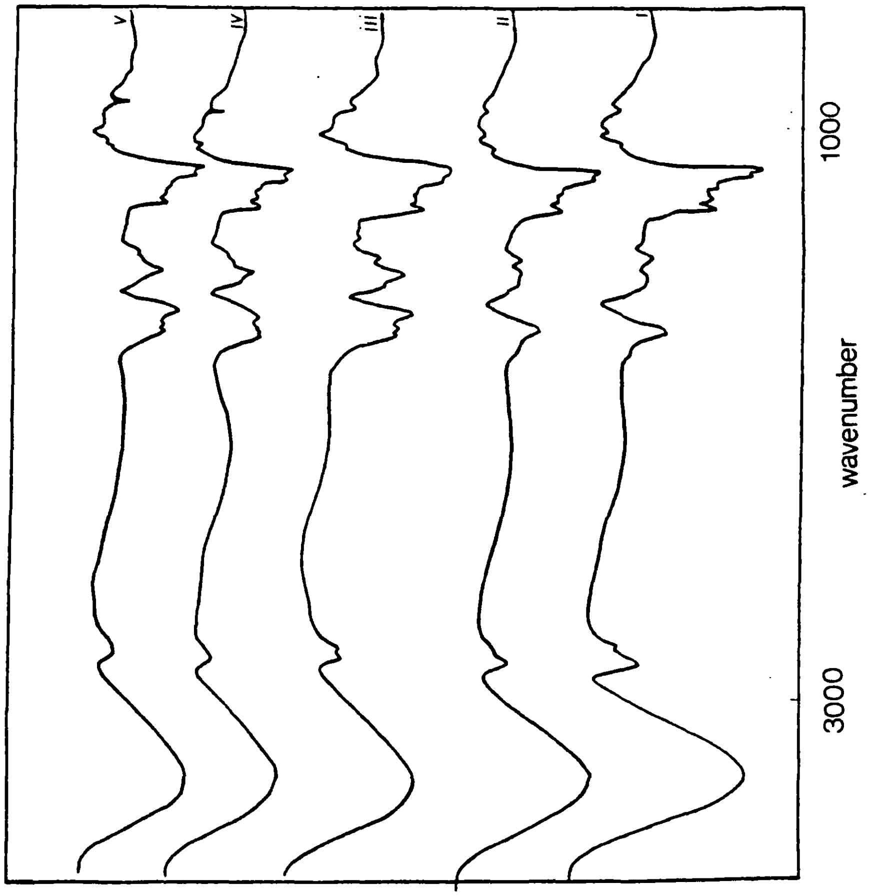
Fractionation of humic substances from a given location, on a G100 Sephadex gel column generates an elution pattern that is visibly different from that observed for either humic acid or fulvic acid (Plate 3.1a). The characteristic two band pattern of the latter materials is not observed for humic substances but, instead, a continuum of macromolecules is observed with a gradation in colour. In the separation of fractions of humic substances, initially paler fractions are eluted followed by gradually darker fractions (Plate 3.2a). The intense colour of the upper end of the sample band observed early in the fractionation procedure decreases slightly as the molecules move down through the gel indicating that a degree of band smearing is occurring but later fractions are darker and this is reflected in their UV absorbance (Plate 3.2b)(Spectrum 3.32). It is important to note that the intense dark band observed in humic or fulvic acid fractionation at the leading edge of the sample band is completely absent and, consequently, no dark coloured fractions are eluted at the exclusion volume (Plate 3.1c). The dark colour of humic materials is reported to be associated with condensed, lignin type structures and on the basis of these observations it is proposed that the source of the intense colour in humic and fulvic acids is an artefact, arising from chemical

**a**



**b**





**Spectrum 3.18** Gel Chromatographic Fractions of Esk 0-5cm Humic Substances using Sephadex G100 Gel
i) - ii) Early Fractions iii) - v ) Later Fractions

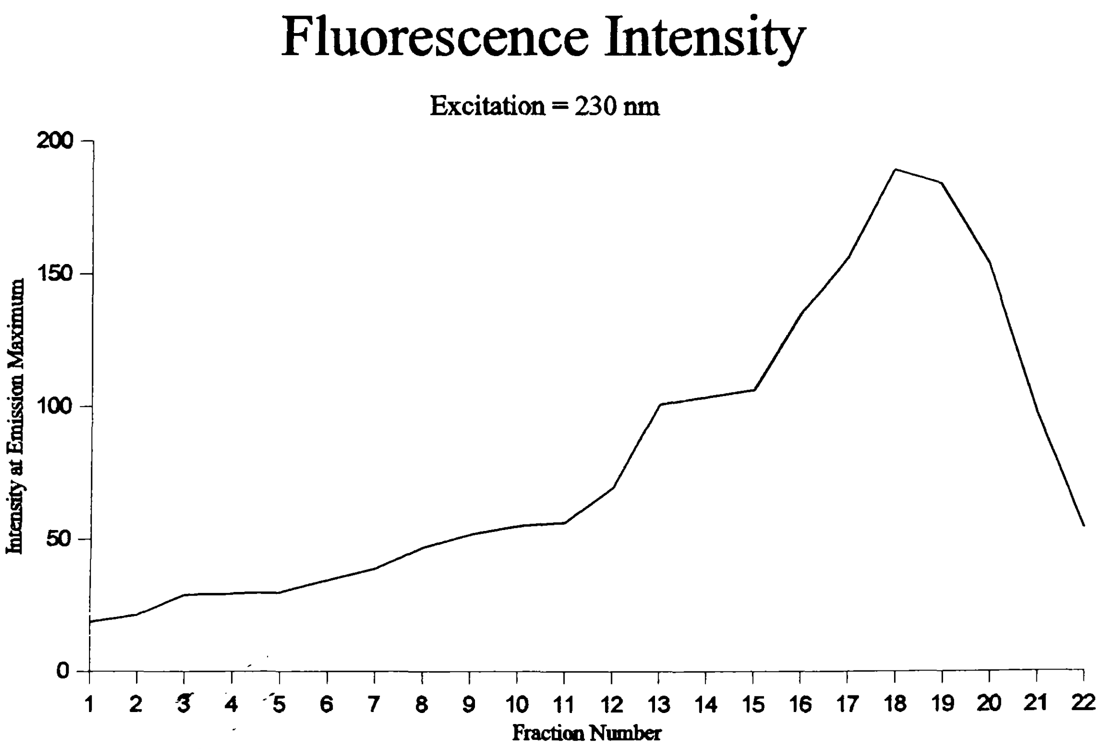
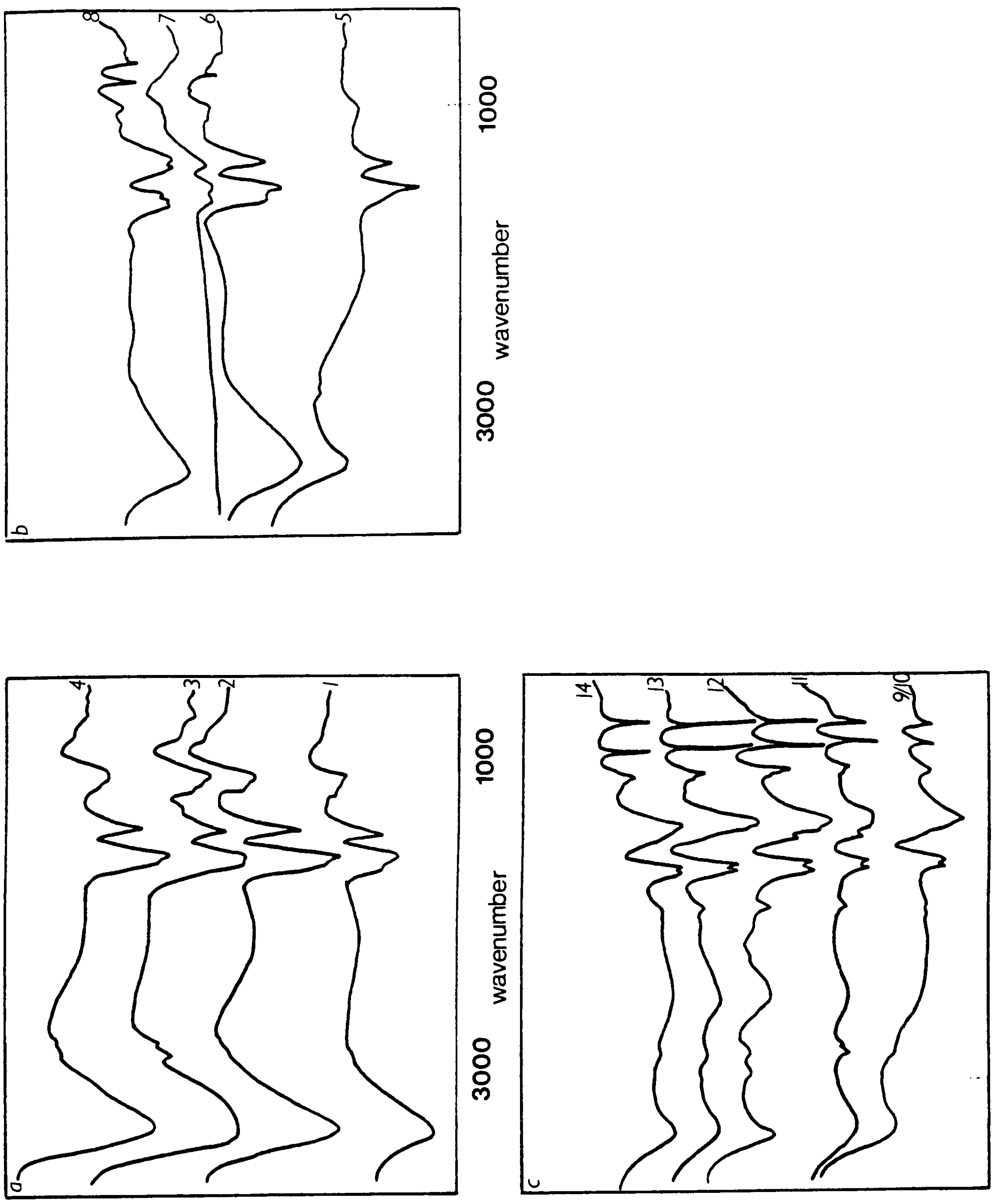
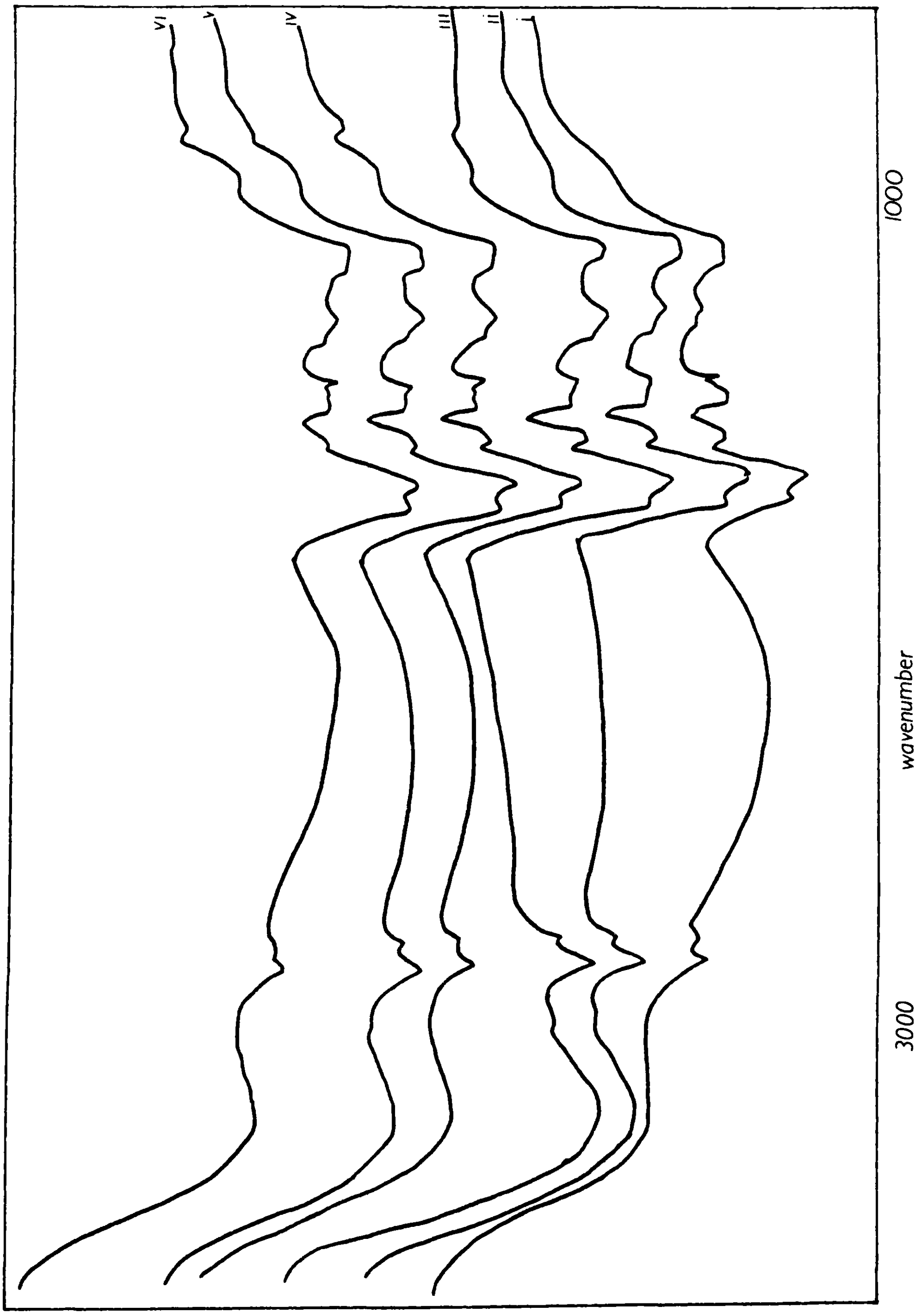


**Figure 3.5 Fluorescence Intensity at Emission Maximum : Gel Chromatographic Fractions (G100 Sephadex) of Humic Substances**

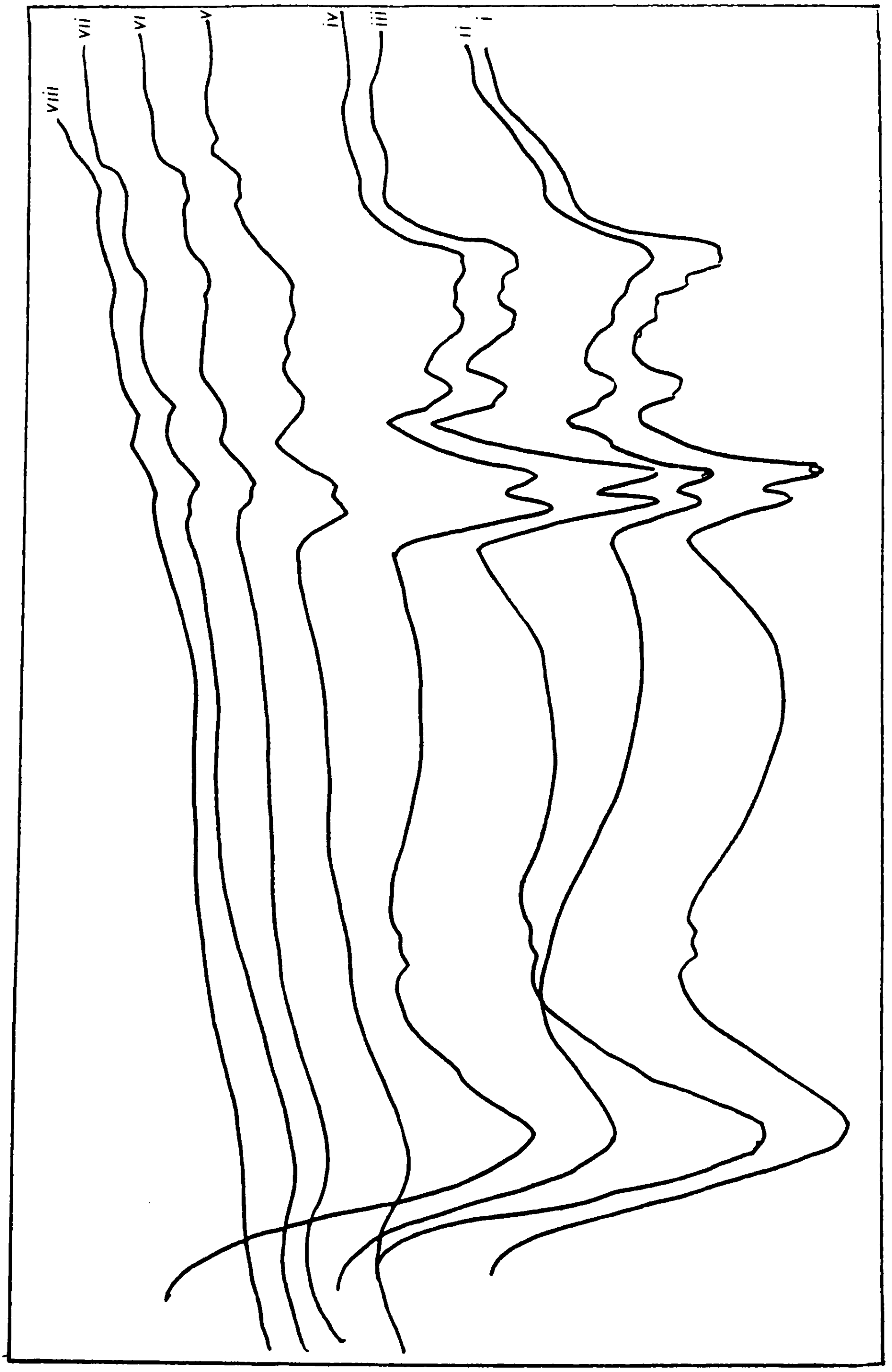


**Spectrum 3.19a b c Gel Chromatographic Fractions of Esk 0-5cm Humic Substances using Sephadex G25 Gel**
**a Fractions 1-4  b Fractions 5-8  c Fractions 9/10, 11-14**

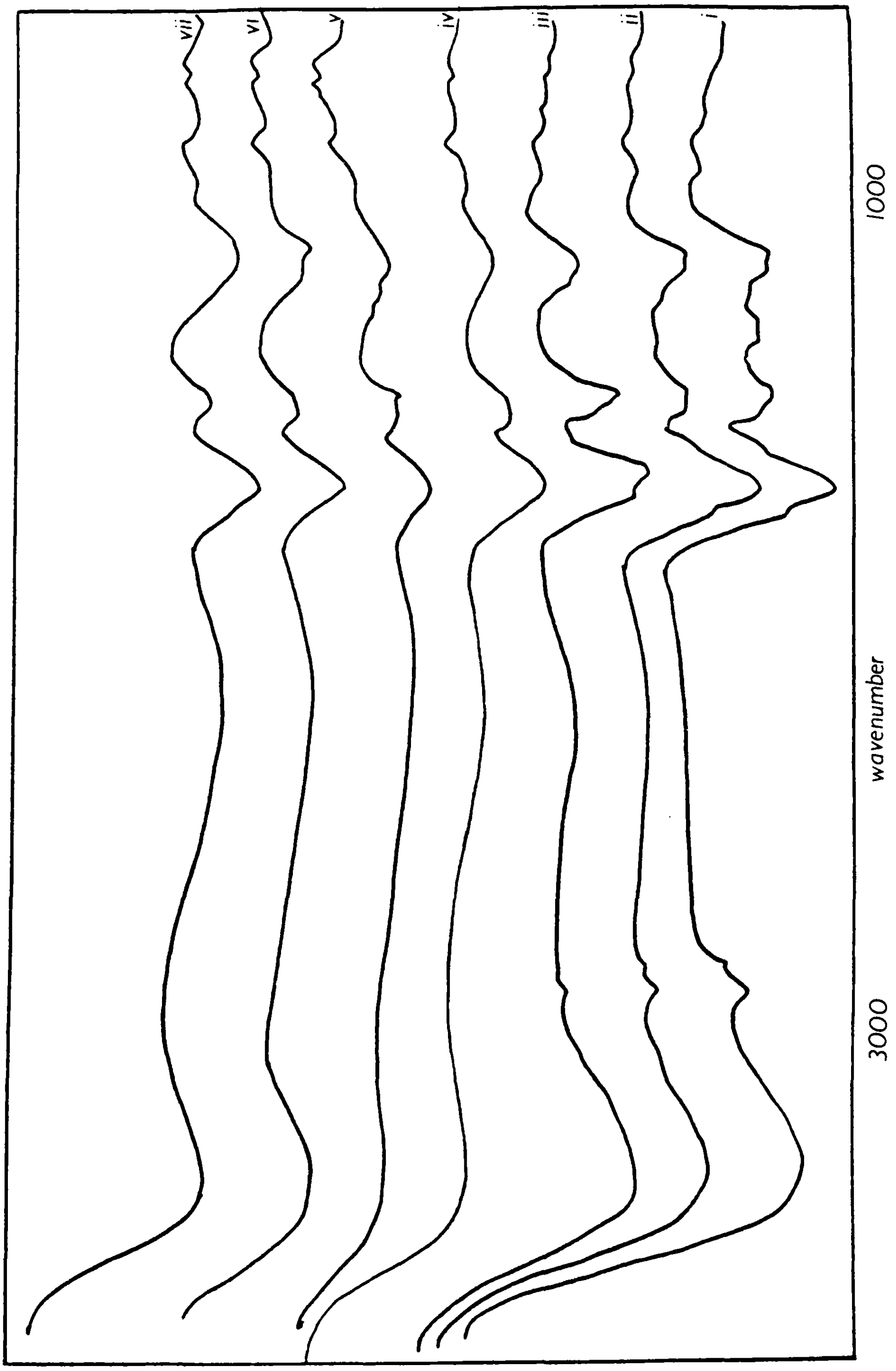


Spectrum 3.20a Gel Chromatographic Fractions of Esk 0-5cm Humic Acid using Sephadex G25 Gel Where Humic Acid and Fulvic Acid were Chemically Separated After Elution of Humic Substances from the Gel Column

i) Fraction 1 ii) Fraction 2 iii) Fraction 3
iv) Fraction 4 v) Fraction 6 vi) Fraction 8



**Spectrum 3.20b Gel Chromatographic Fractions of Esk 0-5cm Humic and Fulvic Acids using Sephadex G25 Gel Where Humic and Fulvic Acids were Chemically Separated After Elution of Humic Substances from the Gel Column**
**i) - iv) Fulvic Acid Fractions 2, 4, 6 and 8**
**v) - viii) Humic and Fulvic Acid Fractions 10, 12, 14 and 16**



Spectrum 3.20c The Effect of pH on the Composition of Humic Acid Precipitated from a 0.1M NaOH Solution

i) pH 7 ii) pH 6 iii) pH 5 iv) pH 4

v) pH 3 vi) pH 2 vii) pH 1

procedures during isolation.

Spectroscopic studies of gel chromatographic fractions of humic substances using Sephadex G100 gel under identical conditions to those used for humic and fulvic acids show that there are compositional differences between fractions (Spectrum 3.18). Moreover, these differences are not related to those observed for humic and fulvic acids. The increased resolution of humic spectra as a result of fractionation has allowed the observation of fine structure in absorptions in the regions 1660-1500cm$^{-1}$ and 1400-1300cm$^{-1}$. The shapes of these peaks are characteristic of aromatic ring structures and these are clearly only present in later fractions. This information together with the presence of a sharp absorption in the region <1000cm$^{-1}$ confirm that these fractions contain substituted aromatic units. Their presence only in later fractions immediately suggests that the fractionation procedure occurring on the gel column may not be simply due to a size separation since it is well-known that simple aromatic compounds are strongly retarded on Sephadex gels (Hayes, 1985). Additional information relating to the presence of aromatic units was obtained from the intensity of fluorescence displayed by macromolecules from these fractions. Figure 3.5 shows that there is a marked increase in the fluorescence intensity for humic macromolecules in later fractions and this correlates well with the presence of the aromatic structures identified by FTIR spectroscopy.

In order to investigate the separation procedures, Sephadex G25 was used to fractionate humic substances from the 0-5cm Esk soil. The smaller pore size of this gel results in the exclusion of a high proportion of the humic material and as a result the spectra of early fractions are relatively broad (Spectrum 3.19a). The humic macromolecules which are not excluded from the gel pores and are eluted at larger volumes are increasingly aromatic in character. This is discussed further in section 3.5.3.

A corresponding experiment involving the separation of humic and fulvic acids following gel fractionation using G25 Sephadex gel showed that the features relating to the aromatic units in spectra of humic substances were again absent in those of humic and fulvic acid (Spectrum 3.20a and b). Half of each fraction was acidifed resulting in the precipitation of humic acid leaving fulvic acid in solution. However, humic and fulvic acid could only be separated from the early fractions due to the small amount of humic material present in later fractions. From the spectra of humic acid it is apparent that the result of humic acid precipitation is a material of fairly uniform composition. Overall, only three significantly different compositions are observed for all spectra in strong contrast to those of humic substances from the same fractions. These compositional differences are identical to those identified for the two bands and intermediate material of fractionated humic and fulvic acids which resulted from the chemical treatment of humic substances prior to gel fractionation. However, the change from carboxyl in early fractions to carboxylate in later fractions was not observed. Spectra of later fractions which contained small amounts of humic and fulvic acid gave rise to some extent to spectra similar to those of band 1. The features of the spectra were simply consistent with those of humic or fulvic acids but not with humic substances.

A number of conclusions can be drawn (or substantiated; see previous sections) from the results of this experiment:

i) the FTIR spectroscopic characteristics of humic substances are not the sum of humic and fulvic acids;

ii) the results provide indisputable evidence of the alteration of humic and fulvic acids;

iii) the mechanism proposed above for the condensation of humic molecules would require to be further tested by the measurement of the average molecular weight of molecules in the fractions containing humic substances and those containing acid treated molecules; however, careful validation of a selected technique to determine accurately molecular weights of humic materials would be required and was beyond the scope of this project;

iv) from the results of this study it is suggested that the two bands observed during the fractionation of both humic and fulvic acids are simply artefacts of the procedures involved in the chemical separation of humic and fulvic acids. More precisely, the observation of only three different spectra for any humic or fulvic acid fraction leads to the explanation of the presence of the two bands. All humic acid molecules giving rise to a spectrum of type I co-elute during gel fractionation of humic acid and lead to the observation of band 1 on the gel column. A similar argument holds for band 2 and also for fulvic acid.

Additional evidence of the alteration of the humic material was obtained using UV/visible spectroscopy. The $E_4/E_6$ ratio (the ratio of the absorbances at 465 and 665nm) for humic acid, fulvic acid and humic substances and their respective gel fractions are shown in Table 3.8. The lowest ratios are observed for humic acids indicating a more highly condensed structure. The highly quenched nature of fluorescence spectra of fractions of humic acid confirm this observation (Spectrum 3.27 a and b). Additionally, the lowest values of the ratio coincide with the position of the two main bands on the gel column and this, together with the information presented in the preceding paragraphs, indicates that a condensation reaction involving substituted aromatic units occurs during the acid precipitation of humic acids. By comparison, humic substances have a substantially less condensed structure than either humic or fulvic acids (Table 3.8 and Spectra 3.27 and 3.28).

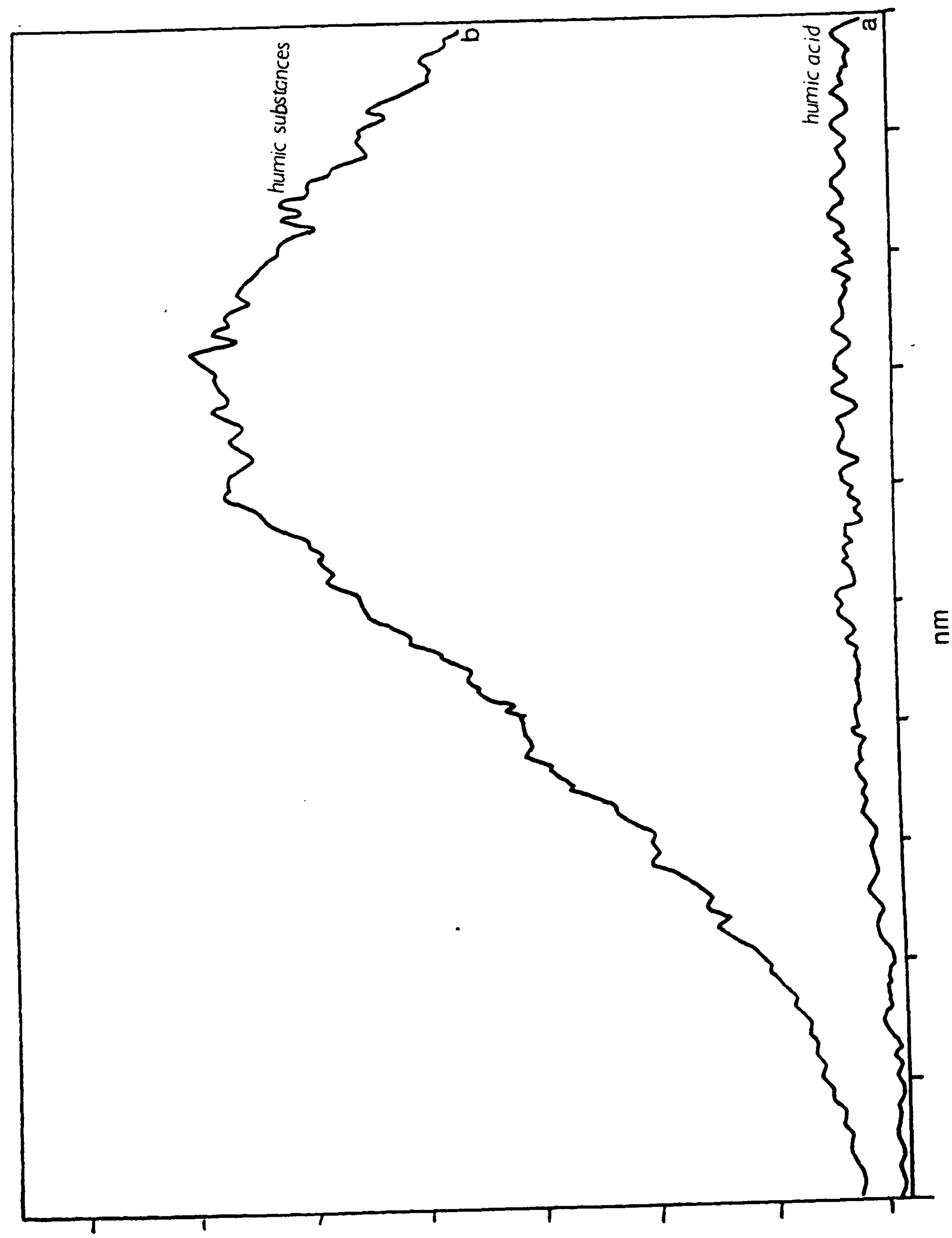
The higher $E_4/E_6$ ratios observed for intermediate fractions between bands 1 and 2 of fractionated humic acid are consistent with the decreased functionality observed in their FTIR spectra.

CHN data for fractions from band 1 of a humic acid also show that significantly higher proportions of oxygen are present in the fractions corresponding to band 1 when compared with the fractions which elute immediately afterwards (Figure 3.6).

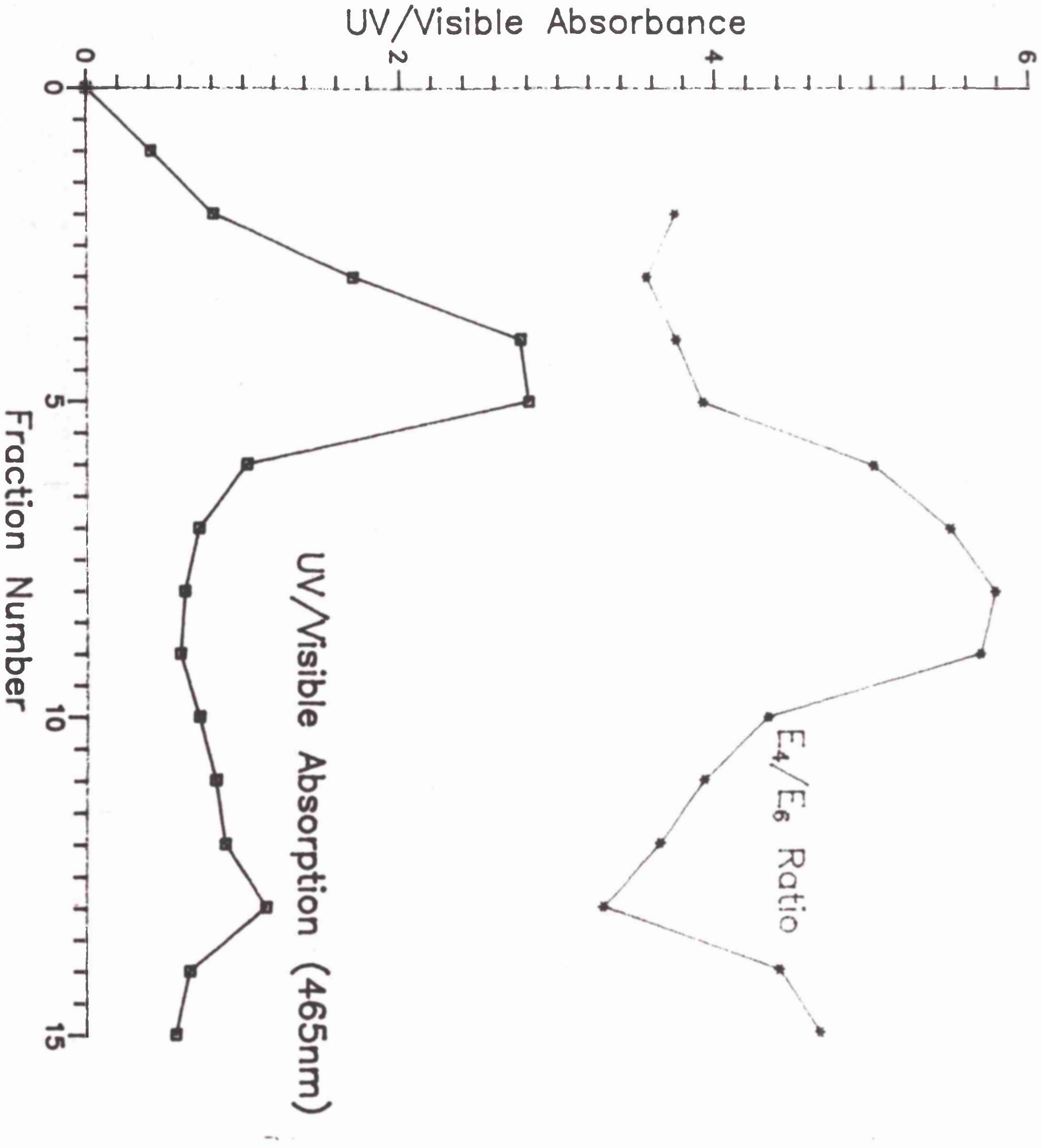
Further conclusions from this additional information are :

| Sample | $E_4/E_6$ Ratio |
|---|---|
| Humic Acid | 4.1 |
| Humic Acid (Gel Fractions) | 3.25-5.75 |
| Fulvic Acid | 6.9 |
| Fulvic Acid (Gel Fractions) | 6.0-8.5 |
| Humic Substances | 9.95 |
| Humic Substances (Gel Fractions) | 4.13-16.1 (av. = 10.15) |

**Table 3.8 $E_4/E_6$ Ratios For Humic and Fulvic Acids and Humic Substances**



Spectrum 3.27 Comparison of Fluorescence Spectra of the Gel Chromatographic Fractions of Humic Acid and Humic Substances



Spectrum 3.28 E$_4$/E$_6$ Ratio for Gel Chromatographic Fractions of Humic Acid

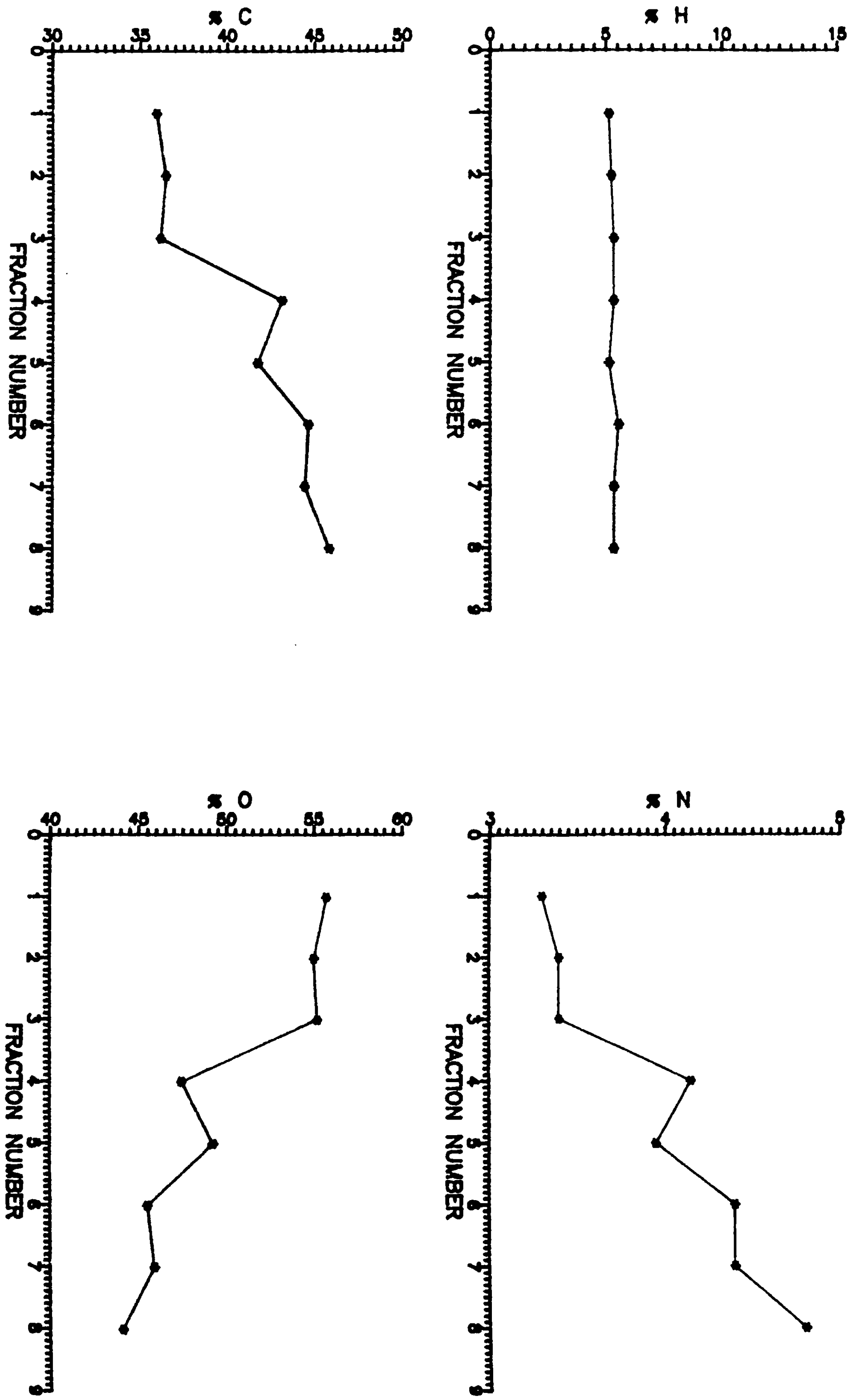


**Figure 3.6 Elemental Data (CHN) for Gel Chromatographic Fractions of Southwick Merse Humic Acid 0-5cm**

i) the molecules of humic acid in bands 1 and 2 have significantly more condensed structures than any fractions of humic substances supporting the mechanism of alteration;

ii) the characteristics of humic substances are clearly not the sum of those of humic and fulvic acids;
iii) the humic acid molecules in fractions between bands 1 and 2 have less condensed structures and this may be related to their lower oxygen content identified using CHN analysis. In particular, these molecules have fewer of the functional groups suitable for condensation reactions and therefore they elute potentially according to their original properties. This would be consistent with the conclusions drawn by Powell and Town (1990) who suggest that some humic molecules undergo irreversible aggregation, others undergo reversible aggregation whilst a proportion are unable to interact in this manner.

### 3.5.3 Molecular Weight Determination of Humic Substances using Sephadex Gels

Studies of the molecular weight distribution of humic and fulvic acids have included the use of gel chromatography. This involves the calibration of the gel column using protein or dextran standards as described in section 1.4.4.7. A number of problems were discussed including the inapplicability of the available standards whose molecular shapes are unsuitable for comparison with humic substances. Additional problems arise where there are interactions of the solute with the gel. The results of the gel chromatographic fractionation of humic substances have clearly shown that in part a chemical separation of components has occurred. The degree of chemical interaction with the gel is affected by the aliphatic/aromatic nature of the humic material and also by the gel type as is illustrated below.

The retardation of humic macromolecules containing aromatic units which was apparent for fractionation on G100 Sephadex gel is greatly enhanced by the use of G25 Sephadex gel. This can be explained in terms of the structure and pore size of the G25 gel since the more rigid gel structure and significantly smaller pore size result in the increased probability of humic interactions with the gel and, in particular, the ether linkages of the gel structure. From this information, it is clear that the Sephadex gels are unsuitable for the determination of the average molecular weight or molecular weight distributions of humic substances which have a significant proportion of aromatic units (eg. terrigenous humic materials).

The effect of humic composition on the resultant separation is discussed further in section 3.7

### 3.5.4 The Effect of pH on Actinide Distribution between Humic and Fulvic Acids

The effect of pH on U and Th distribution between humic and fulvic acids was investigated for :

i) Esk 0-5cm

ii) Needle's Eye 0-5cm.

The precipitation of a representative humic acid is generally accepted to occur at pH<2, so this study involved the analysis of humic acids precipitated at pH values from 3 down to 0.4 (Figures 3.7 and 3.8). The ratios under consideration here are mass ratios. A trend of increasing U/Th ratio for FA and decreasing U/Th ratio for HA with decreasing pH is observed for both samples. The larger ratios for Needle's Eye humic acids are consistent with the enhanced levels of U associated with the humic fraction due to the binding of leached U from nearby mineralisations at this location. This is consistent with the observation of Th values in excess of U for early gel fractions of humic acid whilst the total U value remains in excess of Th. This is important since it is the first indication that the alteration of the distribution of actinide elements occurs during the chemical separation of humic and fulvic acids. Further evidence of such alterations is presented in the sections which follow.

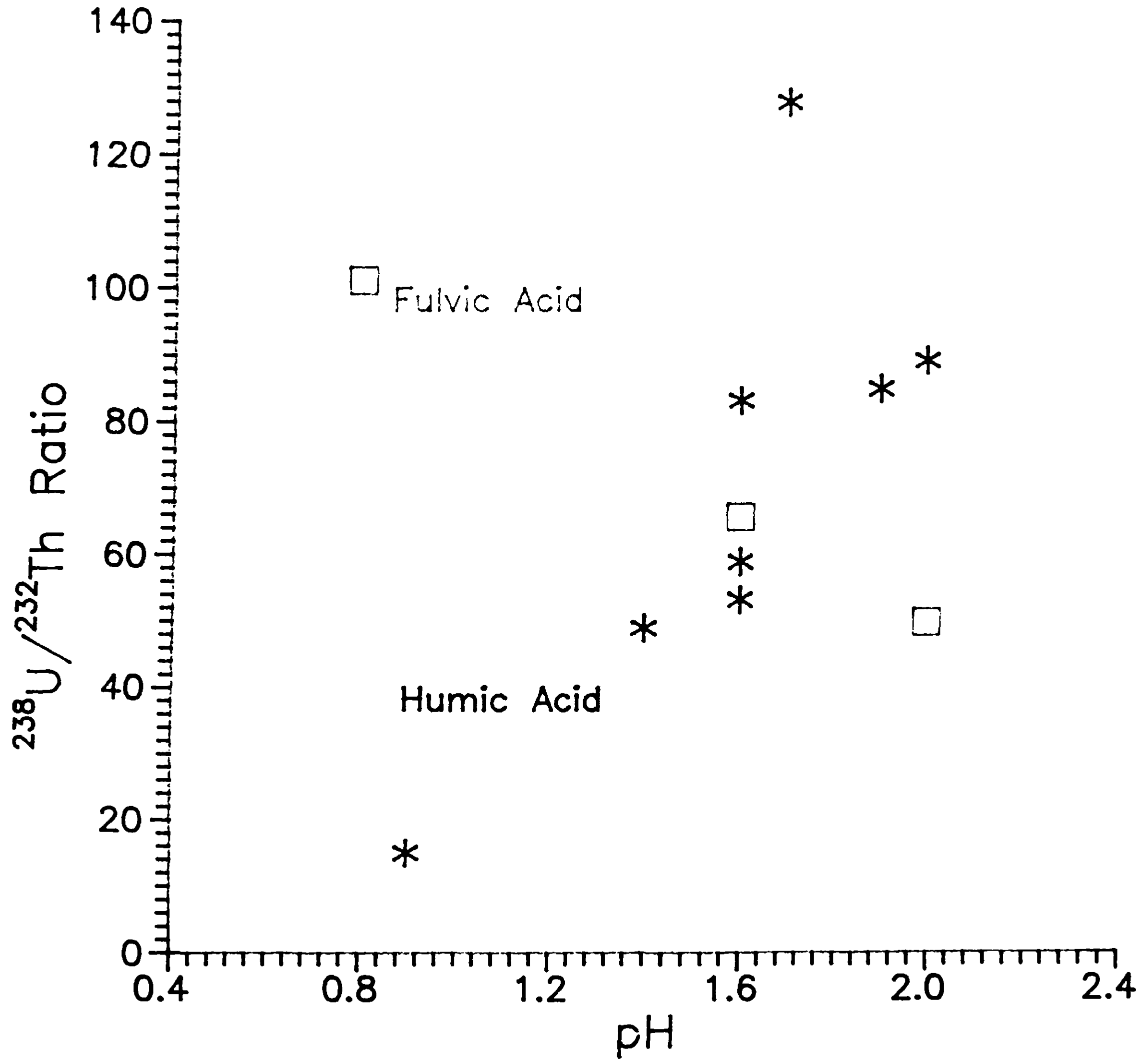


**Figure 3.7 Needle's Eye Humic Material : Variations in $^{238}U/^{232}Th$ Mass Ratio with Changes in pH**

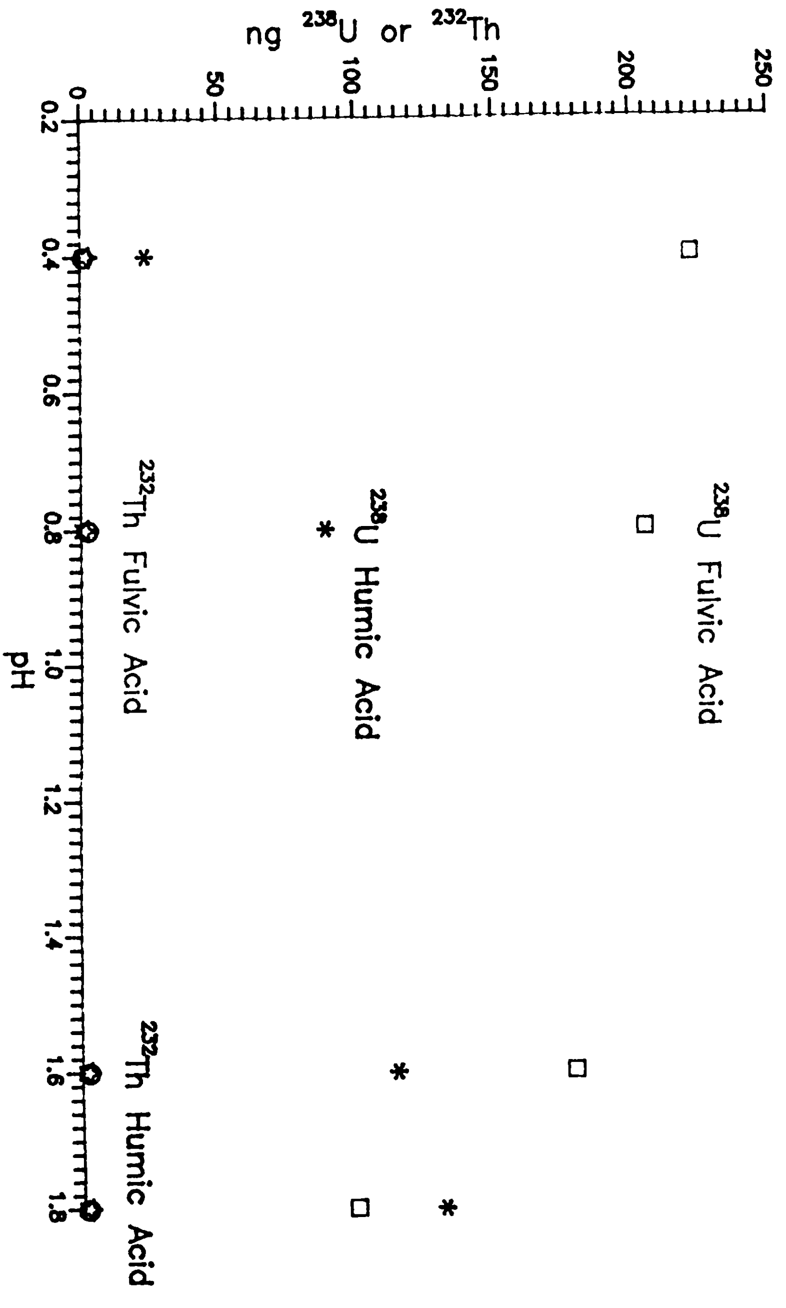


**Figure 3.7a Variations in the Distribution of $^{238}$U and $^{232}$Th between Humic and Fulvic Acids with Changing pH**

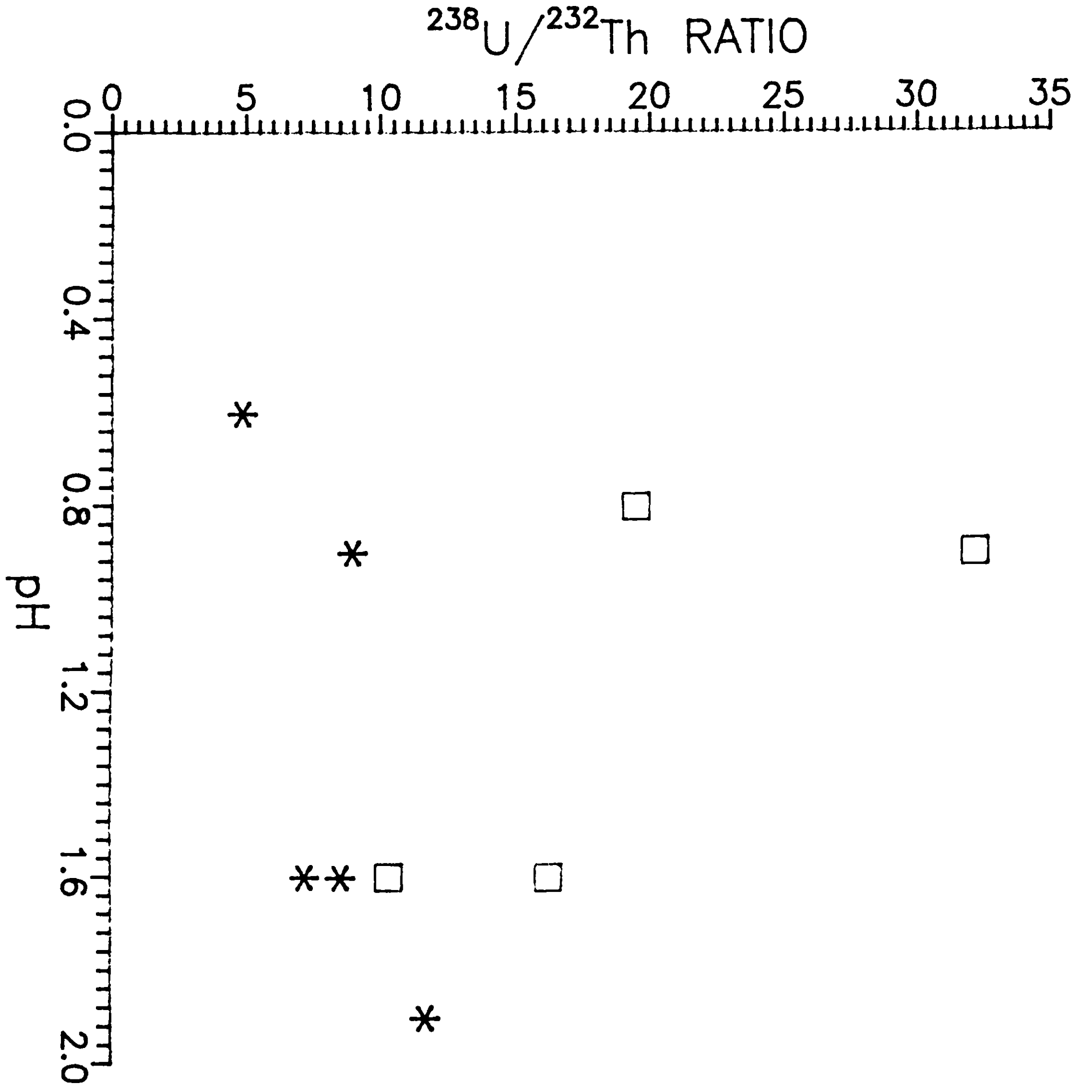


**Figure 3.8 Esk Humic Material : Variations in $^{238}U/^{232}Th$ Mass Ratio with Changes in pH**

This observation may also be related to the behaviour of U observed by Rao (1994) who proposed that two types of U binding exist : weak and strong binding. He also concludes that weak binding is more prevalent at lower pH values and, although this experiment (Rao *et al.*, 1994) involved the addition of metal ions to humic acid, perhaps it could be concluded that this is reflected in the observed associations of U with humic acid in the present study. From the results obtained in this study, the effect of decreasing pH is therefore to release the more weakly bound U which is immediately complexed by fulvic acid in solution. The effect of redistribution with increasing time was not established during the present study since actinide species were not added to the humic materials.

## 3.6 Characterisation of Actinide Interactions with Humic Substances

The total radionuclide content of humic substances was obtained for a selection of samples and a range of actinides, with the results being presented in Table 3.9. A comparison with sequential leaching data for U, Pu and Np (Table 3.10) shows that, for a given soil type, there is good agreement between sequential leaching and alkaline extraction techniques. The data show that the organic fraction is important in binding significant proportions of these actinides. For the Muncaster 0-5cm sample, sequential leaching shows that 56% Pu and 54% Np are associated with the organic fraction (Hursthouse, 1990) and this study, using alkaline extraction, obtained a 54% and 50% association, of Pu and Np respectively, with the humic fraction from a similar floodplain soil. There is also a strong positive correlation between organic/humic content and the proportion of actinide elements associated with the organic fraction. The following order with respect to organic content is observed for samples from the locality of the Irish Sea :

offshore sediment (0.5%) < saltmarsh sediment (5%) < floodplain soil (8%) < organic soil (12%) < organic bog (90%).

The percentage of Pu associated with these sample types from sequential leaching

| Humic Substance | $^{238}U$ (Bqkg$^{-1}$) | $^{239,240}Pu$ (Bqkg$^{-1}$) | $^{237}Np$ (Bqkg$^{-1}$) | $^{241}Am$ (Bqkg$^{-1}$) |
|---|---|---|---|---|
| Esk 0-5cm | 1500 | 3750 | 2.59 | 3058 |
| Esk 10-15cm | 920 | - | - | - |
| Muncaster 0-5cm | 2050 | 5740 | 0.032 | 5146 |
| Muncaster 10-15cm | 695 | - | - | - |
| Southwick Merse 0-5cm | 2120 | - | - | - |
| Southwick Merse 40-45cm | 940 | - | - | - |
| Southwick Merse 65-70cm | - | - | - | - |

**Table 3.9 Actinide Associations with Humic Substances in Soils and Sediments (Bqkg$^{-1}$ humic substances)**

| Method | Organic Soil | | | Floodplain Soil | | Sediment |
|---|---|---|---|---|---|---|
| | % Assoc. of $^{238}U$ | % Assoc. of $^{239,240}Pu$ | %Assoc. of $^{237}Np$ | % Assoc. of $^{239,240}Pu$ | % Assoc. of $^{237}Np$ | % Assoc. of $^{239,240}Pu$ |
| Sequential Extraction (0.1M $Na_4P_2O_7$) | 94 | | | 56 | 54 | 40 |
| Alkaline Extraction (0.1M NaOH) | 90 | 84 | 84 | 54 | 50 | 38 |

**Table 3.10 Comparison of the Percentage Actinide-Organic Association for Soils and Sediments Determined by Sequential and Alkaline Extraction Techniques**

data is as follows :

offshore sediment (20%) < saltmarsh sediment (40%) < floodplain soil (54%) < organic soil (84%)

and alkaline extraction data for Pu and Np gives similar percentage associations for the samples used in this study :

Muncaster 0-5cm [floodplain soil] (54 % Pu, 50% Np)
< Esk 0-5cm [organic soil] (84% Pu, 87% Np)

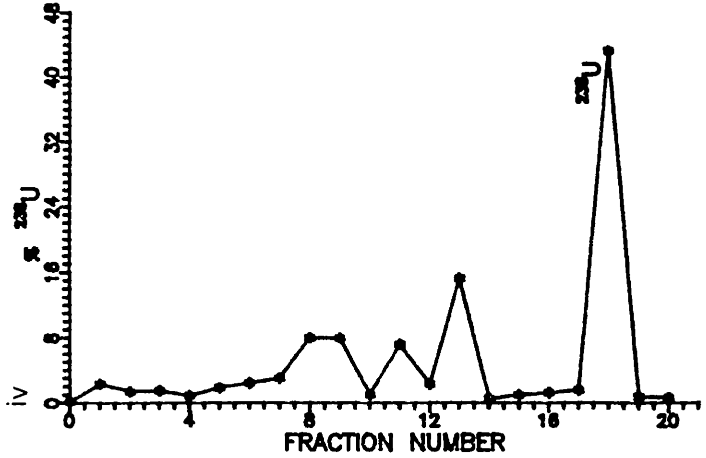
Criticism of sequential leaching techniques focussed on the method dependence of the order of importance of the different phases and that reagents used in initial leaching solutions caused the alteration of the associations of the actinides with the remaining soil components. It was proposed that these observed differences were the result of the co-extraction of material from different phases or that the reagents used to extract one phase did so incompletely. In particular, it was suggested that the order of extraction of secondary Fe/Mn minerals and the organic phases resulted in differences in the perceived importance of these phases. In this study, it has been shown that the actinide-organic associations for the extraction of humic substances using sodium hydroxide solution were comparable with those for the organic fraction extracted as part of a sequential leaching scheme.

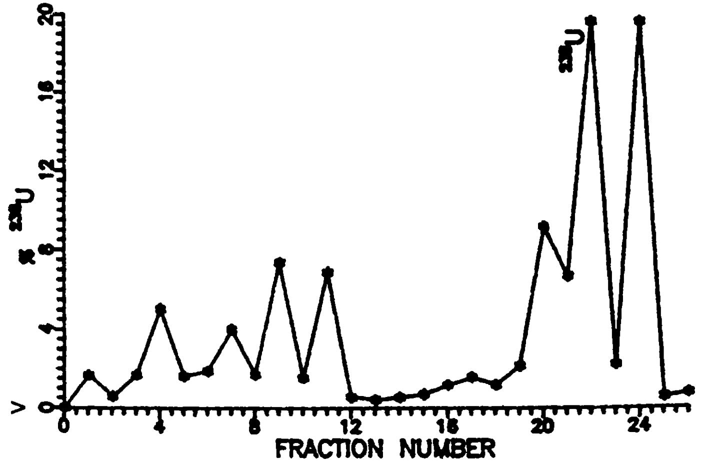
### 3.6.1 Characterisation of Actinide Interactions with Gel Chromatographic Fractions of Humic Substances

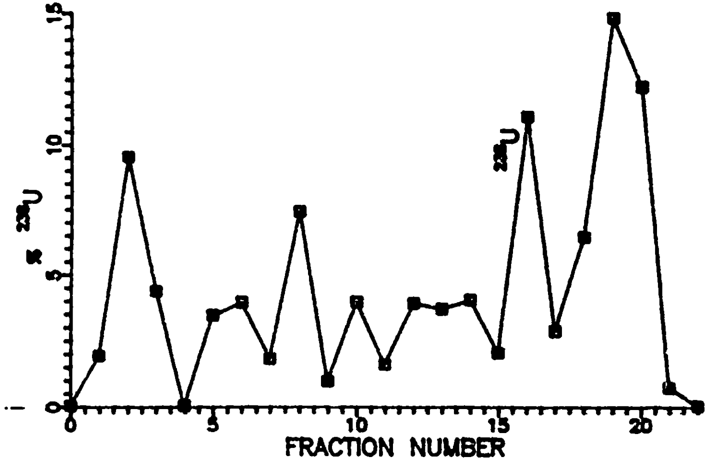
The three locations will be discussed in the following order :

Esk; Muncaster; Merse

U and Th data for all sampling locations will be discussed in terms of
i) varying distributions with depth at individual locations


iv
% $^{238}$U
0
8
16
24
32
40
48
$^{238}$U
0
4
8
12
16
20
FRACTION NUMBER


v
% $^{238}$U
0
4
8
12
16
20
$^{238}$U
0
4
8
12
16
20
24
FRACTION NUMBER


i
% $^{238}$U
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5
10
15
$^{238}$U
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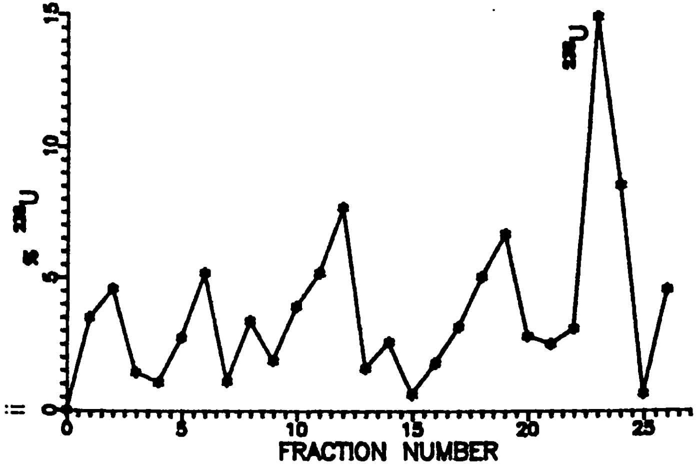
5
10
15
20
FRACTION NUMBER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% $^{238}$U
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15
$^{238}$U
0
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10
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20
25
FRACTION NUMBER

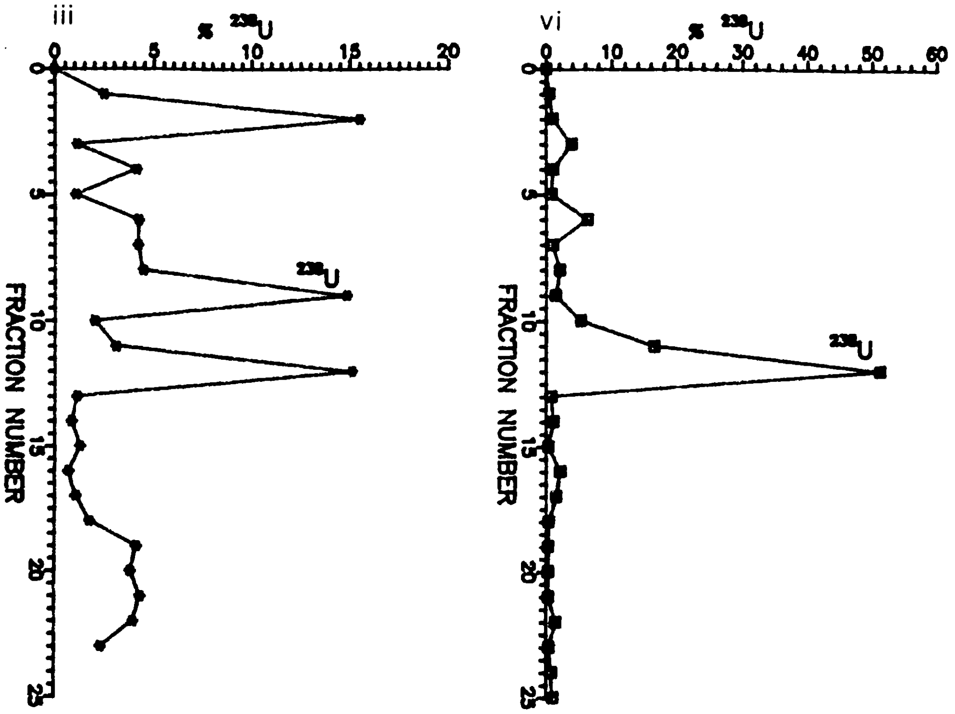
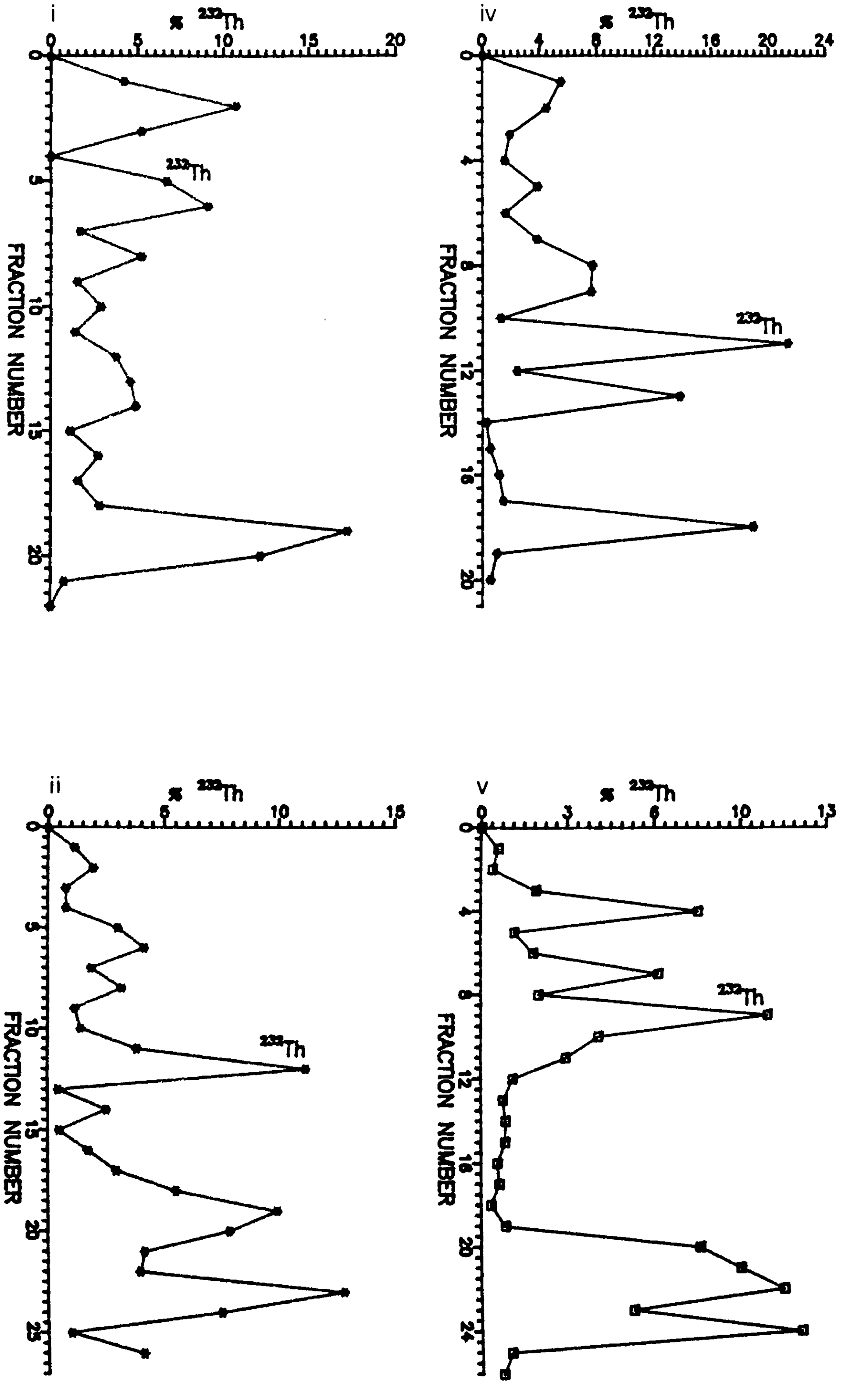


**Figure 3.9 Humic Substances : $^{238}$U Percentage Distribution i) Esk 0-5cm ii) Muncaster 0-5cm iii) Southwick Merse 0-5cm iv) Esk 10-15cm v) Muncaster 10-15cm vi) Southwick Merse 40-45cm**


i
% ²³²Th
0
5
10
15
20
²³²Th
FRACTION NUMBER
iv
% ²³²Th
0
4
8
12
16
20
24
²³²Th
FRACTION NUMBER
ii
% ²³²Th
0
5
10
15
25
²³²Th
FRACTION NUMBER
v
% ²³²Th
0
3
6
10
13
4
8
12
16
20
24
²³²Th
FRACTION NUMBER

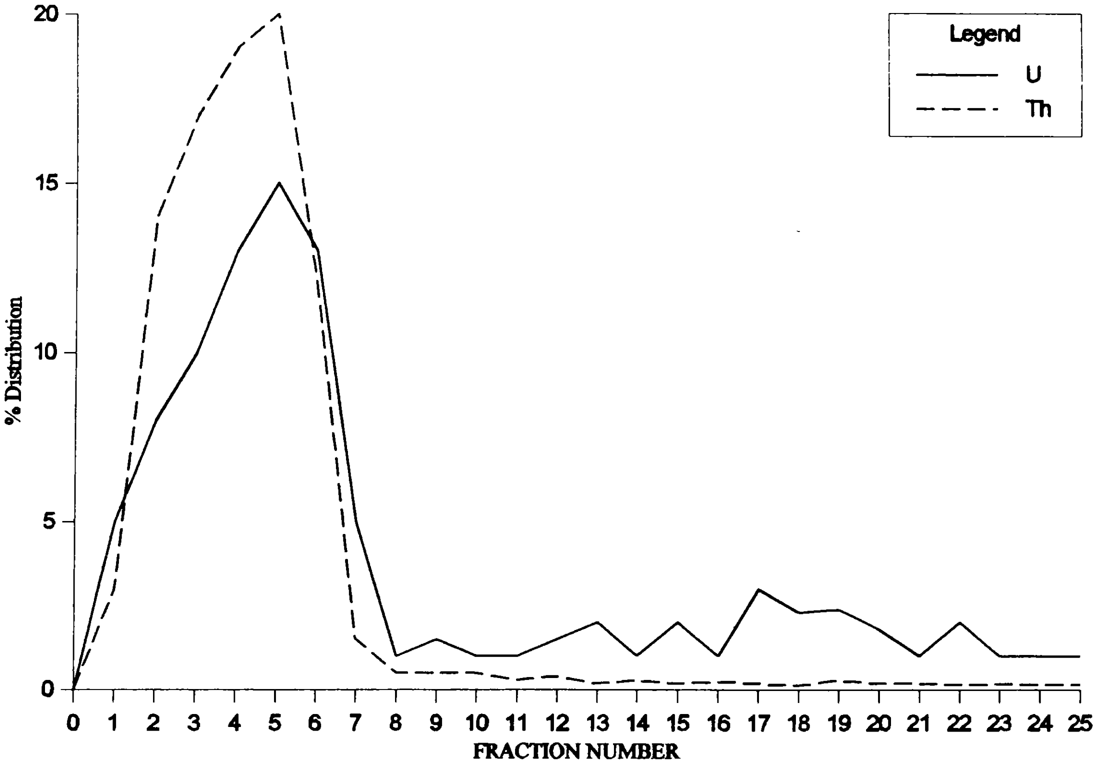


**Figure 3.11b Esk 0-5cm Humic Substances which had been Acidified, Dialysed and Freeze Dried : Percentage Distribution of $^{238}$U and $^{232}$Th**

ii) differing distributions relating to the origins and subsequent prevailing conditions following formation of the humic material.

Pu, Np and Am data will be discussed for specific locations. In addition, spectroscopic information will be used to further the interpretation of the data. Figures 3.9 and 3.10 show U and Th data for surface and depth samples from each location. The elution patterns were subdivided into six regions relating to groups of three or four fractions (dependent on total number of fractions collected; different gel types result in different elution volumes as described in section 2.5). Peaks in these regions were classified on the basis of percentage association as very low (<5%), low (5-10%), medium (10-20%), high (20-40%) or very high (>40%) and the assignments for each elution pattern can be found in Tables 3.11-3.40) together with the percentage association of each actinide in this region. Columns 4 and 5 in these tables represent grouped data in order to show percentage associations with early, middle and late fractions or with the first half and second half of fractions. These groupings are also used in the interpretation of actinide-humic interactions together with spectroscopic data.

### 3.6.1.1 U Distribution : Esk 0-5cm

The U distribution pattern resulting from gel fractionation of 0-5cm Esk humic substances (Figure 3.11) is clearly complex with a number of discrete associations occurring throughout the elution pattern. The six main associations of U are classified as :

medium : low : medium : medium : medium : high

There is a significant association of U with humic material in later fractions. 34% of U is associated with fractions comprising region 6. Moreover, 51% of U is eluted in fractions comprising regions 5 and 6. The broad nature of the later U peaks is a result of the longer time spent by the respective macromolecules on the gel column. This distribution pattern which was obtained from analysis by ICP-MS was reproduced from a replicate fractionation of the humic material by $\alpha$-spectrometry and there is indeed good agreement between the two data sets

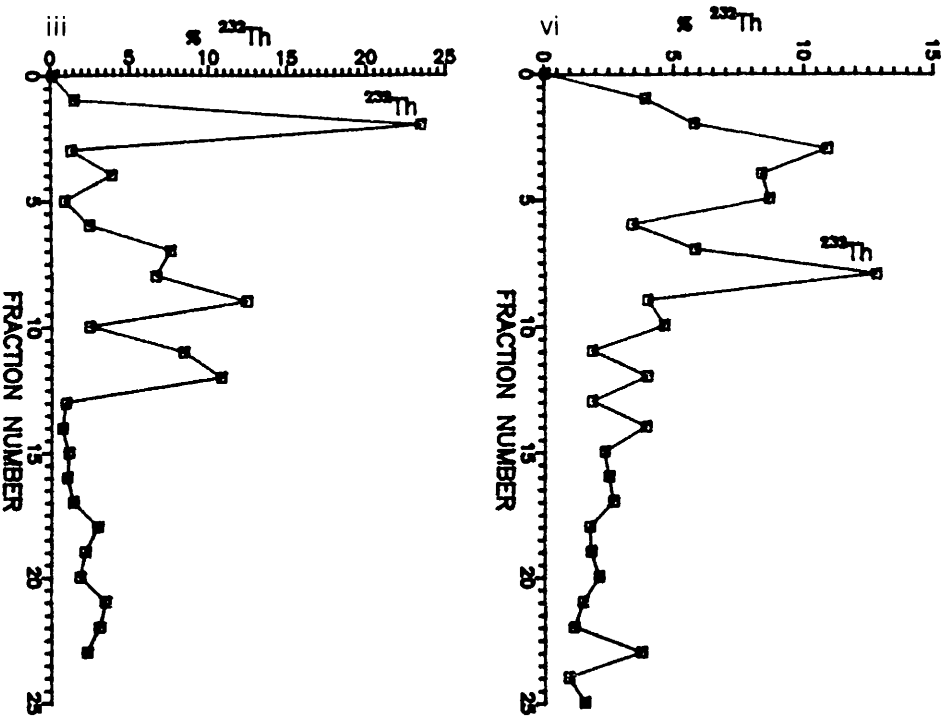


Figure 3.10 Humic Substances : $^{232}$Th Percentage Distribution i) Esk 0-5cm ii) Muncaster 0-5cm iii) Southwick Merse 0-5cm iv) Esk 10-15cm v) Muncaster 10-15cm vi) Southwick Merse 40-45cm

(Eluent $NH_4OH$ except for 3.12 Uranium Determination by Alpha Spectrometry)

(All errors on individual data points were <3%)

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | medium | 16 | 24 | 35 |
| 2 | low | 8 | | |
| 3 | medium | 11 | 24.5 | |
| 4 | medium | 13.5 | | 64.5 |
| 5 | medium | 17 | 51 | |
| 6 | high | 34 | | |
| Activity = 0.15Bq per 100mg | | Eluted = 0.14Bq = 93% | | |

**Table 3.11 Esk 0-5cm Humic Substances Uranium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | high | 22.5 | 50 | 65 |
| 2 | high | 27.5 | | |
| 3 | medium | 16 | 24.5 | |
| 4 | low | 8.5 | | 34 |
| 5 | low | 6.2 | 25.5 | |
| 6 | medium | 19.3 | | |
| Activity (Eluted) | 0.155Bq | | | |

**Table 3.12 Esk 0-5cm Humic Substances Uranium G100 (Determination by Alpha Spectrometry)**

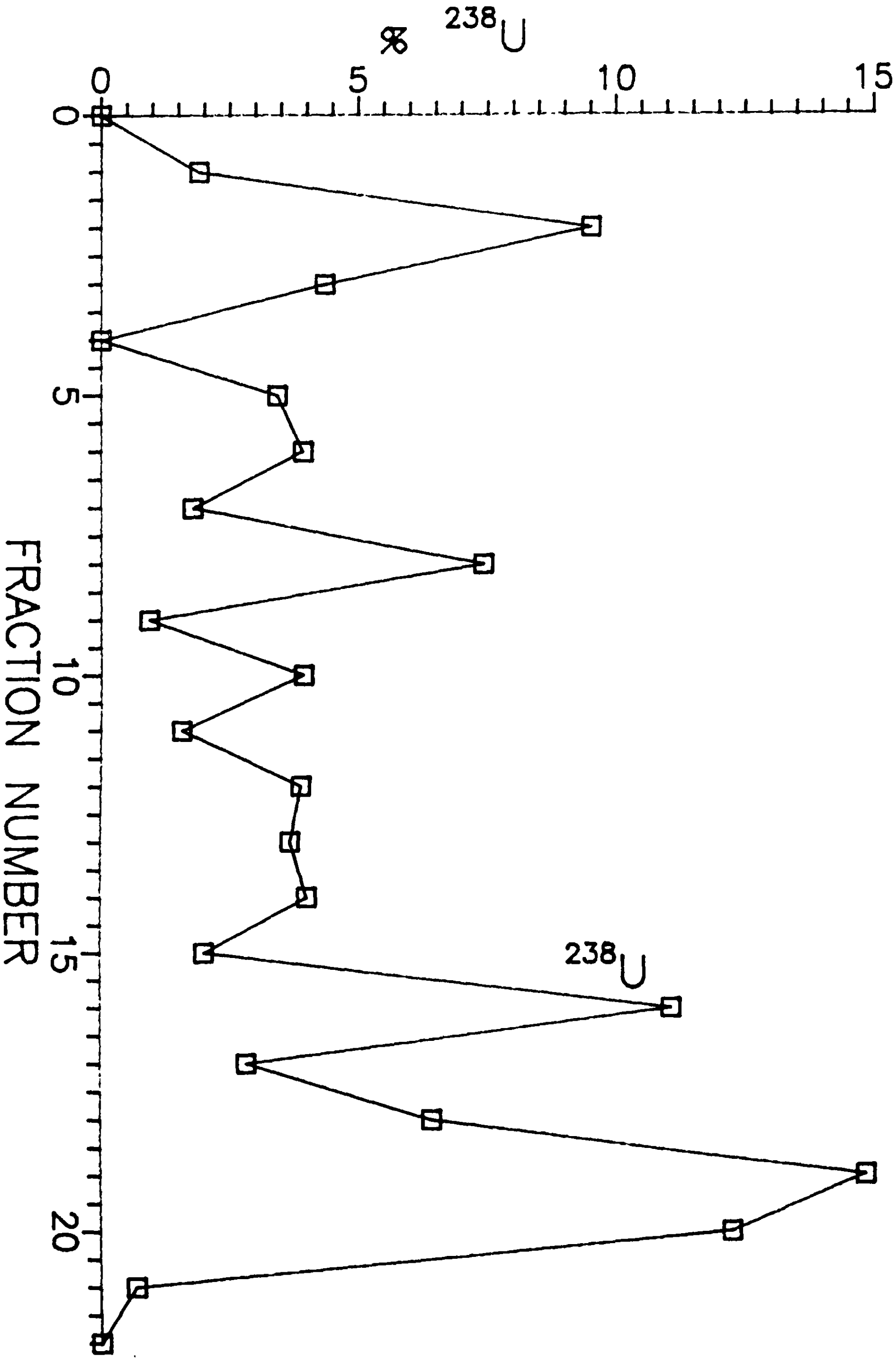


Figure 3.11 Esk 0-5cm Humic Substances : Percentage Distribution of $^{238}$U

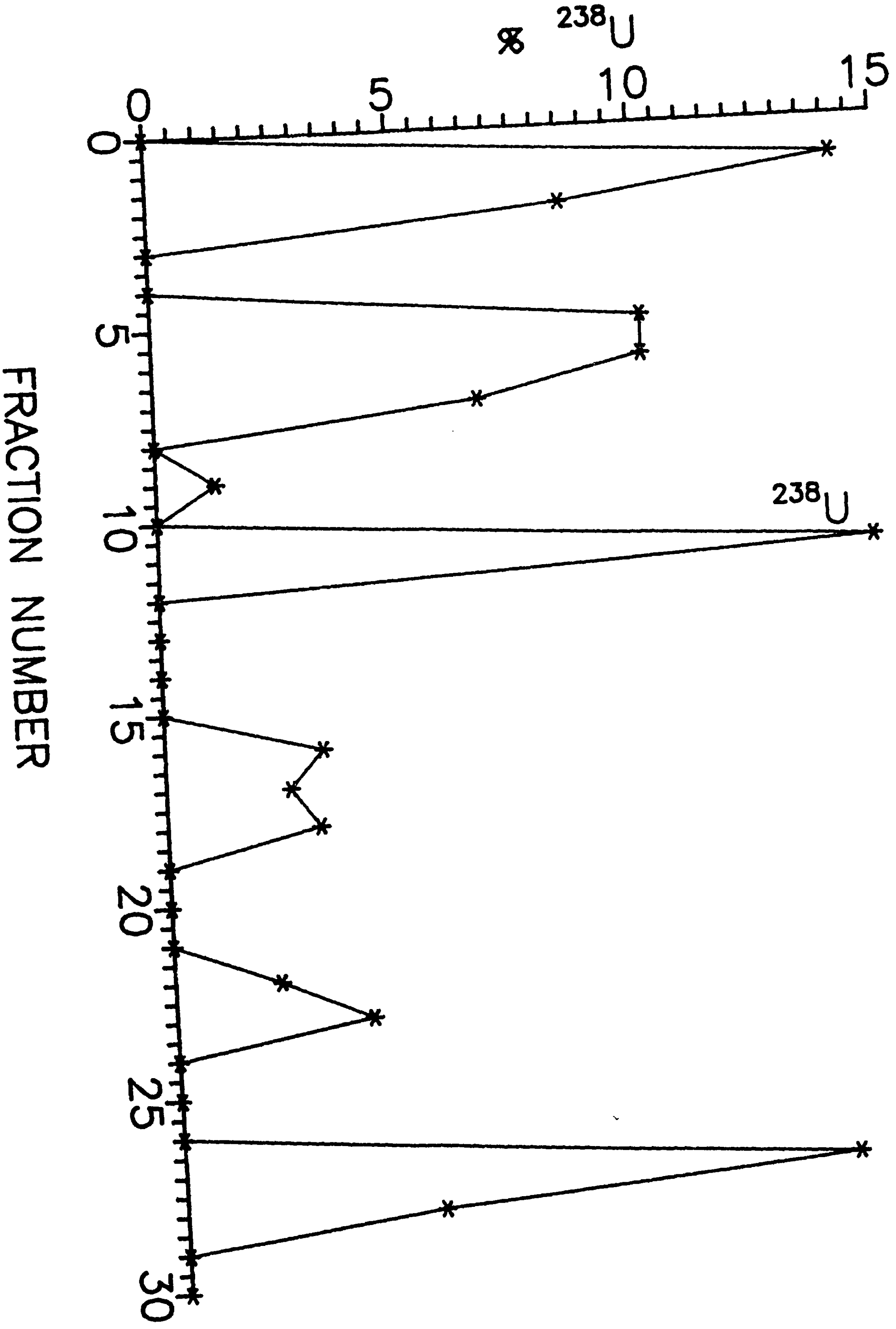


**Figure 3.11a Esk 0-5cm Humic Substances : Percentage Distribution of $^{238}$U Data Obtained by Alpha Spectrometry**

(Figure 3.11a). The peaks obtained for the replicate fractionation matched those from the first elution pattern in terms of relative position, shape and concentration (Table 3.11 and 3.12) demonstrating the reproducible fractionation of humic substances using gel chromatography can be achieved on a run-to-run basis and also the accuracy of the analyses of actinide elements in the absence of suitable reference materials.

On the basis of the humic acid elution pattern, a relatively simple association for U would be predicted. In particular a stronger association of U with humic material in early fractions and very little with those in later fractions would have been expected. An additional elution pattern for acidified humic substances (humic + fulvic acids) which were dialysed but not separated also resulted in a distribution pattern similar to that obtained for humic acid (Figure 3.11b). The pattern of discrete associations of U with humic substances in comparison with the elution patterns obtained for humic and fulvic acids show that the distribution of U observed for humic substances can not be accounted for in terms of combination of humic and fulvic acid. This is, again, strong evidence of alterations in the associations of U with humic substances where humic and fulvic acid have been separated in the analysis.

### 3.6.1.2 U Distribution : Muncaster 0-5cm

The pattern of U distribution obtained from the analyses of gel chromatographic fractions by ICP-MS is again complex (Figure 3.12 and Table 3.13). The observed peaks were classified as follows :

medium : low : high : very low : medium : high

The general pattern observed for Esk 0-5cm humic substances was observed also for Muncaster 0-5cm humic substances in that there was a significant association of U with humic material in later fractions. For this location, 43% of the U was associated with humic molecules in fractions comprising region 5 and 6. However, some differences were also apparent in that 22%, as compared with 11% for 0-5cm Esk humic substances, of the U was found in fractions comprising region 3.

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | medium | 10 | 18 | 40 |
| 2 | low | 8 | | |
| 3 | high | 22 | 25 | |
| 4 | very low | 3 | | 48 |
| 5 | medium | 15 | 43 | |
| 6 | high | 28 | | |
| Activity = 0.21Bq per 100mg | | Eluted = 0.195Bq = 93% | | |

**Table 3.13 Muncaster 0-5cm Uranium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | high | 23 | 50 | 70 |
| 2 | high | 27 | | |
| 3 | high | 20 | 25 | |
| 4 | low | 5 | | 24 |
| 5 | low | 8 | 19 | |
| 6 | medium | 11 | | |
| Activity = 0.21Bq per 100mg | | Eluted = 0.2Bq = 95.1% | | |

**Table 3.14 Merse 0-5cm Uranium G100**

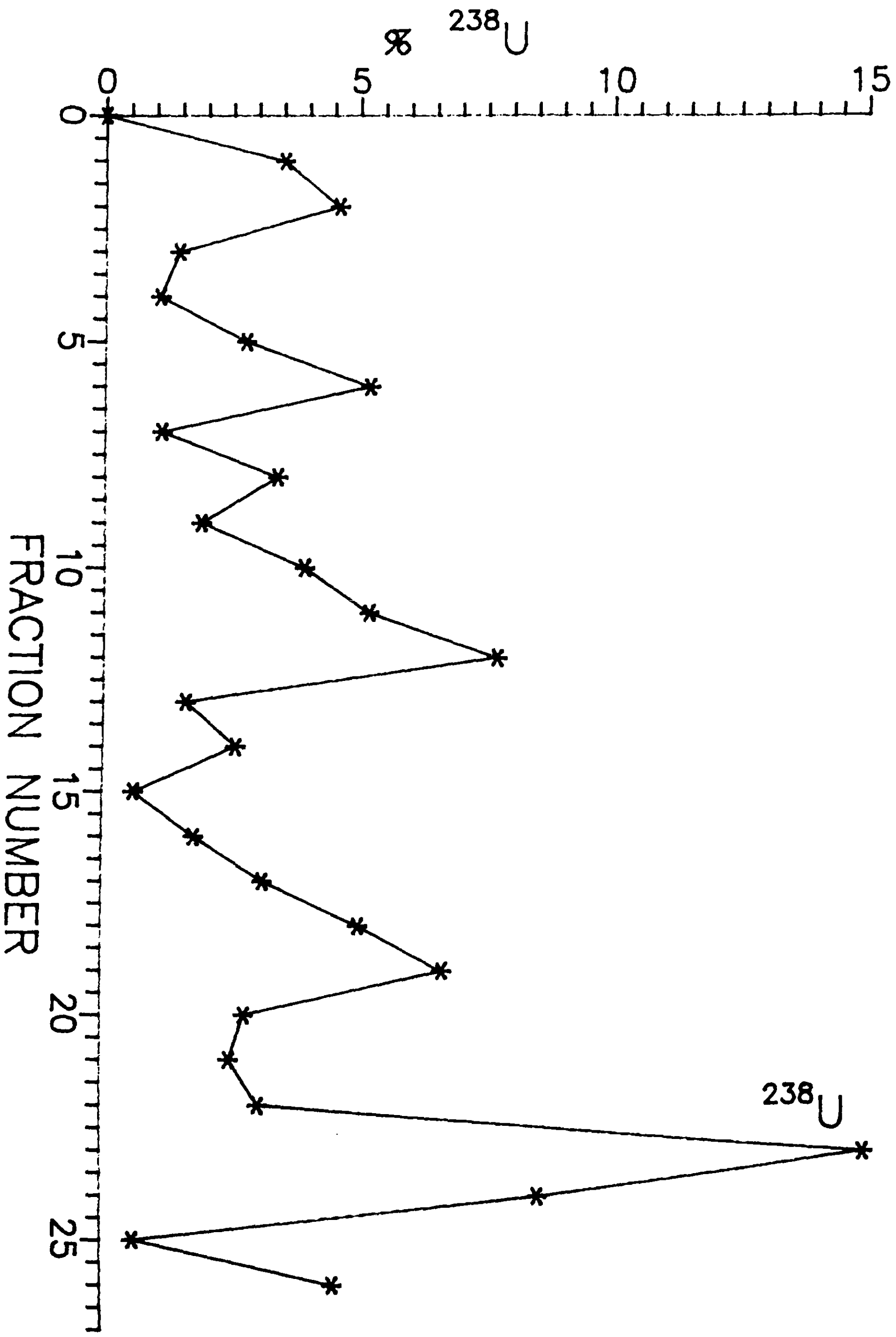


Figure 3.12 Muncaster 0-5cm Humic Substances : Percentage Distribution of $^{238}$U

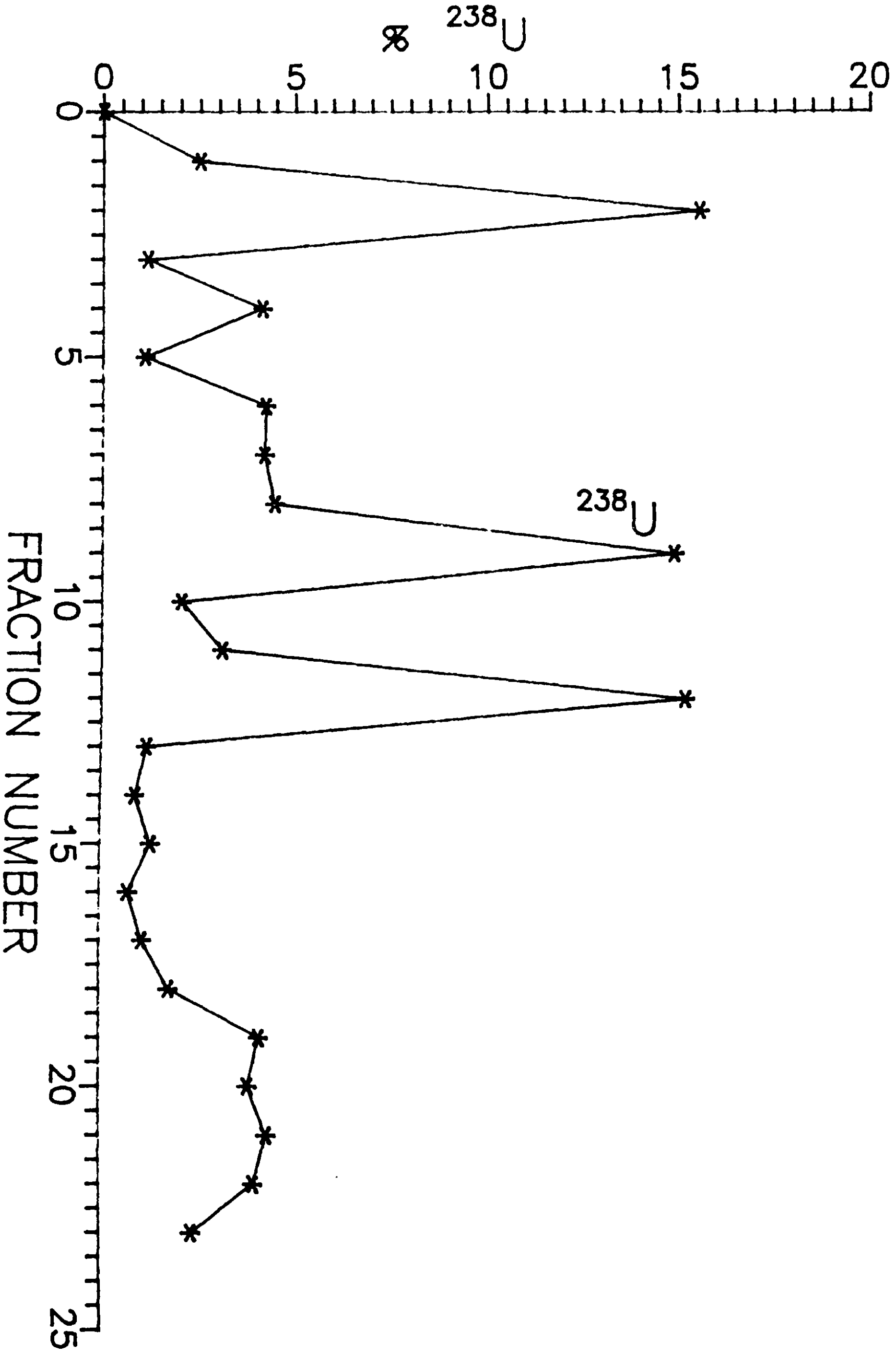


Figure 3.13 Southwick Merse 0-5cm Humic Substances : Percentage Distribution of $^{238}$U

These similarities and differences will be discussed further in section 3.6.1.7.

### 3.6.1.3 U Distribution : Merse 0-5cm

The U distribution pattern for the Merse 0-5cm humic substances was significantly different from those observed for either the Esk 0-5cm or the Muncaster 0-5cm humic substances (Figure 3.13 and Table 3.14). In particular, the predominant association of U was with humic material eluted in the first half of fractions (column 5, Table 3.14). 70% of U was associated with these humic molecules as compared with 35% for Esk 0-5cm or 40% for Muncaster 0-5cm.

The elution pattern was again strongly contrasting to the simple elution patterns observed for either humic or fulvic acid from the same location. For humic substances, a number of discrete assocations were observed. The classification on the same basis of those above were as follows :

high : high : high : low : medium : medium

These distributions will be referred to again in the sections 3.6.1.6 and 3.6.1.7.

### 3.6.1.4 U distribution : Esk 10-15cm

Fewer major peaks were observed in the distribution pattern of U (Figure 3.14 and Table 3.15) with the overall classification being as follows :

very low : very low : medium : high : very low : very high

Again, as was observed for 0-5cm Esk and 0-5cm Muncaster humic substances, a significant proportion of U was associated with humic molecules in fractions comprising region 6.

In comparison with Esk 0-5cm humic material, a number of differences were observed. Fractions comprising regions 1 and 2 contained humic molecules with significantly less associated U for 10-15cm compared with 0-5cm humic substances (8% as compared with 24%). Similarly, only 2% of U was associated with humic material in 10-15cm humic fractions comprising region 5 as compared with 17% for 0-5cm humic substances. Increased associations were therefore

| Region 1 | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very low | 3 | 8 | 27 |
| 2 | low | 5 | | |
| 3 | medium | 19 | 33 | |
| 4 | high | 24 | | 72 |
| 5 | very low | 2 | 48 | |
| 6 | very high | 46 | | |
| Activity = 0.09Bq per 100mg | | Eluted = 0.07Bq | | |

**Table 3.15 Esk 10-15cm Uranium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very low | 4 | 13 | 35 |
| 2 | low | 9 | | |
| 3 | high | 22 | 23 | |
| 4 | very low | 1 | | 63 |
| 5 | low | 6 | 62 | |
| 6 | very high | 56 | | |
| Activity = 0.07Bq | | Eluted = 0.055Bq | | |

**Table 3.16 Muncaster 10-15cm Uranium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | low | 7 | 18 | 88 |
| 2 | medium | 11 |  |  |
| 3 | very strong | 70 | 74 |  |
| 4 | very low | 4 |  | 6 |
| 5 | very low | 1 | 2 |  |
| 6 | very low | 1 |  |  |
| Activity = 0.094Bq |  | Eluted = 0.09Bq |  |  |

Table 3.17 Merse 40-45cm Uranium G100

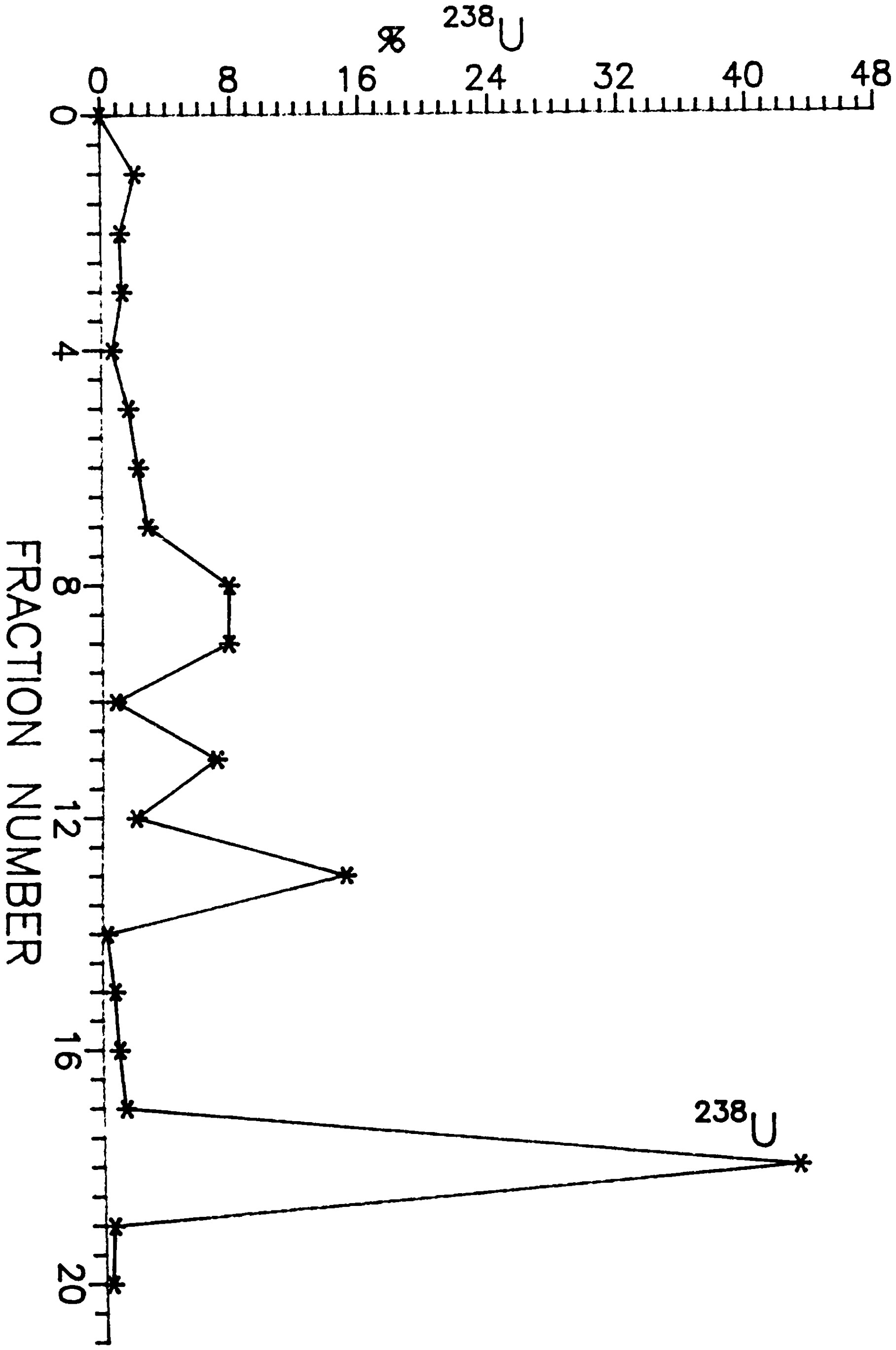


Figure 3.14 Esk 10-15cm Humic Substances : Percentage Distribution of $^{238}$U

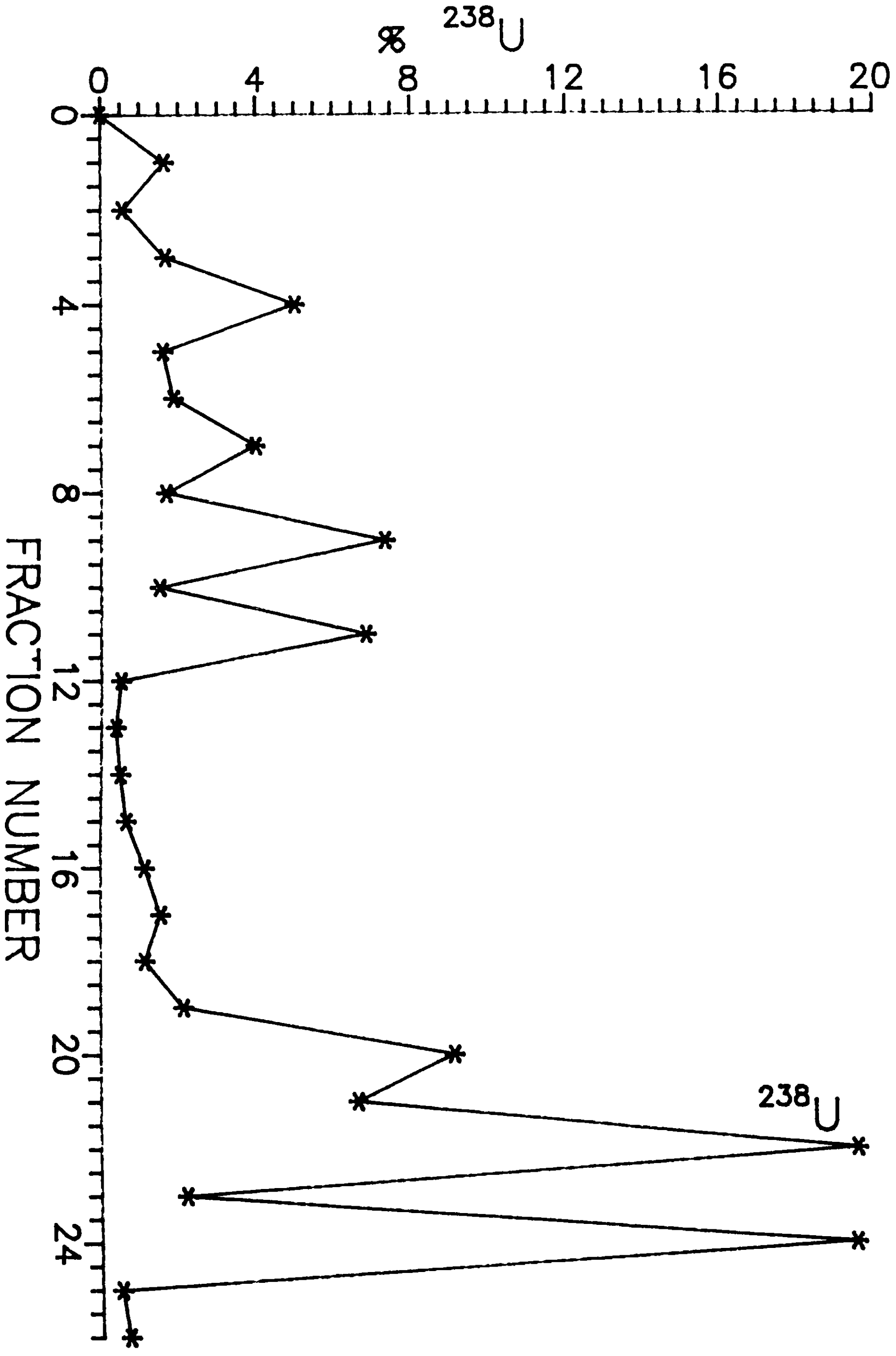


**Figure 3.15 Muncaster 10-15cm Humic Substances : Percentage Distribution of $^{238}$U**

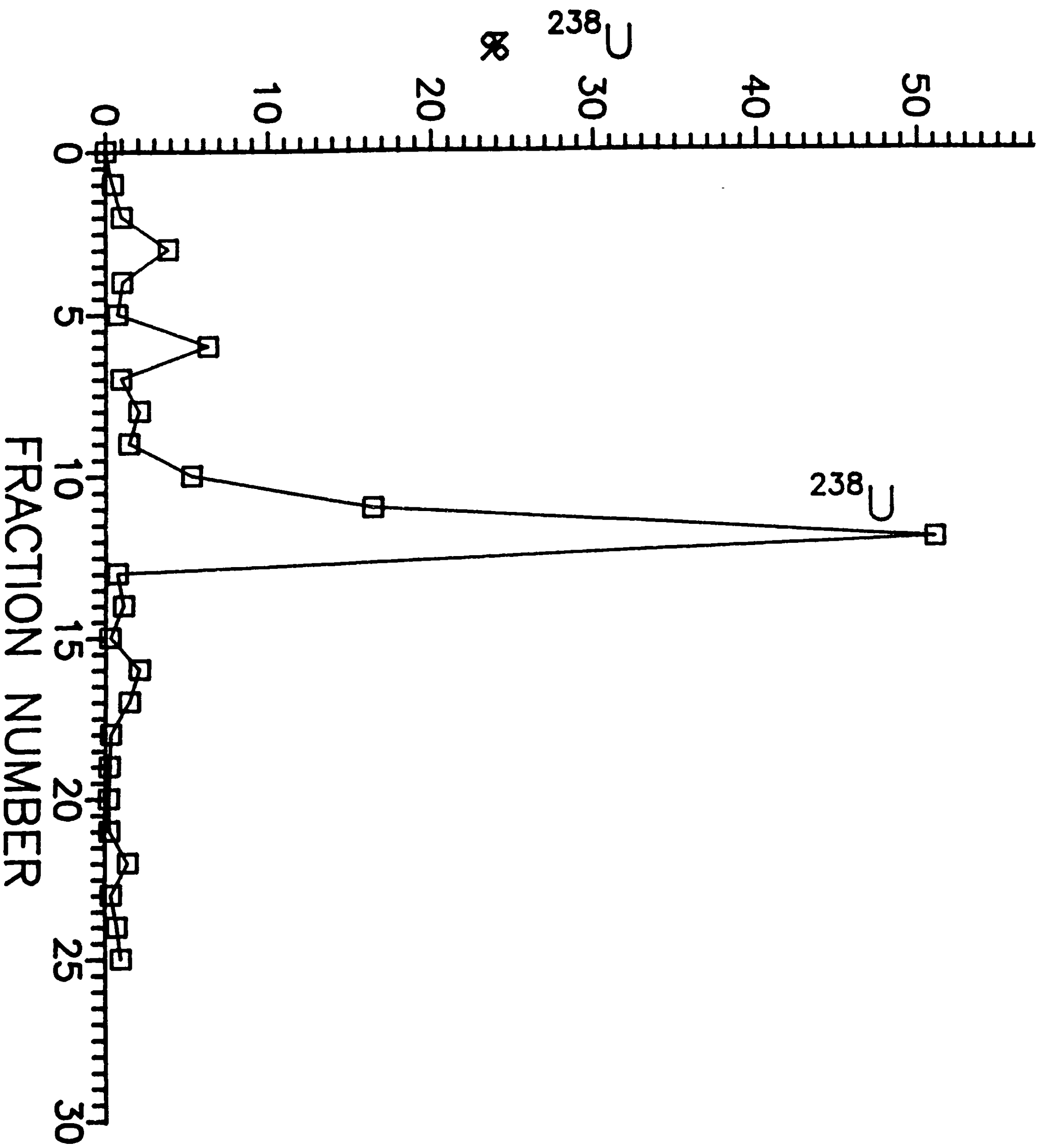


Figure 3.16 Southwick Merse 40-45cm Humic Substances : Percentage Distribution of $^{238}$U

observed for material in fractions comprising regions 3 (19% as compared with 11%), 4 (24% as compared with 14%) and region 6 (46% as compared with 34%). The differences between these elution patterns, in particular the perceived loss of binding capacity of components of the humic fraction (a proportion of molecules in fractions comprising regions 1, 2 and 5), is consistent with the degradation ie. loss of functionality undergone by the organic fractions during early diagenetic alterations.

### 3.6.1.5 U Distribution : Muncaster 10-15cm

The U distribution amongst fractions of 10-15cm Muncaster humic substances (Figure 3.15 and Table 3.16) led to the classifications of peaks as follows :

very low : low : high : very low : very low : high

The pattern observed for 10-15cm Muncaster humic substances (ie. the predominance of U association with humic molecules in fractions comprising regions 3 and 6) is slightly more accentuated but in general similar to that of 0-5cm humic compounds. This is consistent with the highly reducing conditions prevailing at this location which lead to a relatively slower degradation of the organic fraction. In comparison with the alterations with depth observed for Esk humic substances, the degree of change to the U distribution pattern is much smaller although a number of similarities are apparent. A slight decrease in percentage associations is observed for regions 1 and 2 (18% to 15%) but the major change is observed in region 5 with only 6% as opposed to 18% U being associated with molecules in the 10-15cm humic fractions.

### 3.6.1.6 U Distribution : Merse 40-45cm

The U distribution pattern observed for Merse 40-45cm humic substances (Figure 3.16 and Table 3.17) is distinctly different from those observed for Esk 10-15cm or Muncaster 10-15cm humic materials. These materials are comparable on the basis of the accumulation rates at the respective locations. In addition, the differences observed are explicable in terms of the differences described above for the distribution patterns of U associated with 0-5cm humic substances from these locations.

The observed peaks were classified as follows :

low : medium : high : very low : very low : very low

As observed for 0-5cm Merse humic substances, the predominant association of U was with humic molecules eluting in the first half fractions (88%). The diagenetic alteration of the humic material is observed in that only one of the three regions identified for 0-5cm humic substances is of major importance for the 40-45cm humic substances. These results are discussed further in section 3.6.1.7.

It is also apparent that the U distributions for humic substances from 40-45cm Southwick Merse sediments are again different from those observed for humic and fulvic acids and in particular combination of humic acid and fulvic acid elution patterns does not result in the pattern observed for humic substances (Figure 3.16a). The alteration of the distribution of actinide elements has been discussed in section 3.6.1.1 and 3.6.1.3. It is, however, evident from the distribution patterns observed for the 0-5cm Esk humic substances, 0-5cm and 40-45cm Sothwick Merse humic substances that the composition of the humic fraction is an important factor in determining the nature of these alterations.

### 3.6.1.7 U Associations with Humic Substances

The distribution of U within the humic fraction changes with depth and is consistent with compositional changes identified in section 3.5.1. The changing distribution with depth is characteristic of the humic material from each location and can be related to the origin of the humic material and prevailing conditions.

0-5cm Esk humic material has a characteristic U distribution pattern as described above with a strong association with later fractions. These discrete associations can tentatively be related to the changing position of the emission maximum in

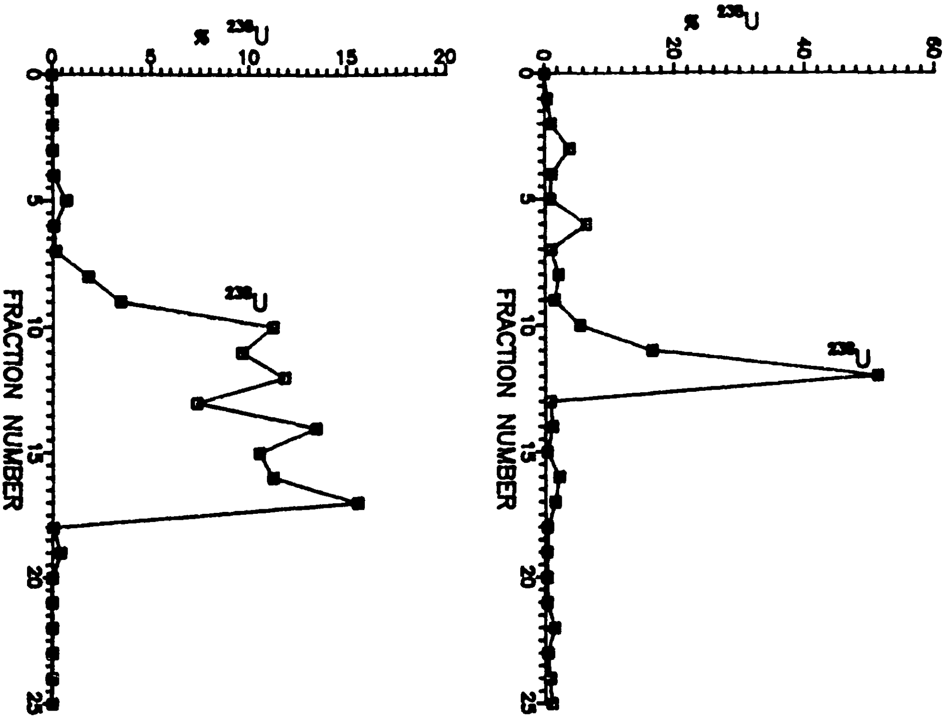
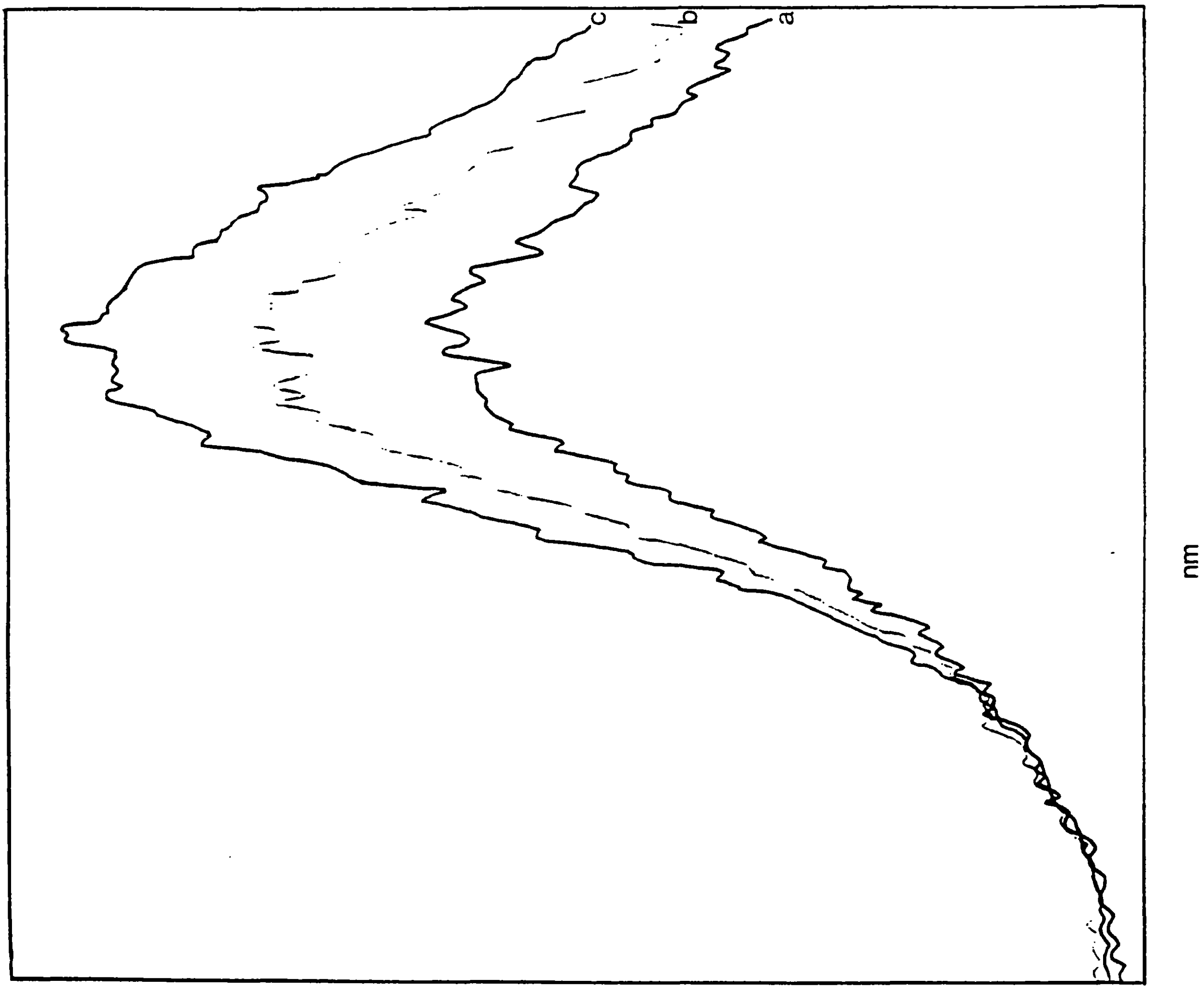


Figure 3.16a Comparison of Southwick Merse Humic Acid and Humic Substances : Percentage Distribution of $^{238}U$

| Fraction Number | $\lambda_{max}$ (emission) | Intensity |
|---|---|---|
| Region 1 Fr. 1 (a) | 446nm | 18.7 |
| Fr. 2 (b) | 446nm | 21.5 |
| Fr. 3 (c) | 446nm | 29.0 |
| Region 2 Fr. 5 (d) | 420-440nm (broad) | 30.0 |
| Fr. 7 (e) | 420-440nm (broad) | 39.0 |
| Fr. 8 (f) | 420-440nm (broad) | 47.0 |
| Region 3 Fr. 9 (g) | 436-446nm | 52.0 |
| Fr. 10 (h) | 436-446nm | 55.0 |
| Fr. 11 (i) | 436-446nm | 56.5 |
| Region 4 Fr. 12 (j) | 420-446nm | 69.5 |
| Fr. 13 (k) | 420-446nm | 101.0 |
| Fr 15 | 446nm | 106.5 |
| Region 5 Fr. 16 (l) | 420-440nm | 135.0 |
| Fr. 17 (m) | 420-442nm | 156.0 |
| Fr. 18 (n) | 420-442nm | 189.4 |
| Region 6 Fr. 19 (o) | 420-450nm | 184.1 |
| Fr. 20 (p) | 420-430nm | 154.0 |
| Fr.21 | 420nm | 97.5 |
| Fr.22 | 415-425nm | 54.0 |

**Table 3.41 Position of Emission Maximum in Fluorescence Spectra of Gel Chromatographic Fractions of Humic Substances (letters in brackets correspond to spectra labels in Spectrum 3.26a)**



Spectrum 3.26a Fluorescence Spectra of Gel Chromatographic Fractions of Humic Substances

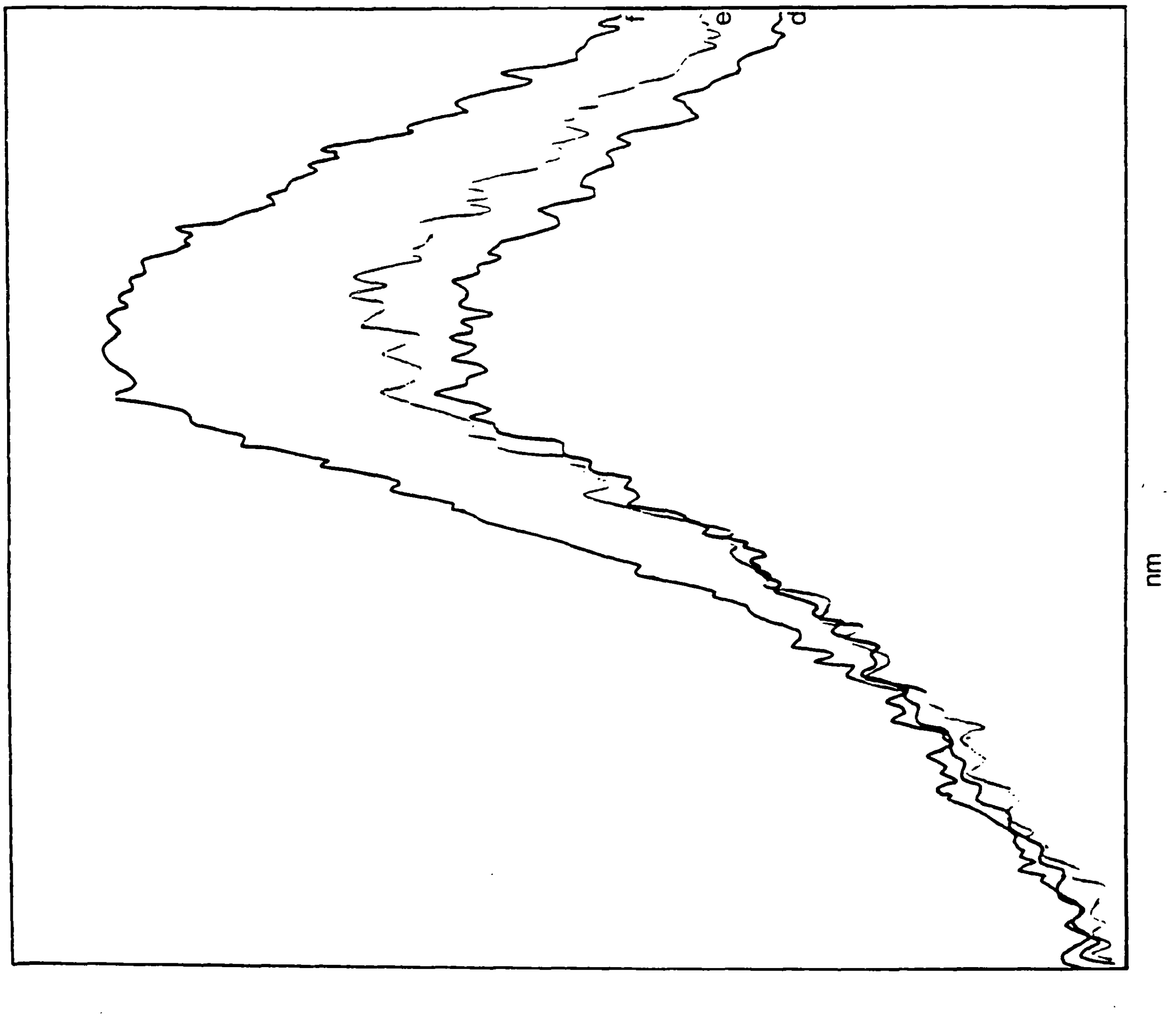


Figure 3.26a Fractions d, e and f : Fluorescence Spectra of Gel Chromatographic Fractions

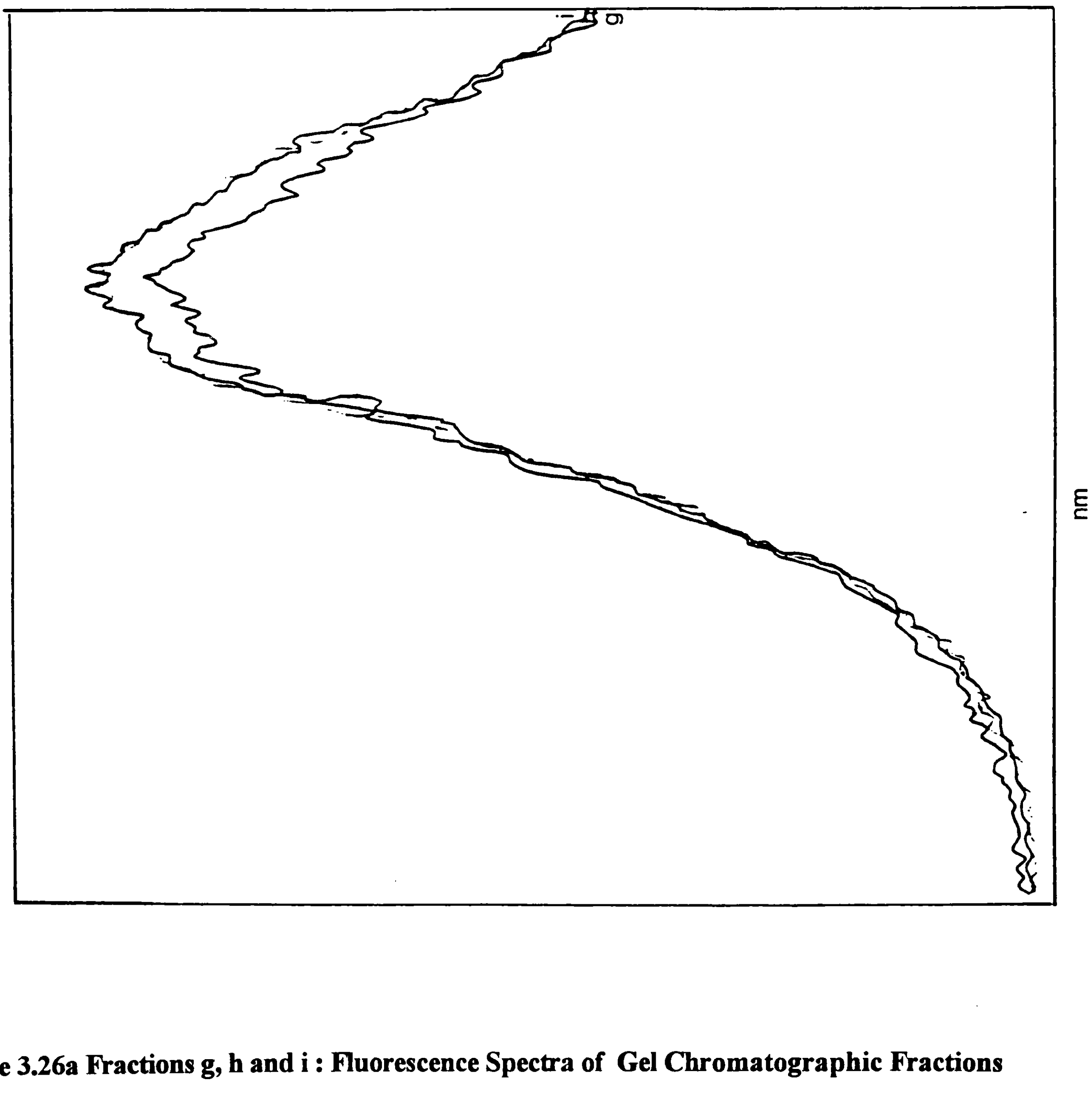


Figure 3.26a Fractions g, h and i : Fluorescence Spectra of Gel Chromatographic Fractions

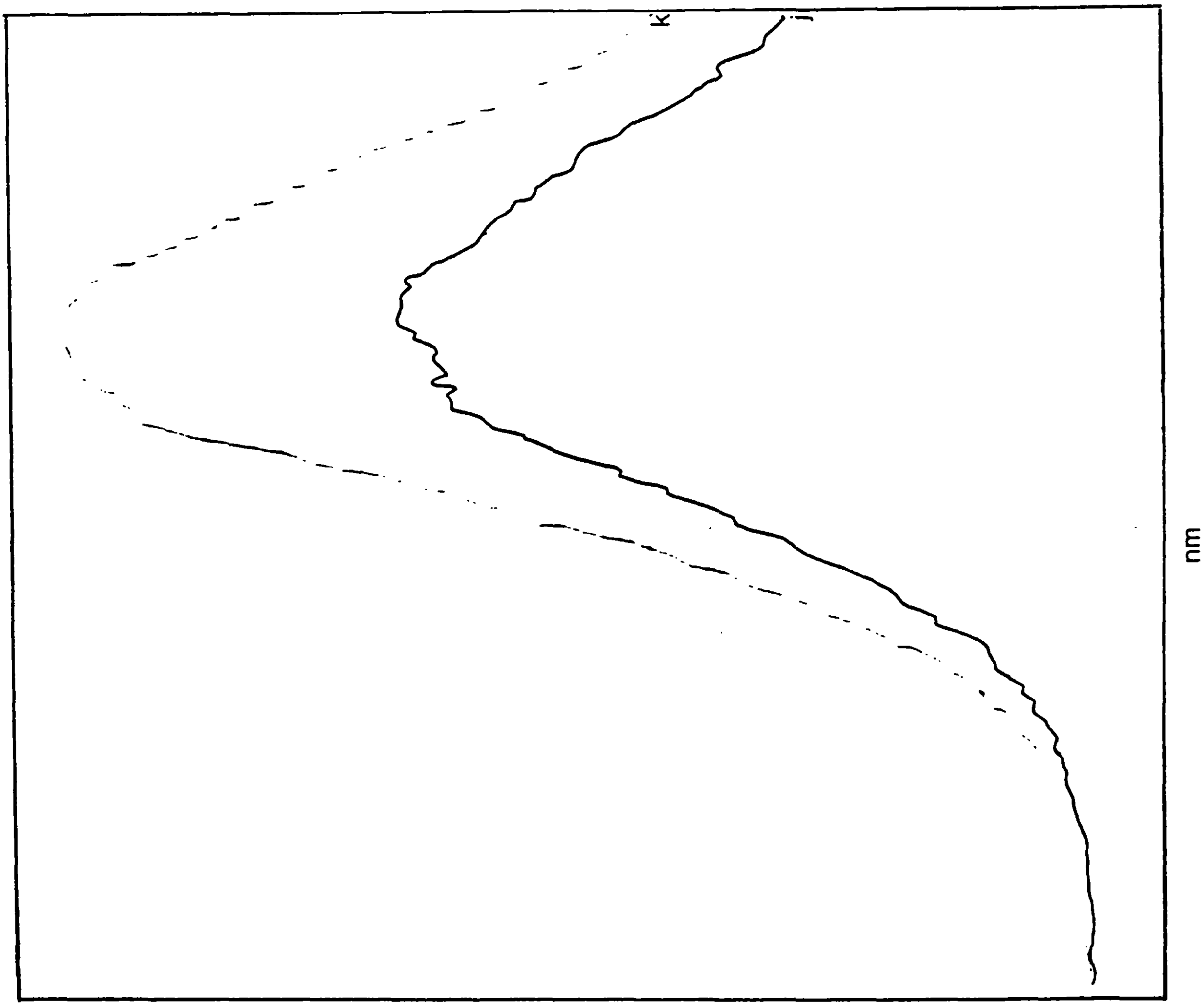


Figure 3.26a Fractions j and k : Fluorescence Spectra of Gel Chromatographic Fractions

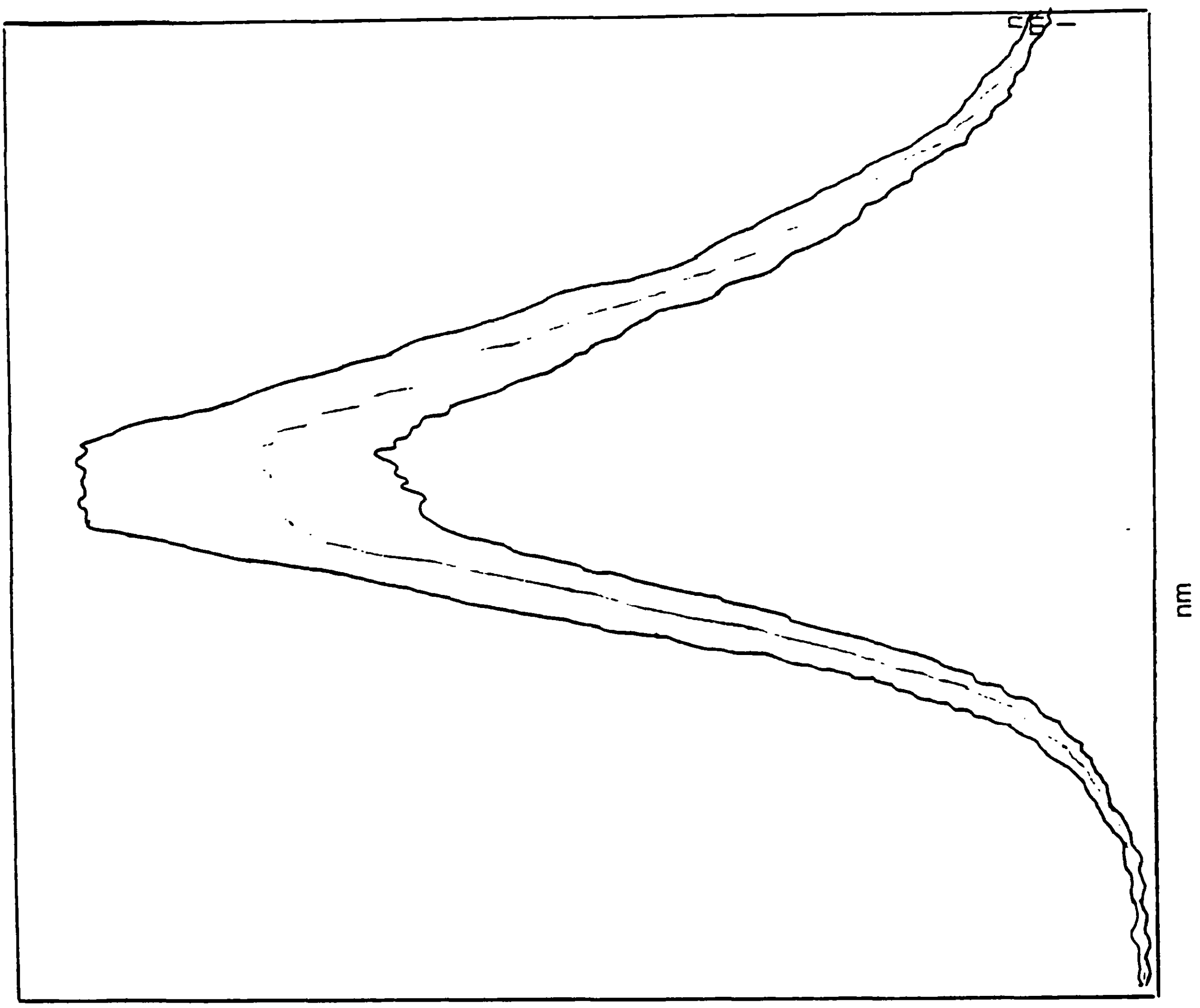


Figure 3.26a Fractions l, m and n : Fluorescence Spectra of Gel Chromatographic Fractions

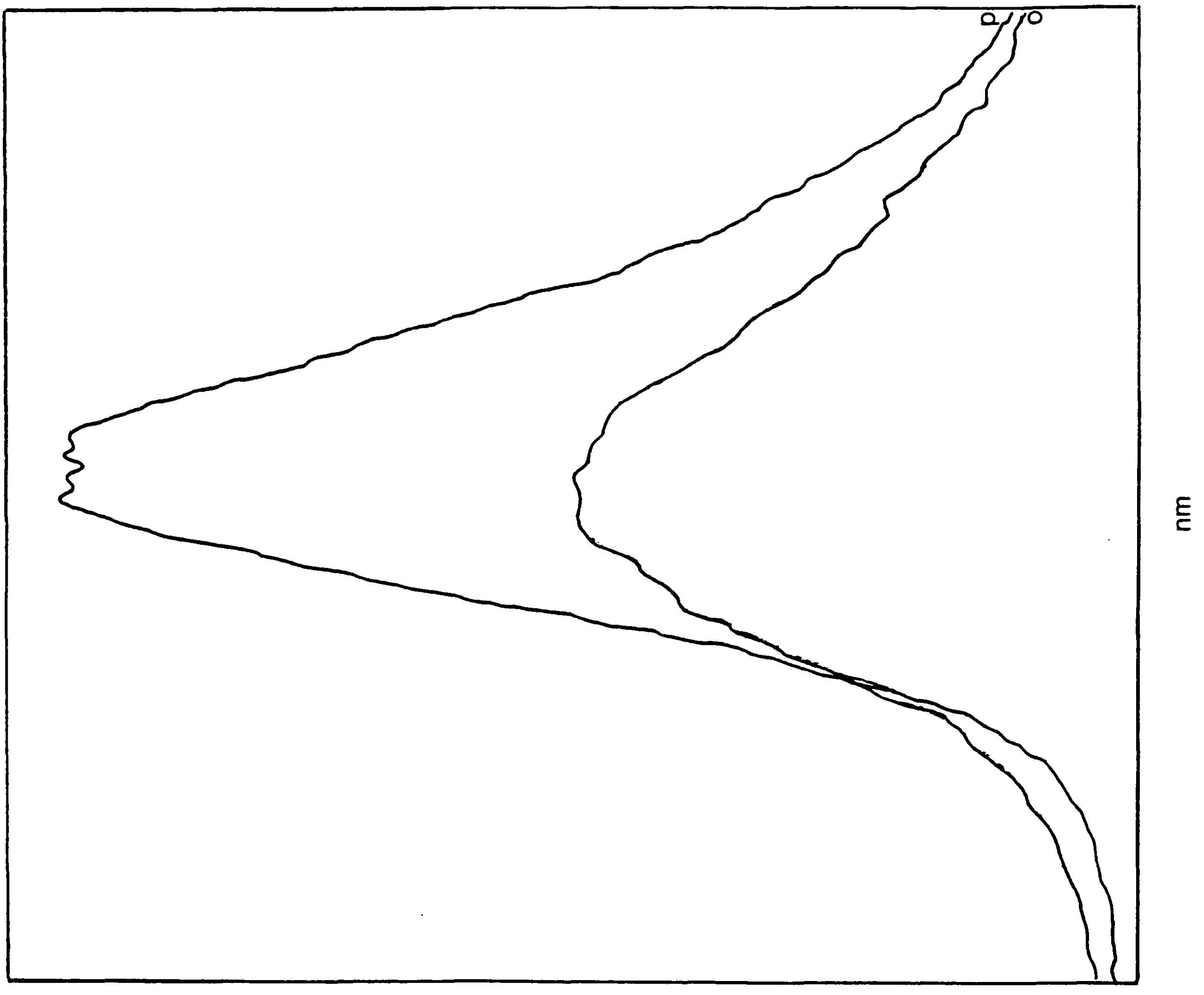


Figure 3.26a Fractions o and p : Fluorescence Spectra of Gel Chromatographic Fractions

fluorescence spectra of fractions (where effects of concentration have been taken into account) which suggests that each discrete association is related to an interaction of U with compositionally different humic macromolecules (Table 3.41). At depth for both Muncaster and Esk locations, later fractions are substantially more important in binding U than early fractions and this suggests that the humic material in these fractions is highly stable since the associations of radionuclides have remained intact over the time period of burial ie. no loss of functionality (particularly oxygen-containing functional groups) from the humic molecules in these fractions. This is consistent with the following observations:

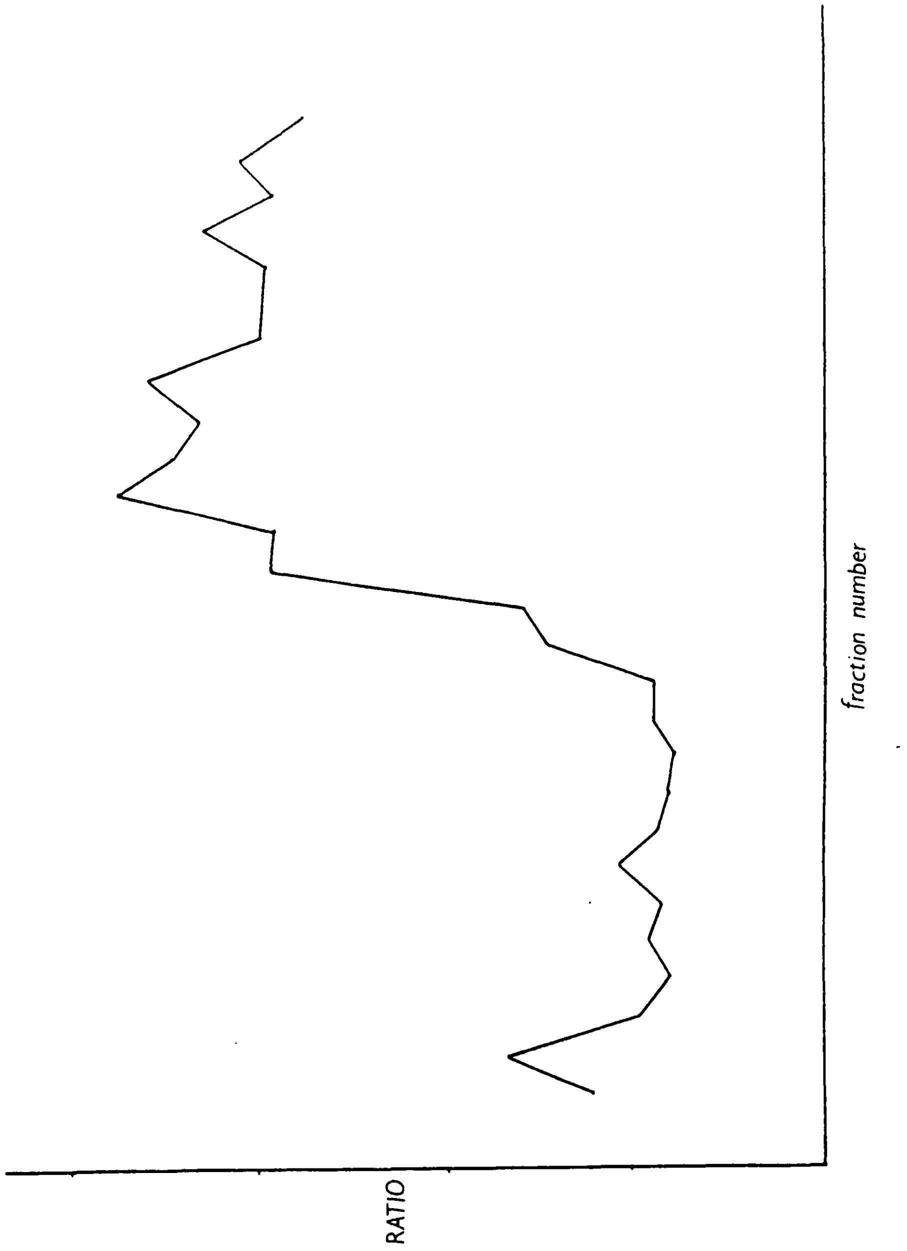
i) aromatic nature identified in FTIR spectra (Spectrum 3.13) which is consistent with a predominantly non-marine origin of this component,

ii) a high $E_4/E_6$ ratio resulting from a strong absorbance at 465nm which is indicative of the presence of quinones (Spectrum 3.34).

iii) a significant increase in the intensity of fluorescence in later fractions confirming the presence of aromatic units which give rise to strong emissions in fluorescence spectra.

It is not implied that these features relate to the specific associations of the U binding to humic macromolecules but only to the inherent stability of the macromolecules in these fractions. In other words it is not implied that U is directly bound to the aromatic rings since the related absorptions in the IR region are not the only feature indicated in the spectrum. A range of oxygen-containing functional groups are also indicated to occur from the spectra of these fractions.

Fractions 14-17 (region 5) which contained U for 0-5cm humic substances contain very little U for 10-15cm humic substances and spectra show that there is a loss of oxygen containing functional groups from humic substances extracted from increasing depths. This is in agreement with the proposal that the degradation of



**Spectrum 3.34 $E_4/E_6$ Ratio : Muncaster 10-15cm**

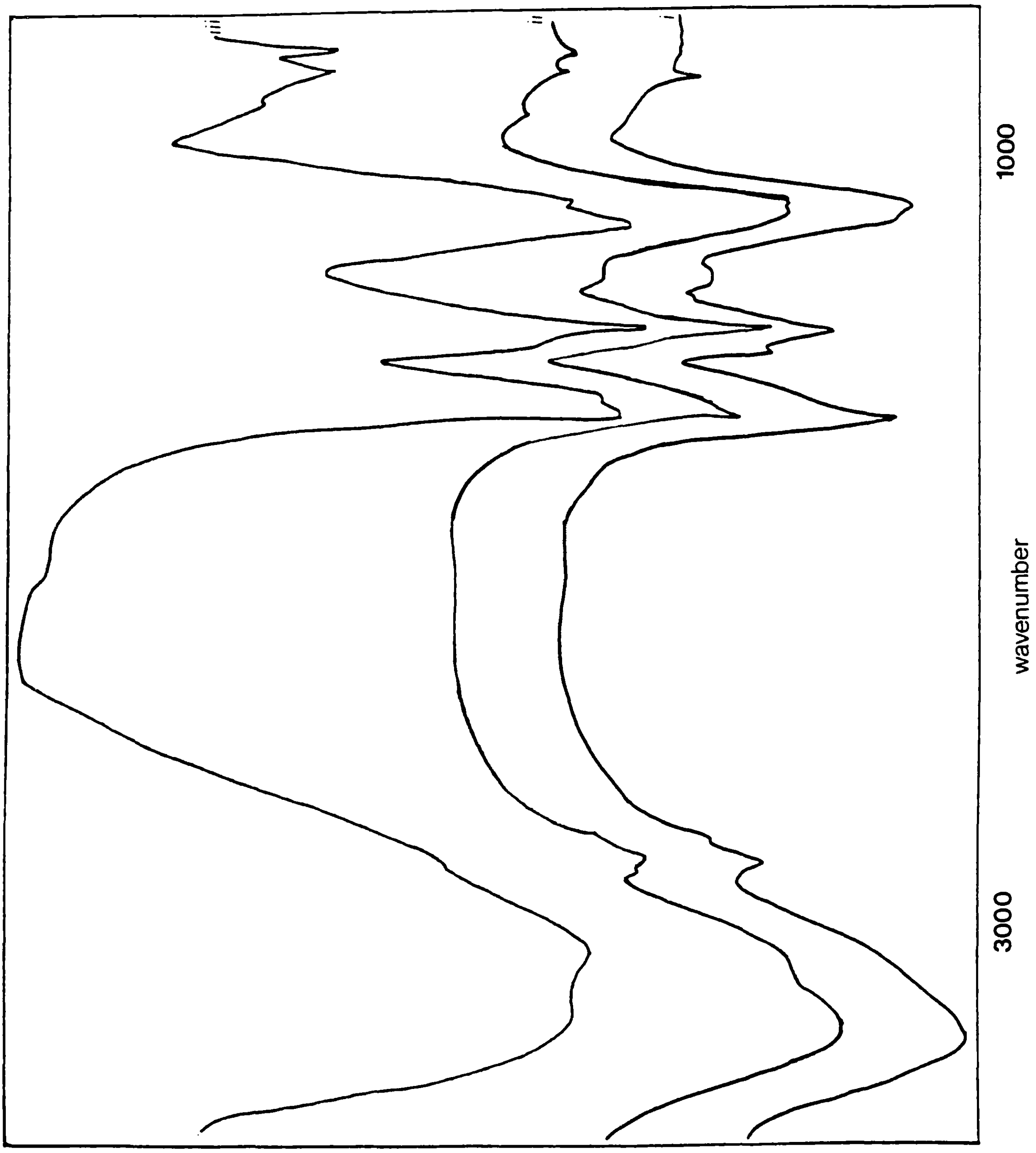
the humic fraction involves the loss of oxygen containing functional groups which are of known importance in actinide binding (Katz, Seaborg and Morss, 1986).
The U distribution pattern for fractionated 0-5cm humic substances from the highly reducing, periodically marine- inundated Muncaster soil bears some significant similarities to that of the 0-5cm Esk humic material. U displays a number of discrete associations and again a particularly strong association with later fractions. Stronger associations with early fractions indicate an increased important of more aliphatic components due to a lower contribution of organic debris from wooded plants. Less significant changes are observed with depth as a result of the highly reduced nature of this soil (Livens, 1985).
A strikingly different U distribution pattern is observed for 0-5cm humic substances from Southwick Merse which is highly marine influenced. A comparison of 0-5cm and 40-45cm U distribution patterns show a marked redistribution of U with increasing depth consistent with the degradation of the humic fraction. FTIR spectroscopic studies further suggest that the degradable humic material is predominantly of marine origin and the remaining association of U at 40-45cm occurs with humic material of terrigenous origin (Spectra 3.21 and 3.22)
Comparison of the distribution of U amongst gel chromatographic fractions of humic substances from different locations therefore highlights a relationship between

i) the degree of marine influence at a particular location following the order :

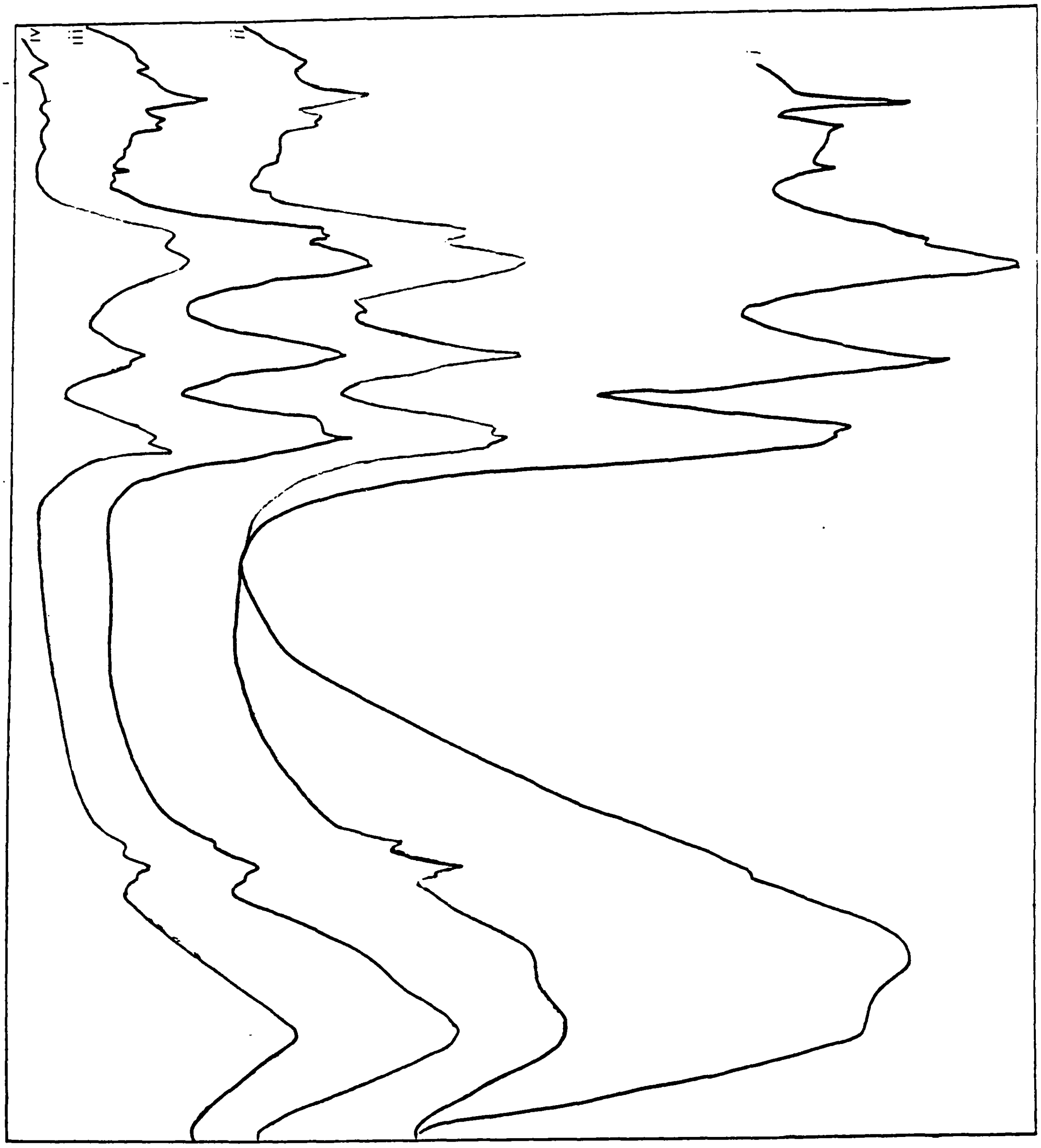
Esk < Muncaster < Southwick Merse,

ii) the prevailing vegetation cover (biomass inputs)
and the U distribution pattern although the prevailing conditions (oxidising or reducing) are also influential.

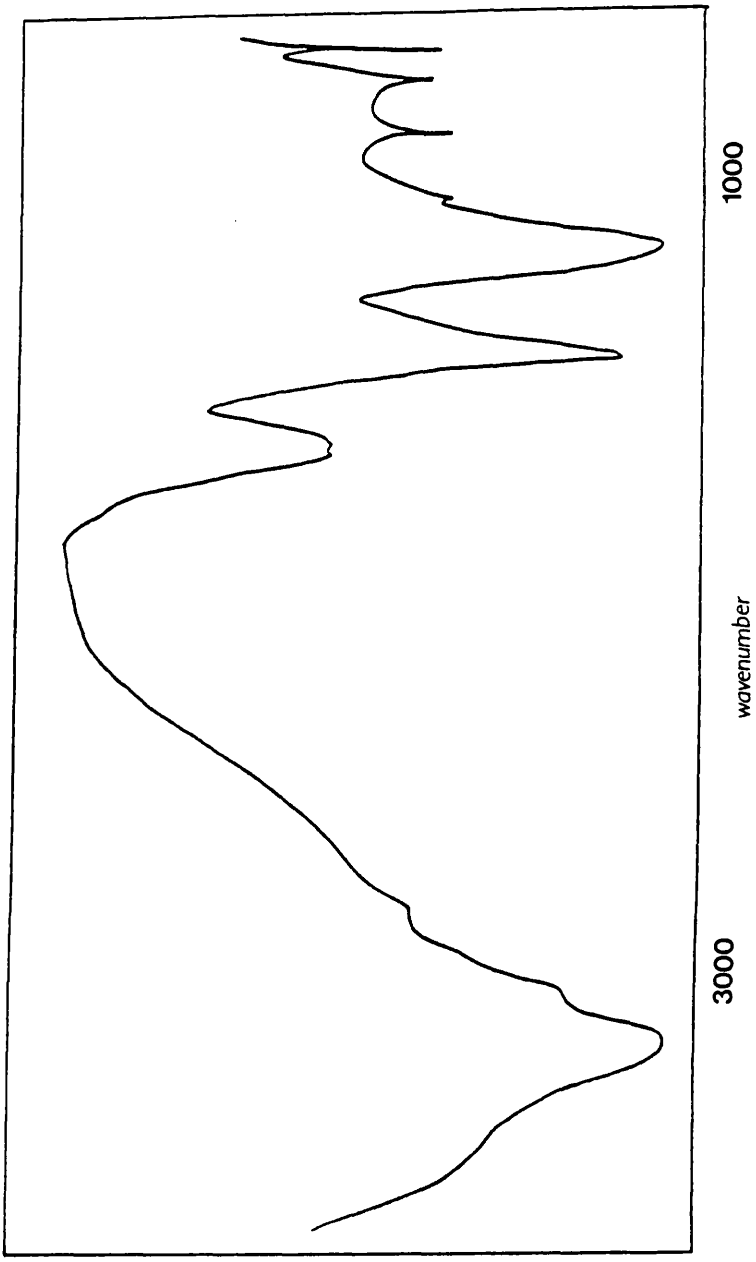
The Esk sample contains the most highly terrigenous humic material and the Southwick Merse 0-5cm humic material has two main components, one of which



**Spectrum 3.21a Gel Chromatographic Fractions of Southwick 0-5cm Humic Substances using Superdex 75 Gel**
**i) Fraction 2 ii) Fraction 4 iii) Fraction 6**



Spectrum 3.21b Gel Chromatographic Fractions of Southwick 0-5cm Humic Substances using Superdex 75 Gel
i) Fraction 9 ii) Fraction 12
iii) Fraction 15 iv) Fraction 18



Spectrum 3.22 A Typical Gel Chromatographic Fraction of Humic Substances Isolated from an Irish Sea Offshore Sediment using Superdex 75

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | high | 20 | 36 | 46 |
| 2 | medium | 16 | | |
| 3 | medium | 10 | 25 | |
| 4 | medium | 15 | | 50 |
| 5 | very low | 3 | 35 | |
| 6 | high | 32 | | |

**Table 3.18 Esk 0-5cm Thorium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very low | 4 | 19 | 29 |
| 2 | medium | 15 | | |
| 3 | high | 20 | 23 | |
| 4 | very low | 3 | | 61 |
| 5 | high | 33 | 58 | |
| 6 | high | 25 | | |

**Table 3.19 Muncaster 0-5cm Thorium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | high | 31 | 58 | 80 |
| 2 | high | 27 | | |
| 3 | high | 22 | 25 | |
| 4 | very low | 3 | | 17 |
| 5 | low | 6 | 14 | |
| 6 | low | 8 | | |

**Table 3.20 Merse 0-5cm Thorium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | medium | 10 | 16 | 36 |
| 2 | low | 6 | | |
| 3 | high | 20 | 58 | |
| 4 | high | 38 | | 50 |
| 5 | very low | 2 | 22 | |
| 6 | high | 20 | | |

**Table 3.21 Esk 10-15cm Thorium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very low | 3 | 21 | 38 |
| 2 | medium | 18 | | |
| 3 | medium | 17 | 20 | |
| 4 | very low | 3 | | 54 |
| 5 | very low | 4 | 51 | |
| 6 | very high | 47 | | |

**Table 3.22 Muncaster 10-15cm Thorium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | high | 38 | 69 | 75.5 |
| 2 | high | 31 | | |
| 3 | low | 6.5 | 16 | |
| 4 | low | 9.5 | | 18.5 |
| 5 | very low | 4 | 9 | |
| 6 | low | 5 | | |

**Table 3.23 Merse 40-45cm Thorium G100**

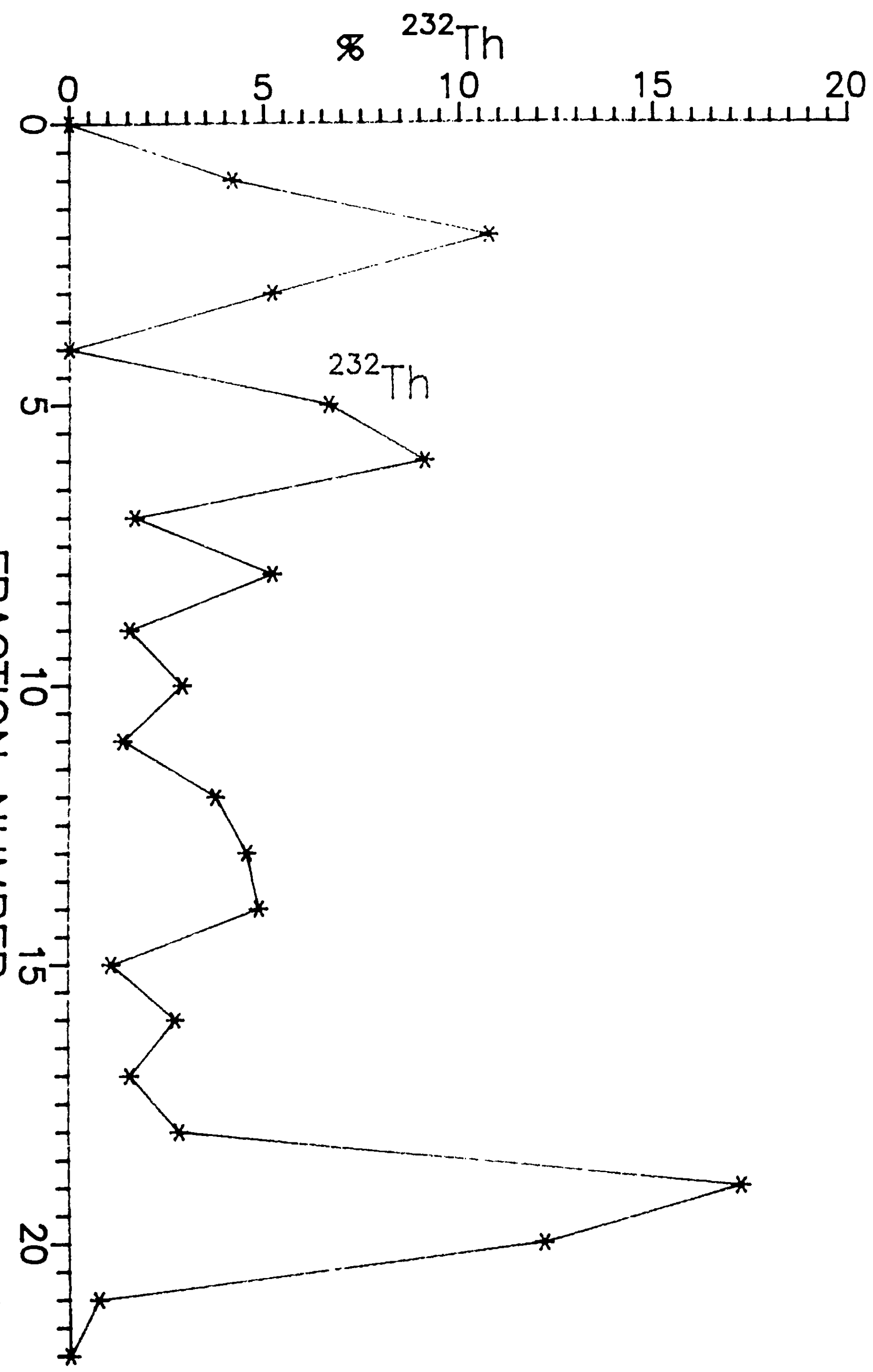


**Figure 3.17 Esk 0-5cm Humic Substances : Percentage Distribution of $^{232}$Th**

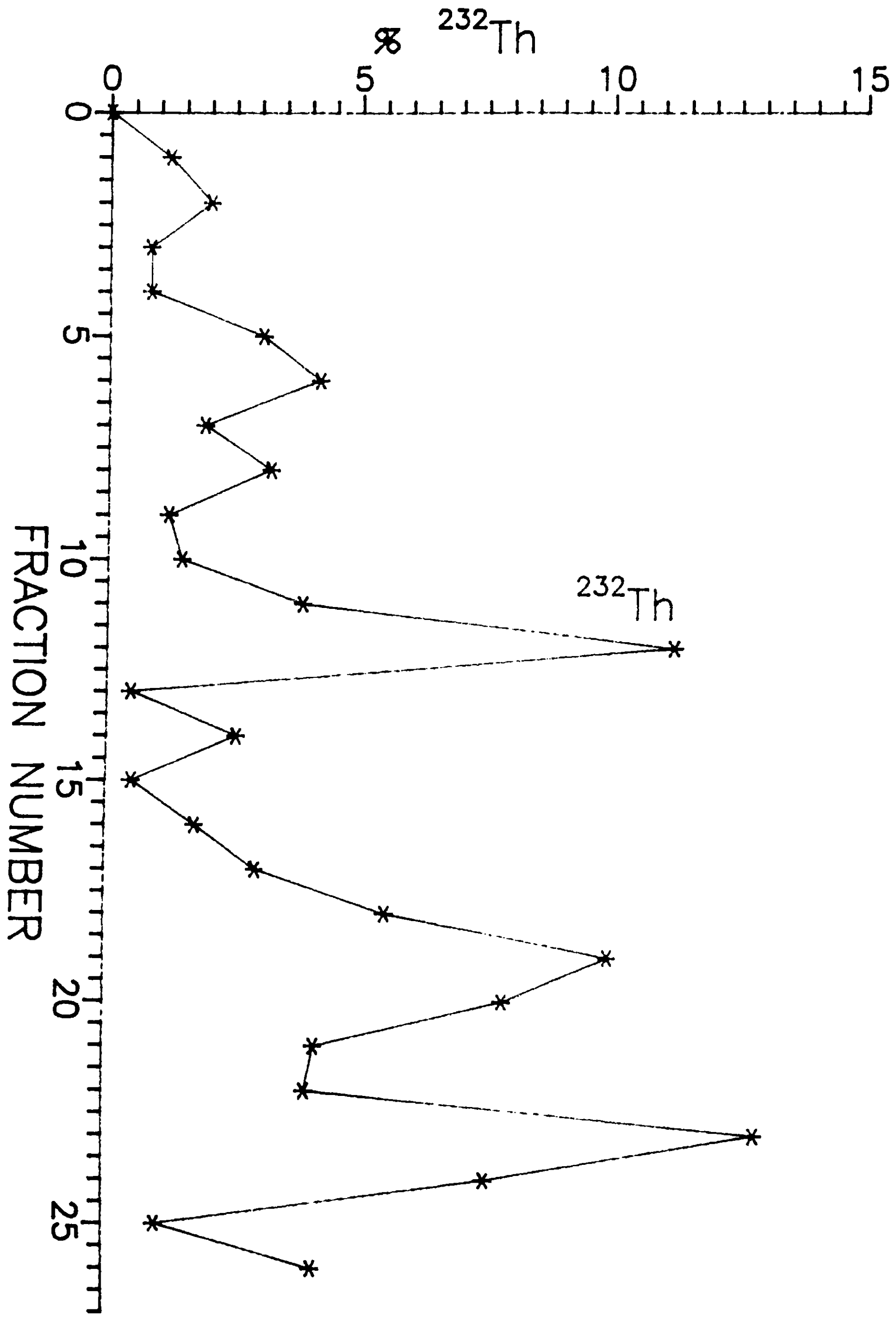


**Figure 3.18 Muncaster 0-5cm Humic Substances : Percentage Distribution of $^{232}$Th**

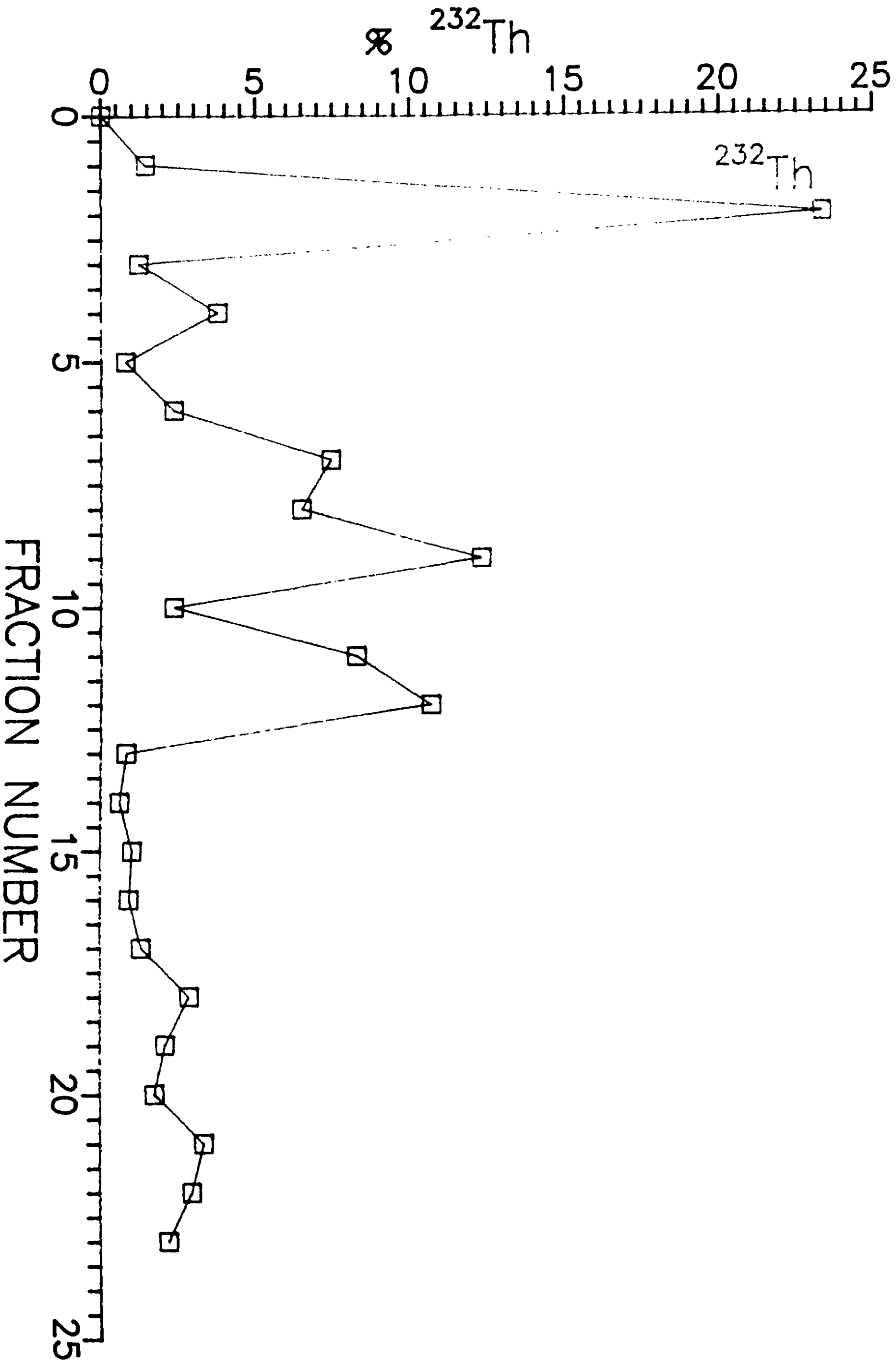


**Figure 3.19 Southwick Merse 0-5cm Humic Substances : Percentage Distribution of $^{232}$Th**

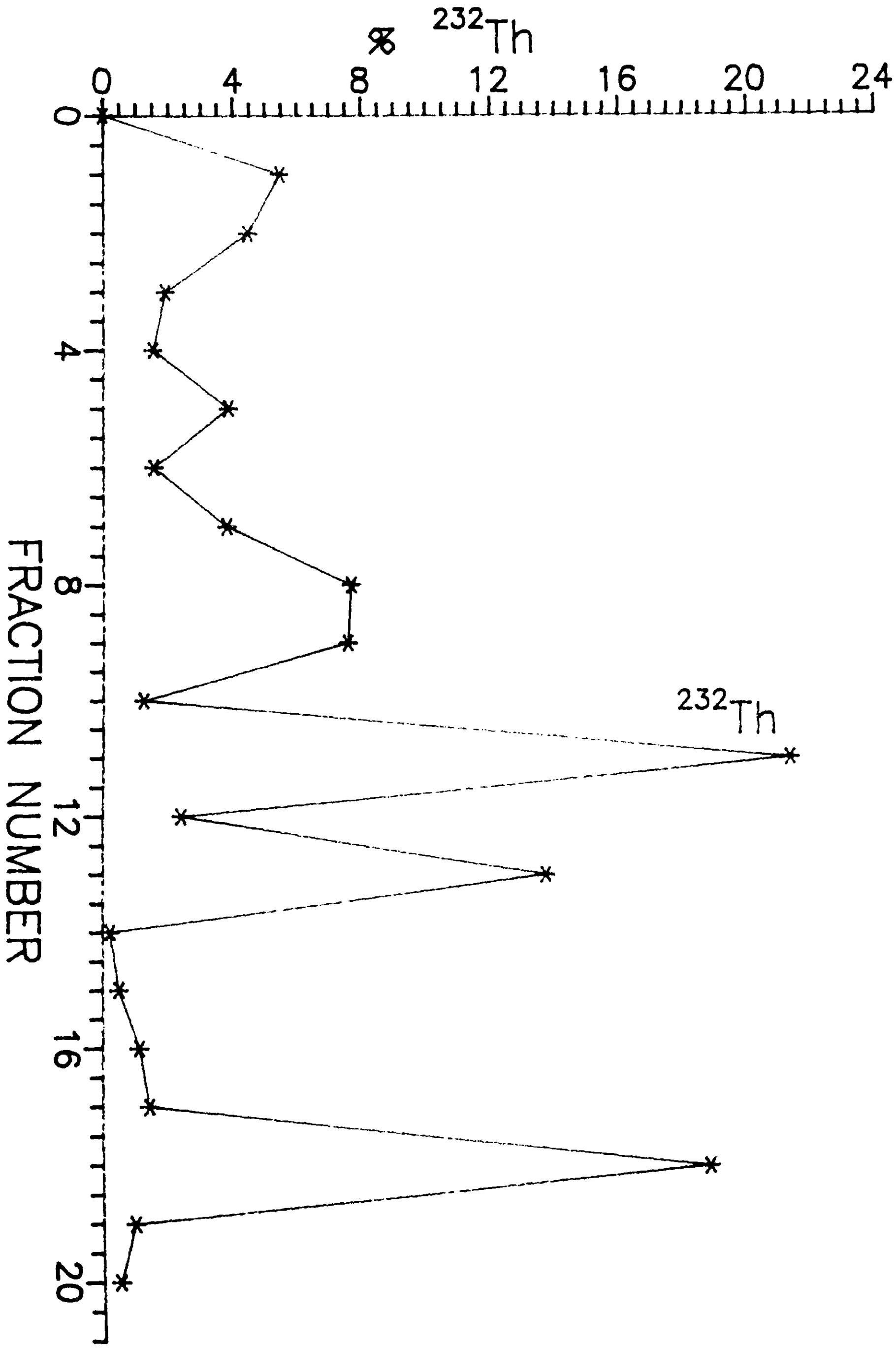


Figure 3.20 Esk 10-15cm Humic Substances : Percentage Distribution of $^{232}$Th

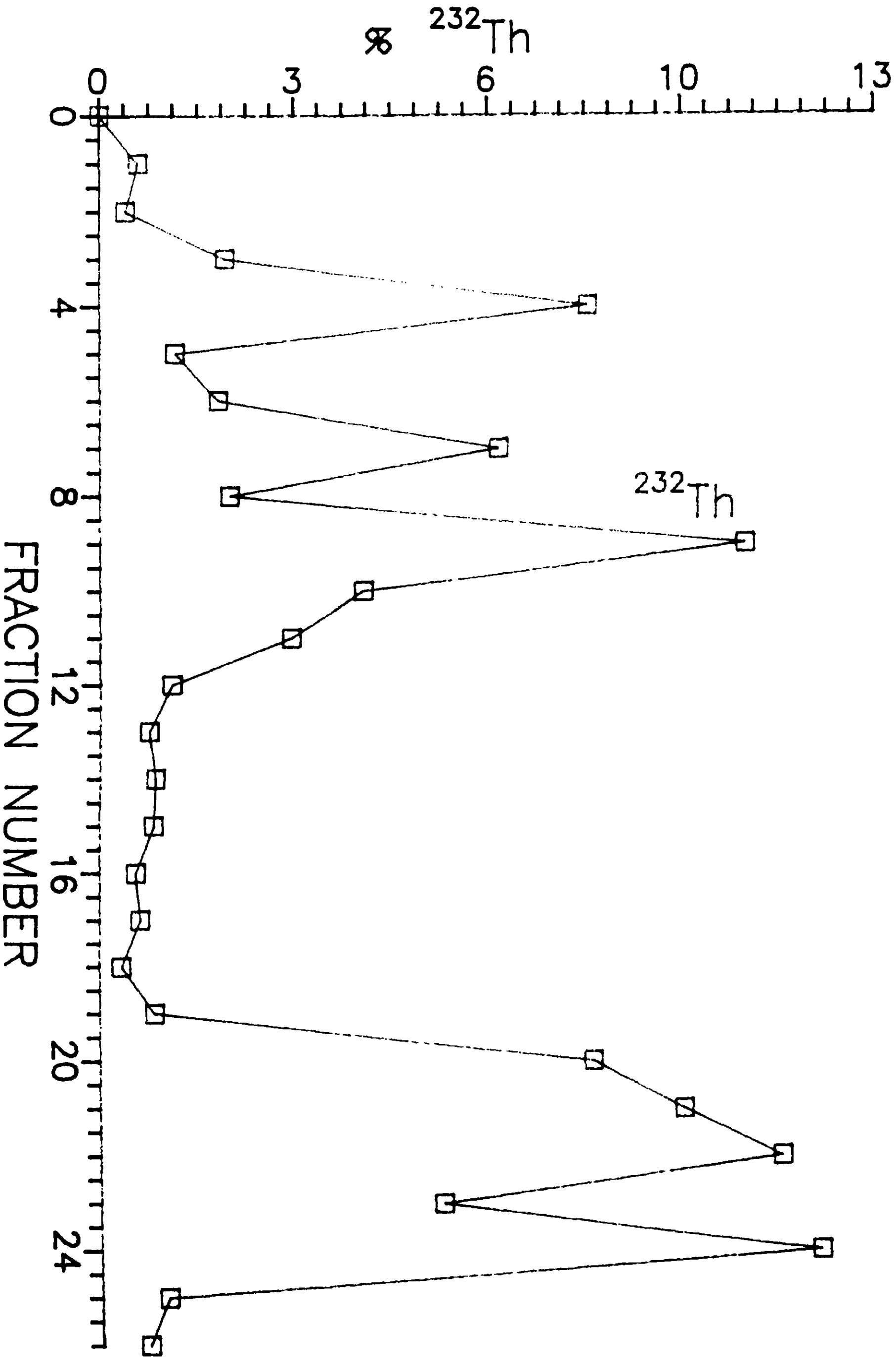


Figure 3.21 Muncaster 10-15cm Humic Substances : Percentage Distribution of $^{232}$Th

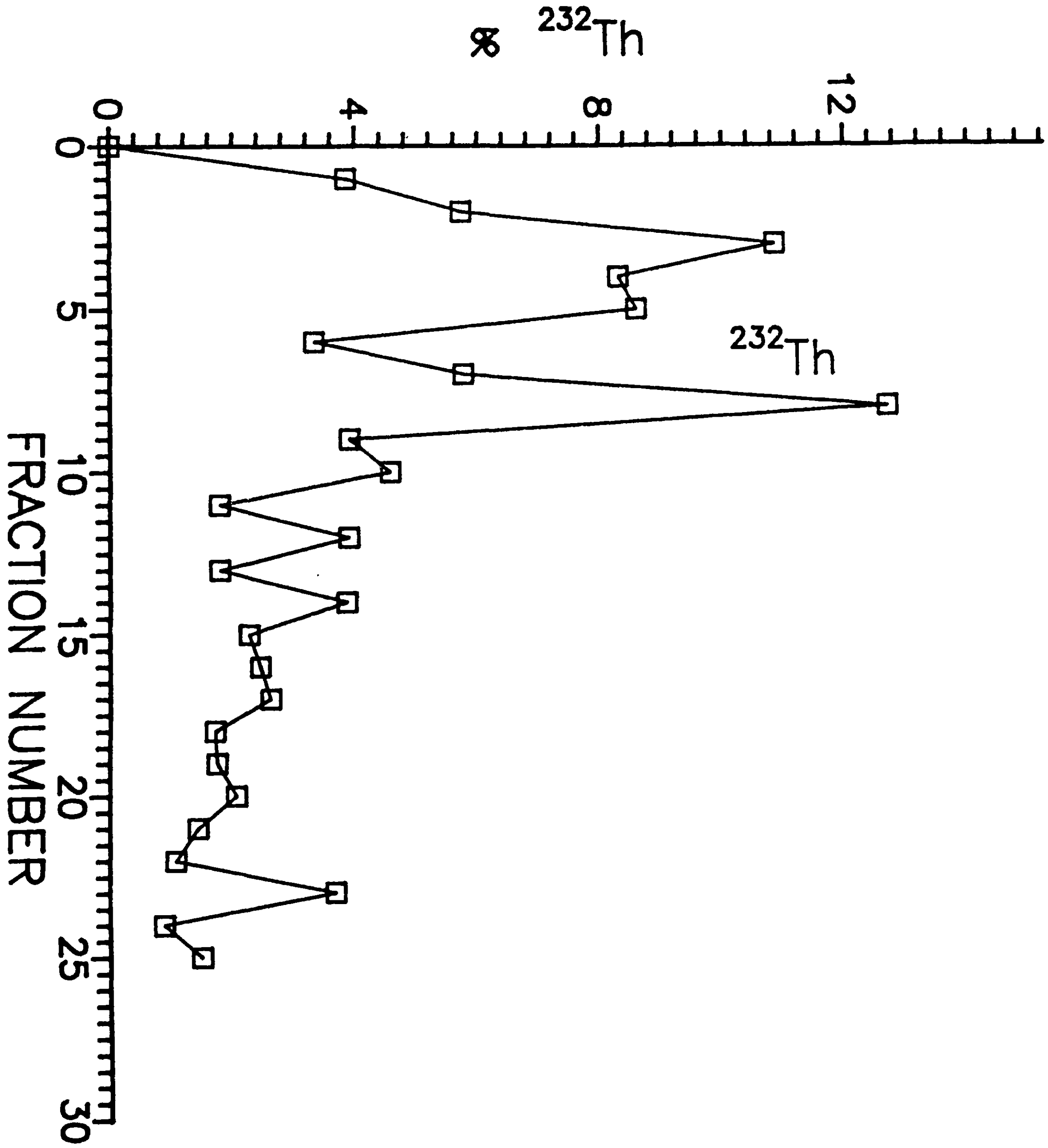


**Figure 3.22 Southwick Merse 40-45cm Humic Substances : Percentage Distribution of $^{232}$Th**

is marine derived.

### 3.6.1.8 Th Distribution : Esk 0-5cm

As for U, discrete associations of Th are observed in the distribution between components of humic substances (Figure 3.17 and Table 3.18). The six main peaks were classified as follows:

high : medium : medium : medium : very low : high

Although the overall distributions of U and Th initially appear very similar, with a significant proportion of Th being associated with the humic material in fractions comprising region 6 (32%), a number of significant differences are observed. In particular, the ratio of percentage associations of U and Th in the six main regions is not constant. Th is found to have a preferential association with the humic molecules in fractions comprising regions 1 and particularly region 2. Contrastingly, U has a strong preferential association with the humic molecules in fractions comprising region 5. In all other regions, U and Th have a very similar distribution. This is quantified below in terms of the %U / %Th ratio for each region :

0.8 : **0.5** : 1.1 : 0.9 : **5.7** : 1.1

### 3.6.1.9 Th Distribution : Muncaster 0-5cm

As was observed for the comparison of U distributions amongst fractions of 0-5cm Esk and Muncaster humic substances (Figure 3.18 and Table 3.19), certain similarities are observed in the distribution pattern of Th when these two humic materials are compared. The underlying trend indicates a strong association of U and Th with the molecules in later fractions as exemplified by the classifications below :

very low : medium : high : very low : high : high

However, there are again differences between the distributions of U and Th which are quantified in terms of the %U / %Th ratio for each of the defined regions:

2.5 : **0.53** : 1.1 : 1.0 : **0.45** : 1.16

U is preferentially associated with humic molecules eluting in region 1 whereas Th is preferentially associated with humic molecules eluting in regions 2 and 5.

### 3.6.1.10 Th Distribution : Merse 0-5cm

The distribution of Th amongst fractions of humic substances from the Merse 0-5cm sample (Figure 3.19 and Table 3.20) shows some similarities to that of U for the same material in that the predominant association (80%) is with molecules eluting in fractions comprising regions 1-3 :

high : high : high : very low : low : low

This is again significantly different from the distribution patterns observed for both Esk and Muncaster humic materials.

Differences between U and Th distributions are reflected in the value of the %U / %Th ratio for the first region only in that Th is preferentially associated with humic molecules eluting in region 1:

**0.74** : 1.0 : 0.91 : - : - : -

### 3.6.1.11 Th Distribution : Esk 10-15cm

The distribution pattern for Th for 10-15cm humic material (Figure 3.20 and Table 3.21) shows a strong association of Th with molecules eluting in region 6 but a predominant association with molecules eluting in region 4. The classifications indicate that, in comparison with the distribution observed for Th in 0-5cm humic substances, the Th distribution has become more localised involving predominantly molecules in regions 3, 4 and 6 :

medium : low : high : high : very low : high

The distribution of Th amongst fractions of humic substances from the Esk 10-15cm sample again indicates a relatively strong association with molecules in later fractions although not as pronounced as for U. This differs from the observations made for the comparison of U and Th distributions for the 0-5cm Esk humic material where the % association of U and Th with later fractions was similar. The %U / %Th ratio highlights the disimilarities between the distributions of U and Th. In particular, Th is preferentially associated with molecules in region 4 whereas U is preferentially associated with molecules in region 6 :

**0.3** : 0.83 : 0.95 : **0.39** : 1.0 : **2.3**

### 3.6.1.12 Th Distribution : Muncaster 10-15cm

The most striking difference between the Th distribution for Muncaster 0-5cm and 10-15cm humic material (Figure 3.21 and Table 3.22) is the loss of the association of Th with molecules eluting in region 5 and the reultant increase in the association with molecules eluting in region 6. In constrast to the 10-15cm Esk Th distribution the predominant association of Th is with humic molecules eluting in these late fraction. As was observed for U, less marked associative differences are observed between 0-5 and 10-15cm Muncaster humic substances when compared with the alterations with depth observed in U and Th associations with Esk humic substances. The main similarities observed between all four distribution patterns for the 10-15cm humic materials from these locations is the loss of the association of U andTh with molecules eluting in region 5 as exemplified by the classification below :

very low : medium : medium : very low : very low : very high

Some differences between U and Th distributions for Muncaster 10-15cm humic material are also indicated by the %U / %Th ratio which indicated a greater association of Th with molecules eluting in region 2 whilst U showed a greater association with moleucles eluting in region 3 :

1 : **0.5** : **1.29** : 1 : 1 : 1.19

### 3.6.1.13 Th Distribution : Merse 40-45cm

The distribution of Th amonst fractions of 40-45cm Merse humic substances (Figure 3.22 and Table 3.23) again reveals a predominant association of Th with early fractions (76%) and in particular an increased % association with the fractions comprising regions 1 and 2 :

high : high : low : low : very low : low

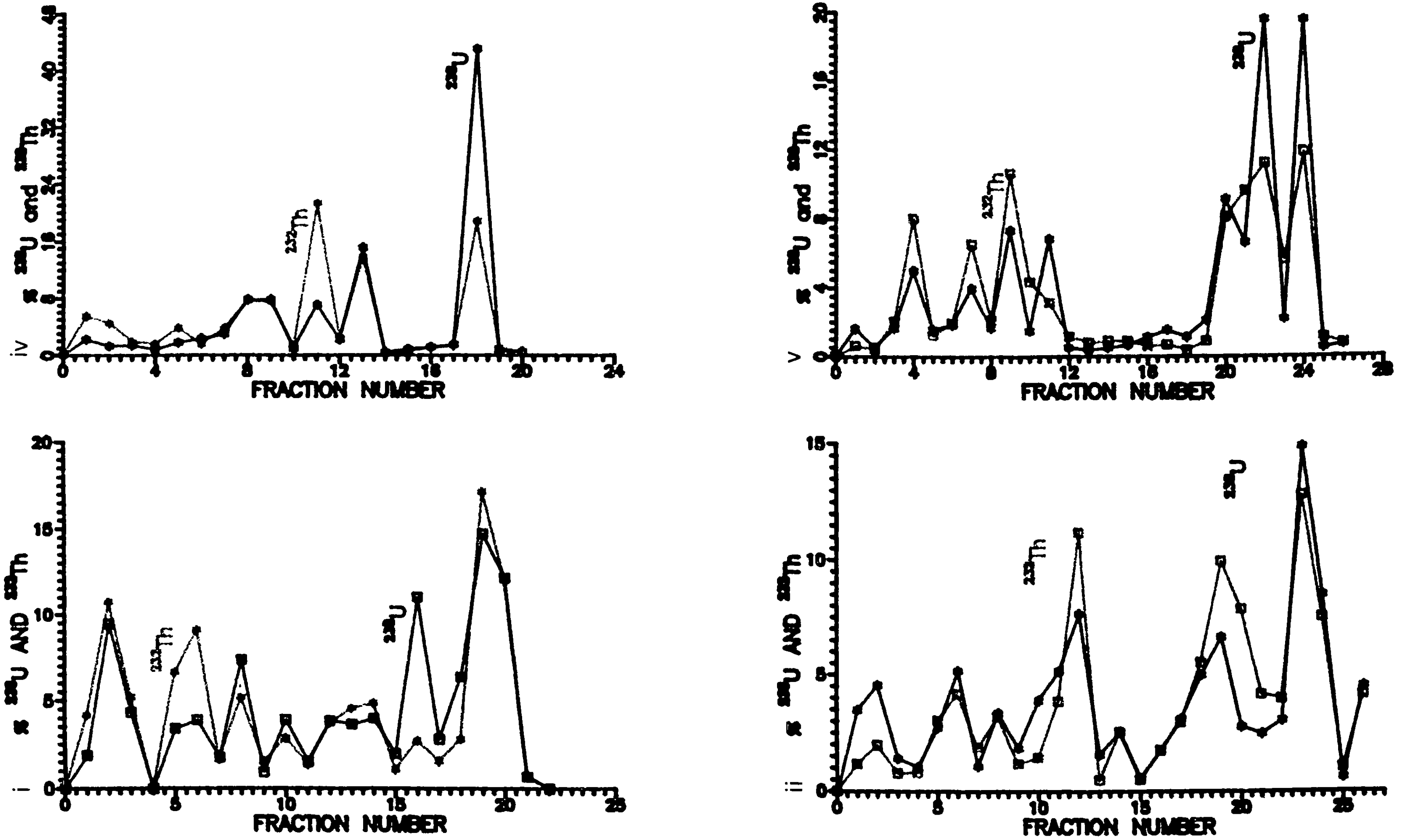
The main difference between the Th distributions for 0-5cm and 40-45cm Merse humic material occur in region 3 with in excess of a three fold loss of binding capacity with respect to Th. %Th present in all later regions is in excess of %U. The differences between U and Th distributions on alteration with depth are most marked for the comparison of 0-5cm and 40-45cm humic material in relation to the respective materials from Esk and Muncaster locations and this is reflected in the variations in the %U / %Th ratio for each of the classified regions for the 40-45cm Merse humic material :

**0.18 : 0.35 : 10.77 : 0.4 : 0.25 : 0.2**

In particular, Th is preferentially associated with molecules in early fractions whereas U is predominantly associated with molecules eluting in region 3 which have a low Th association at this depth. This strongly contrasts with the surface distribution patterns where three strong associations were observed for both U and Th.

### 3.6.1.14 Th Associations with Humic Substances

Discrete associations are observed for Th with humic fractions for all locations. The Th distribution patterns for each location are highly specific to each location but again, as for the trends observed for U, a clear difference between the Esk/Muncaster locations and the Merse location is the absence of strong associations of Th with molecules eluting in region 6 for the Merse material, both for 0-5cm and 40-45cm samples. For each location, the Th distribution alters with depth (Figure 3.23) although the alterations of associations observed differ in some ways from those observed for U and are again highly specific to each location. However, the main trend in differences between the U and Th distributions for certain associations at each location indicate that Th is present in excess of U for regions 1-2 in the Esk and Merse 0-5cm and Esk 10-15cm,


iv
% 238U and 232Th
0
8
16
24
32
40
48
232Th
238U
FRACTION NUMBER
0
4
8
12
16
20
24
v
% 238U and 232Th
0
4
8
12
16
20
232Th
238U
FRACTION NUMBER
0
4
8
12
16
20
24
28
i
% 238U AND 232Th
0
5
10
15
20
232Th
238U
FRACTION NUMBER
0
5
10
15
20
25
ii
% 238U AND 232Th
0
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232Th
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FRACTION NUMBER
0
5
10
15
20
25

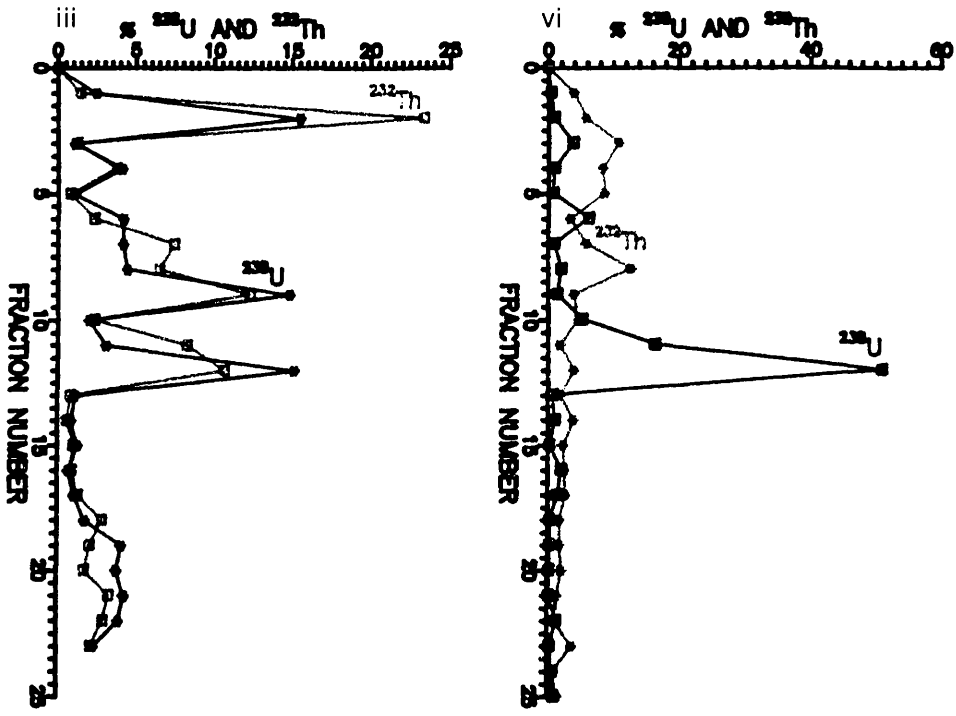


**Figure 3.23 Humic Substances : Percentage Distribution of $^{238}$U and $^{232}$Th i) Esk 0-5cm ii) Muncaster 0-5cm iii) Southwick Merse 0-5cm iv) Esk 10-15cm v) Muncaster 10-15cm vi) Southwick Merse 40-45cm**

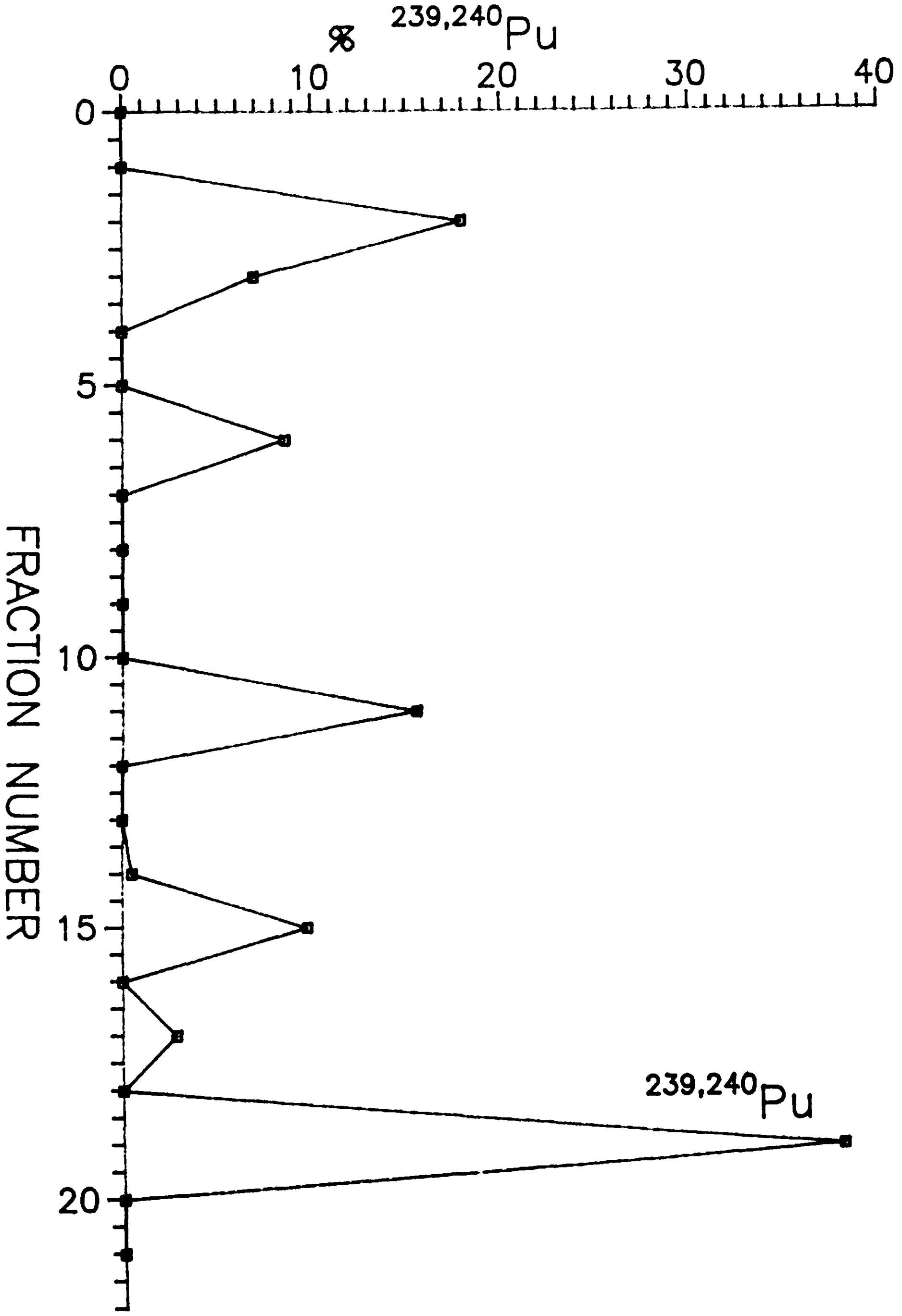


Figure 3.24 Esk 0-5cm Humic Substances : Percentage Distribution of $^{239,240}$Pu

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | high | 24.89 | 33.46 | 48.95 |
| 2 | low | 8.57 | | |
| 3 | medium | 15.49 | 16.0 | |
| 4 | very low | 0.51 | | 51.05 |
| 5 | medium | 12.57 | 50.54 | |
| 6 | high | 37.97 | | |
| Activity = 0.38Bq | | Eluted = 0.35Bq | | |

**Table 3.24 Esk 0-5cm Plutonium G100**

| Muncaster | $^{238}Pu/^{239,240}Pu$ Soil | $^{238}Pu/^{239,240}Pu$ Soil (Hursthouse, 1990) | $^{238}Pu/^{239,240}Pu$ Humic Substances | $^{238}Pu/^{239,240}Pu$ Fraction 2 | $^{238}Pu/^{239,240}Pu$ Fraction 19 |
|---|---|---|---|---|---|
| 0-5cm | 0.2115 | 0.209 ± 0.06 | 0.207 | 0.199 | 0.201 |
| 10-15cm | 0.069 | 0.093 ± 0.028 | 0.071 | - | - |

**Table 3.42 $^{238}Pu/^{239,240}Pu$ Activity Ratios for Soil, Humic Substances and Gel Chromatographic Fractions of Humic Substances (all standard errors on activities were less than 0.05)**

Muncaster 10-15cm and Merse 40-45cm humic substances.

### 3.6.2 Plutonium Associations with 0-5cm Esk Humic Substances

The activity ratio of $^{238}$Pu / $^{239,240}$Pu for 0-5cm humic substances is the same as that for the 0-5cm soil sample within error. The ratio was obtained for only certain of the gel fractions due to the low levels of Pu in remaining fractions. The values of the activity ratio can be found in Table 3.42.

The $^{239,240}$Pu distribution pattern for fractions of 0-5cm Esk humic substances is shown in Figure 3.24 and Table 3.24. There are some similarities between the distributions of this anthropogenic radionuclide and the distribution of U. However, there are several differences which would be expected due to the highly characteristic behaviour of individual actinides and and should reflect differences in chemical speciation prior to complexation, which in turn relate to most stable oxidation states and prevailing chemical form in the environment. Using a similar classification system as for U and Th, percentage associations give rise to the following pattern:

high : low : medium : very low : medium : high

and the ratios of percentage distribution for each of these regions (U/Pu and Th/Pu) are :

U/Pu : **0.64** : 0.93 : **0.71** : **26.4** : **1.35** : 0.90

Th/Pu : **0.80 : 1.87 : 0.65 : 29.4 : 0.24 : 0.84**

On the basis of % associations and the Pu / U and Pu / Th ratios highlight the following it can be concluded that Pu is distributed non-uniformly between fractions of humic substances and again shows a marked association with humic molecules eluting in late fractions (region 6). The relative importance of the discrete associations observed, however, varies from that for U and particularly Th indicating that we have preferential associations of individual actinides with different components of the humic material.

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very low | 2 | 18 | 58 |
| 2 | medium | 16 | | |
| 3 | very high | 40 | 62 | |
| 4 | high | 22 | | 44 |
| 5 | high | 20 | 20 | |
| 6 | - | - | | |

**Table 3.25 Esk 0-5cm Neptunium G100**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | - | - | 55 | 55 |
| 2 | very high | 55 | | |
| 3 | - | - | | |
| 4 | - | - | | 31 |
| 5 | high | 31 | 31 | |
| 6 | - | - | | |

**Table 3.26 Muncaster 0-5cm Neptunium G100**

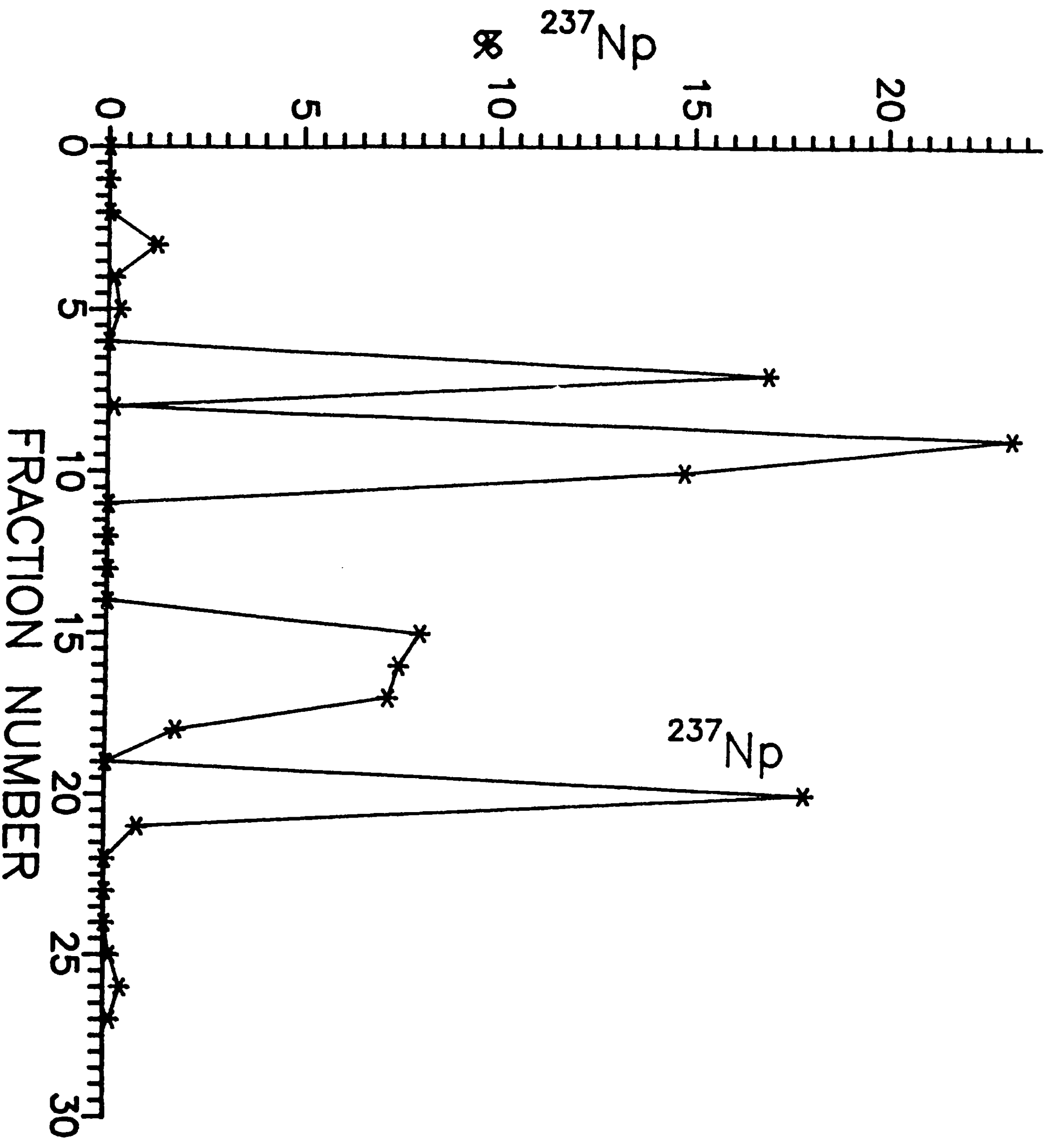


Figure 3.25 Esk 0-5cm Humic Substances : Percentage Distribution of $^{237}$Np

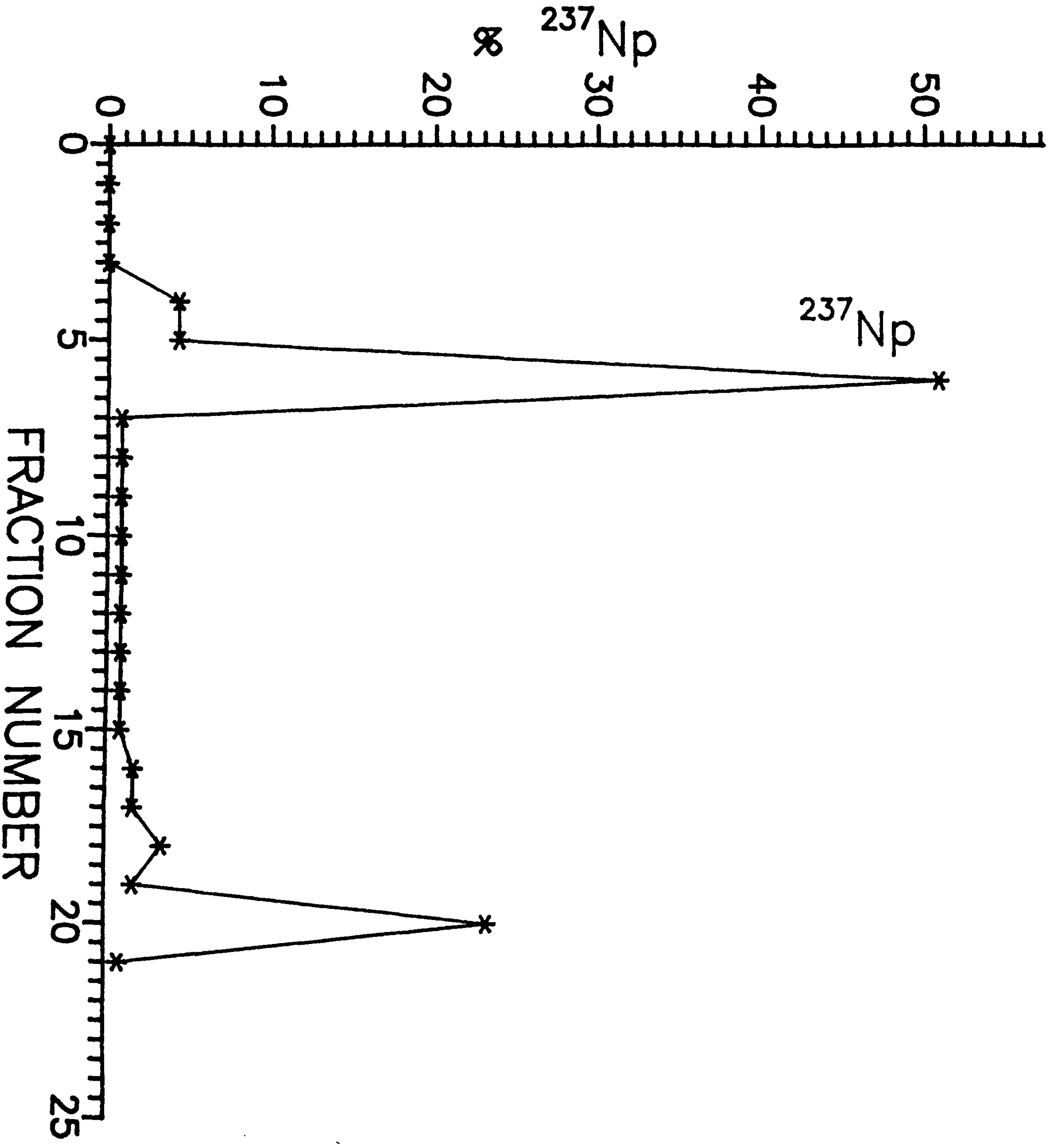


**Figure 3.26 Muncaster 0-5cm Humic Substances : Percentage Distribution of $^{237}$Np**

### 3.6.3.1 Np Associations with 0-5cm Esk Humic Substances

The activity ratio of $^{237}Np/^{239,240}Pu$ was 0.001 for both soil and humic substances which is the same, within error, as that obtained in previous studies (Assinder, 1991; Hursthouse, 1991). Hence, there would not appear to be any preference of humic substances for Pu or Np in the initial interaction of these actinides with humic molecules. However, the distribution of Pu and Np amongst fractions of humic substances (Figure 3.25 and Table 3.25) would appear to indicate that their prevailing associations are highly characteristic of the individual element. The distribution of Np amongst fractions of humic substances is classified as follows :

low : medium : very high : high : high : low

Np is distributed between a smaller number of fractions when compared with Pu, U and Th. In particular, only small associations of Np are observed with fractions comprising regions 1 and 6. The remaining associations occur in two groups separated by a number of fractions for which the concentration of Np was below the detection limit (although chemical yields for the analyses of these samples were high).

### 3.6.3.2 Np Association with 0-5cm Muncaster Humic Substances

The ratio of $^{237}Np/^{239,240}Pu$ was $1 \times 10^{-5}$ for both soil and humic substances indicating again that no preferential association of Pu or Np was being observed.. Although the activity of Np present in this soil is significantly lower than that of 0-5cm Esk sample, it was still possible to do the analyses of fractions of humic substances for Np which gave the following distribution pattern.

very low : very high : very low : very low : very high : very low

Two main associations were observed which were separated by a number of fractions which contained Np at levels below the detection limit.

The Np distribution pattern for fractions of 0-5cm Muncaster humic substances (Figure 3.26 and Table 3.26) provides further information to suggest that the interaction of Np is both highly specific and characteristic of the behaviour of this individual actinide.

## 3.7 An Investigation of the Nature of the Separation Processes Occurring During Gel Chromatographic Fractionation of Humic Substances

In the later part of this study, the observed compositional differences (namely aromatic or aliphatic character) between humic materials from Esk and Merse locations were exploited in the investigation of the separation processes occurring during gel fractionation.

Humic substances from Southwick Merse sediments were observed visually to adsorb less irreversibly to Sephadex G100 gel when compared with humic substances from the Esk soil (the degree of sorption is significantly smaller [approximately 3%] than that observed for any humic acid studied [up to 30%]) and this can again be related to the composition of the two humic materials. It is proposed that the humic material from the 0-5cm at the Merse location is highly marine-influenced which is confirmed by CHN data (C/H = 7.78; C/N = 10.37) and therefore is predominantly aliphatic in composition whereas that from the Esk location is predominantly terrigenous material (low amino acid content) and more aromatic. Interactions of aromatic compounds with Sephadex gels are most pronounced for gels with higher matrix contents and therefore smaller pore sizes. The interaction of these humic materials was therefore studied using various different Sephadex gels and the results of fractionation characterised spectroscopically and radioanalytically. The difference between the two materials was most evident from the distribution patterns obtained for each humic material following fractionation using G25 gel. In both cases, a proportion of the humic material was eluted close to the exclusion volume. However, a marked difference in the distribution of both U and Th for the two materials is apparent (Figure 3.27).

### 3.7.1 U Distribution : G25 Sephadex Gel and 0-5cm Esk Humic Substances

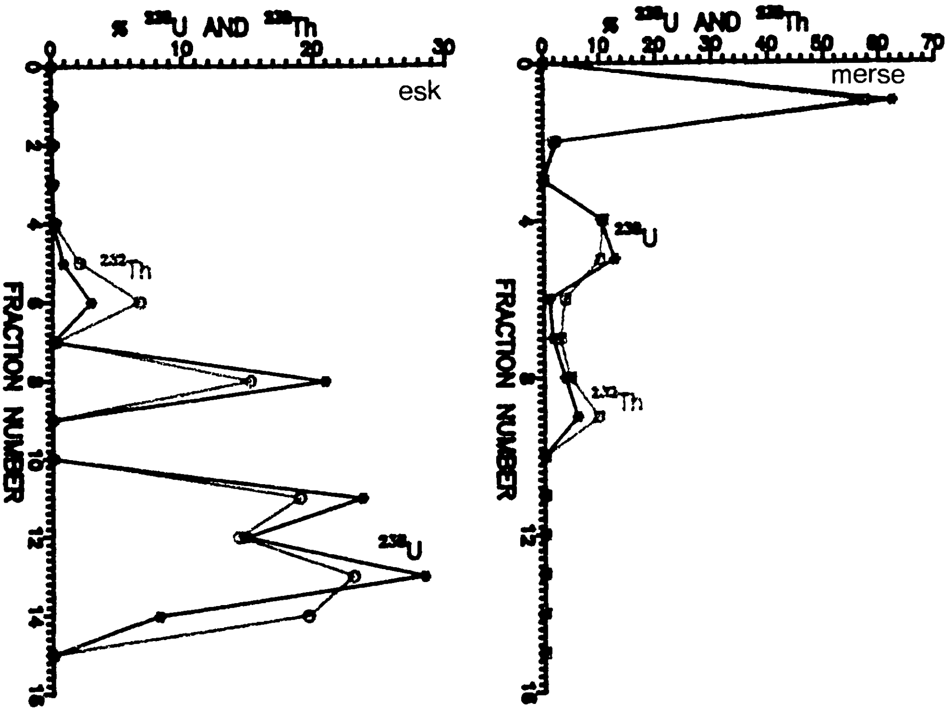


Figure 3.27 Esk 0-5cm and Southwick Merse 0-5cm Humic Substances : Percentage Distribution of $^{238}$U and $^{232}$Th (G25 Sephadex)

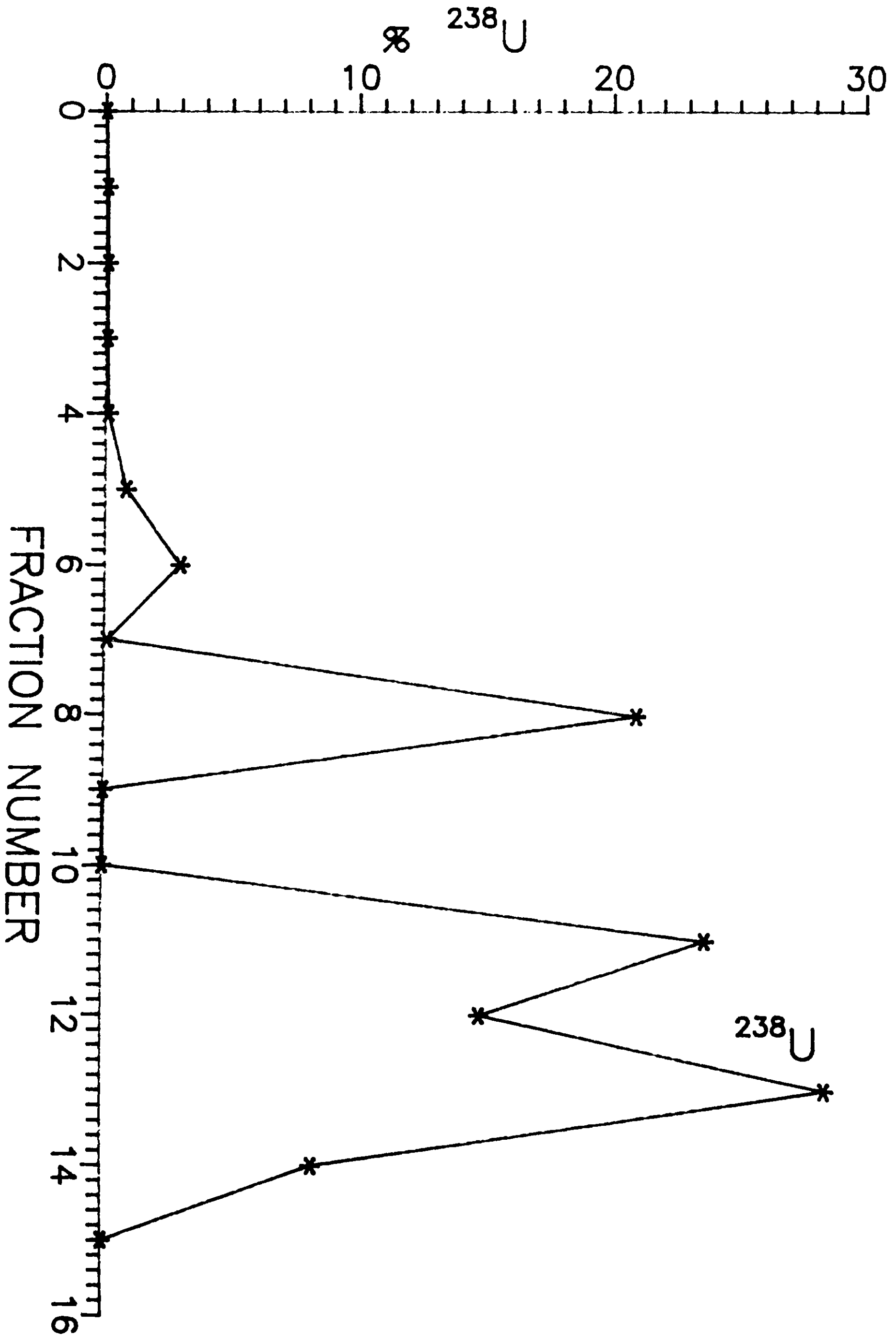


Figure 3.28 Esk 0-5cm Humic Substances : Percentage Distribution of $^{238}$U (G25 Sephadex)

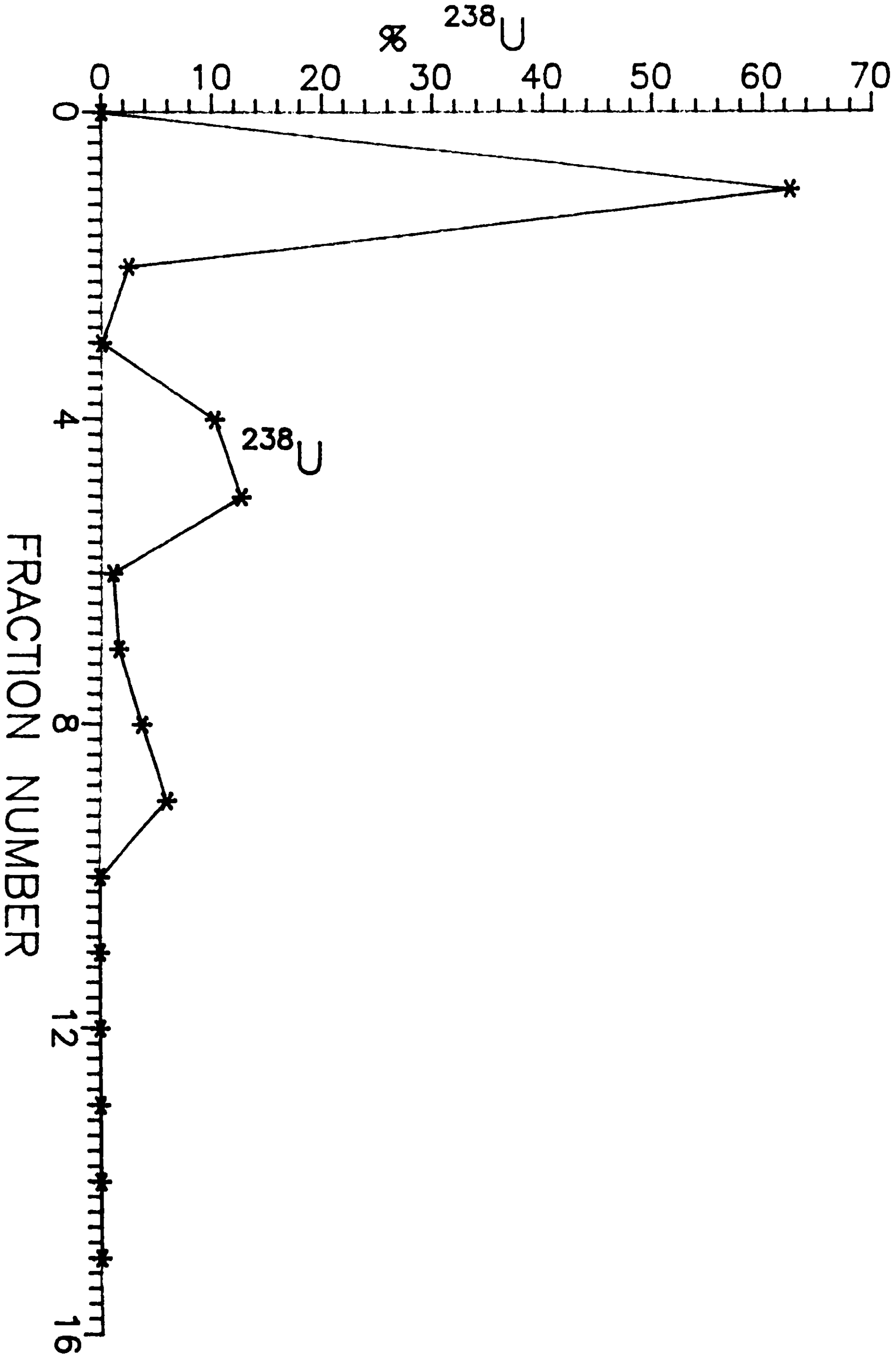


Figure 3.29 Southwick Merse 0-5cm Humic Substances : Percentage Distribution of $^{238}$U (G25 Sephadex)

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | - | - | 0 | 5 |
| 2 | - | - |  |  |
| 3 | low | 5 | 27 |  |
| 4 | low | 22 |  | 95 |
| 5 | high | 36 | 73 |  |
| 6 | high | 37 |  |  |
| Activity = 0.15Bq per 100mg |  | Eluted = 0.13Bq |  |  |

Table 3.27 Esk 0-5cm Uranium G25

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very high | 64 | 66 | 88 |
| 2 | very low | 2 |  |  |
| 3 | high | 22 | 32 |  |
| 4 | medium | 10 |  | 11 |
| 5 | very low | 1 | 1 |  |
| 6 | - | - |  |  |
|  |  |  |  |  |

Table 3.28 Merse 0-5cm Uranium G25

The distribution pattern (Figure 3.28 and Table 3.27) can be classified as follows:

very low : very low : low : high : very high : very high

The Esk distribution pattern shows that 95% of $^{238}U$ is associated with the molecules which are retarded by the gel and eluting in regions 4,5 and 6. This would not be expected on the basis of the U distribution pattern obtained using G100 Sephadex gels which showed that the molecules eluting in regions 1,2 and 3 contained 35% of the U associated with humic substances. Separation on the basis of size would result in the coelution of at least a proportion of the material in these early fractions at the exclusion volume. The increasing actinide content with increasing fraction number is shown spectroscopically to correlate with the predominance of aromatic units in later fractions (Spectrum 3.19) and so it is concluded that a chemical separation is involved in the processes occurring on the gel column during the elution of humic substances. This process is dominant for the G25 gel and occurs to a lesser extent for the G100 gel (Figure 3.11 and Table 3.11).

### 3.7.2 U Distribution : G25 Sephadex Gel and 0-5cm Merse Humic Substances

The distribution pattern (Figure 3.29 and Table 3.28) can be classified as follows:

very high :very low : high : medium : very low : very low

The distribution pattern for fractions of Southwick Merse humic substances indicates that 86% of $^{238}U$ is associated with molecules eluting close to the exclusion volume in fractions comprising regions 1,2 and 3. The elution of high proportions of humic close to the exclusion volume would be expected on the basis of the high molecular weight attributed to humic substances. The sum of the % associations for regions 1 and 2 gives 66% U for G25 compared with 50% for G100.

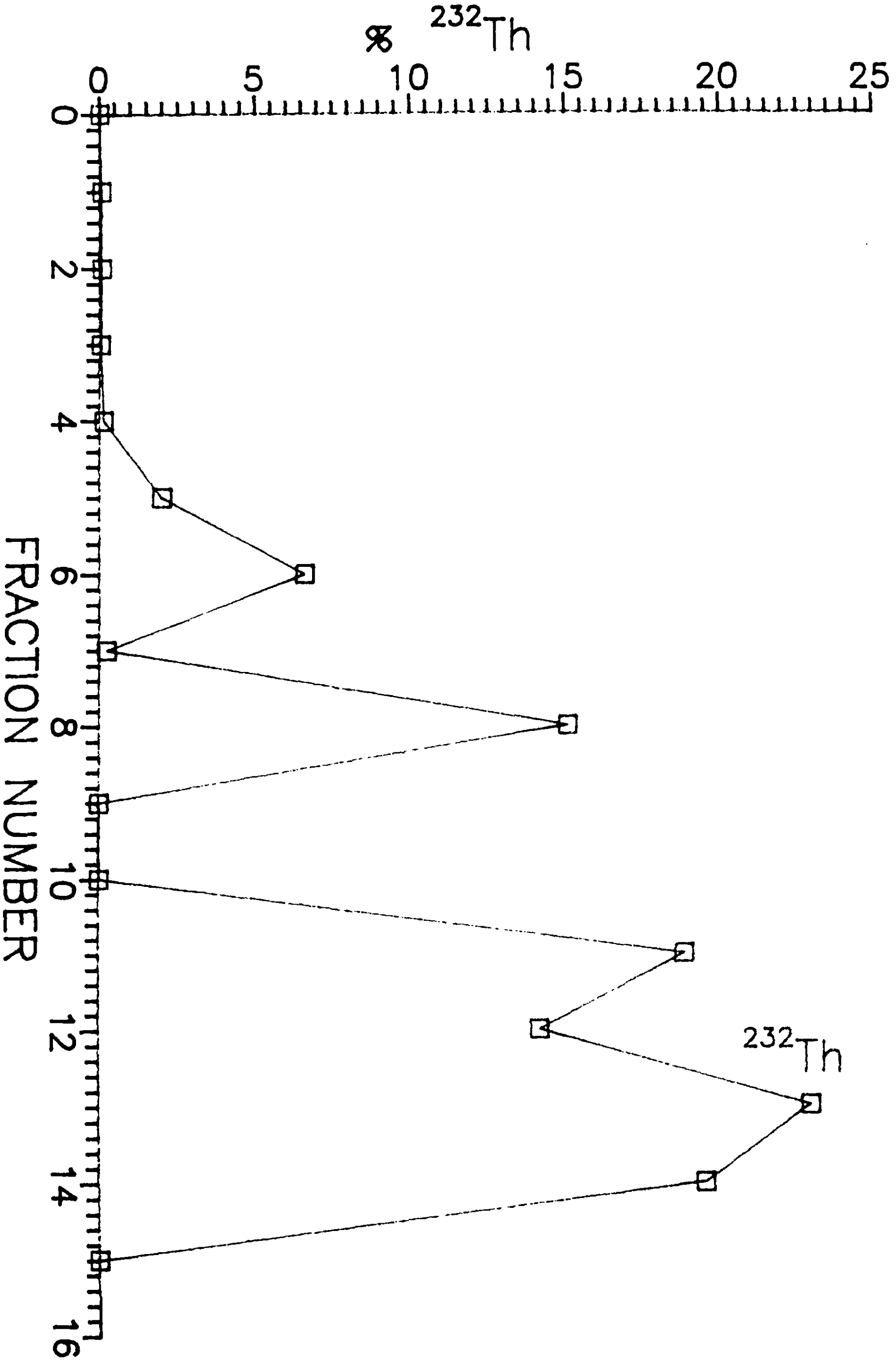


Figure 3.30 Esk 0-5cm Humic Substances : Percentage Distribution of $^{232}$Th (G25 Sephadex)

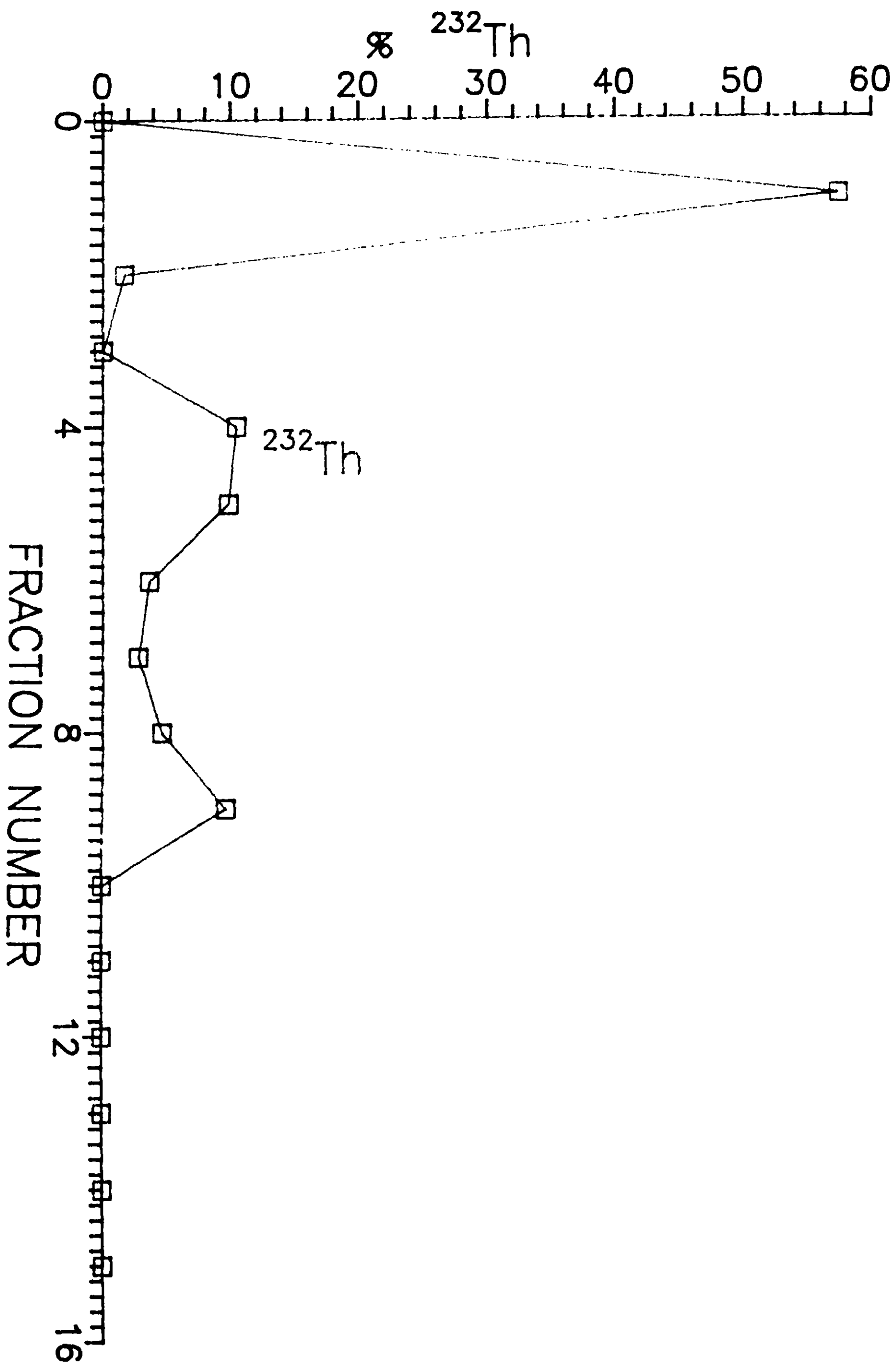


**Figure 3.31 Southwick Merse 0-5cm Humic Substances : Percentage Distribution of $^{232}$Th**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | - | - | 0 | 5 |
| 2 | - | - |  |  |
| 3 | medium | 10 | 27 |  |
| 4 | medium | 17 |  | 93 |
| 5 | high | 32 | 71 |  |
| 6 | high | 39 |  |  |
|  |  |  |  |  |

Table 3.29 Esk 0-5cm Thorium G25

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | very high | 58 | 60 | 81 |
| 2 | very low | 2 |  |  |
| 3 | high | 21 | 37 |  |
| 4 | medium | 16 |  | 17 |
| 5 | very low | 1 | 1 |  |
| 6 | - | - |  |  |
|  |  |  |  |  |

Table 3.30 Merse 0-5cm Thorium G25

### 3.7.3 Th Distribution : G25 Sephadex Gel and 0-5cm Esk Humic Substances

The Th distribution pattern (Figure 3.30 and Table 3.29) again indicates that the predominant associations are with later fractions. In particular, 88% of Th was eluted in fractions comprising regions 4, 5 and 6. The pattern of distribution was classified as follows :

very low : very low : medium : medium : very high : very high

The Th distribution pattern is similar to that of the U pattern indicating that virtually all humic species from this location have a sufficient aromatic nature to be retarded by the gel and so very little difference between U and Th distribution can be observed. The ratio of %U / %Th indicates that Th is preferentially associated with humic molecules from region 3 whilst region 4 contained fractions of humic moleucles which had slightly greater preference for U as indicated below:

- : - : **0.5** : **1.29** : 1.13 : 0.95

### 3.7.4 Th Distribution : G25 Sephadex Gel and 0-5cm Merse Humic Substances

The Th distribution (Figure 3.31 and Table 3.30) shows that 81% of Th was eluted in the fractions comprising regions 1, 2 and 3 and the pattern was classified as follows :

very high : very low : high : medium : very low : very low

Again, the pattern was similar to that of U with only a slight difference being observed in later fractions which showed a preference for Th :

1.1 : - : 1.05 : **0.63** : - : -

The comparison of these elution patterns confirms that the two materials differ significantly in their composition and in particular, the marine-influenced

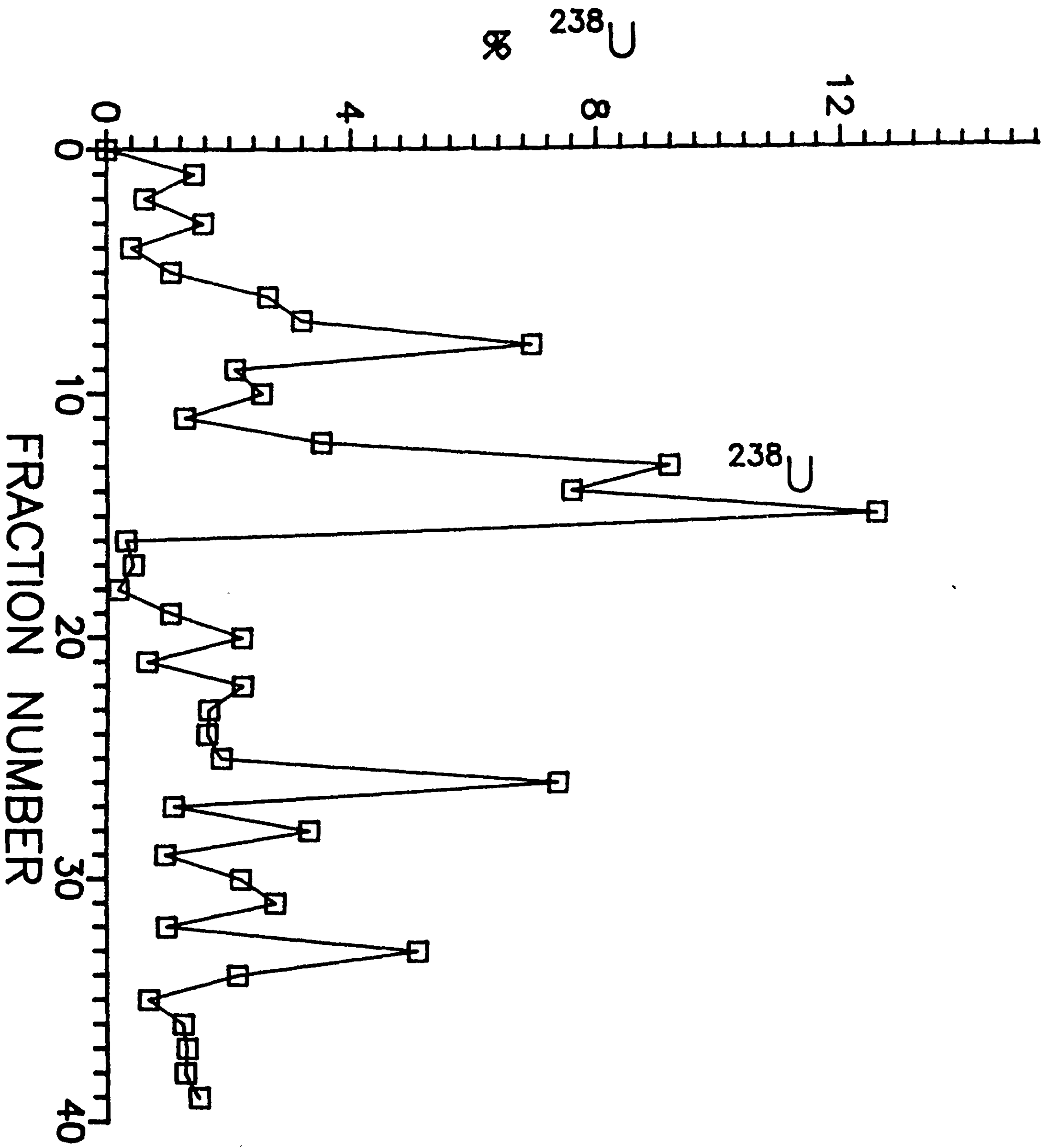


**Figure 3.32 Esk 0-5cm Humic Substances : Percentage Distribution of $^{238}$U (G200 Sephadex)**

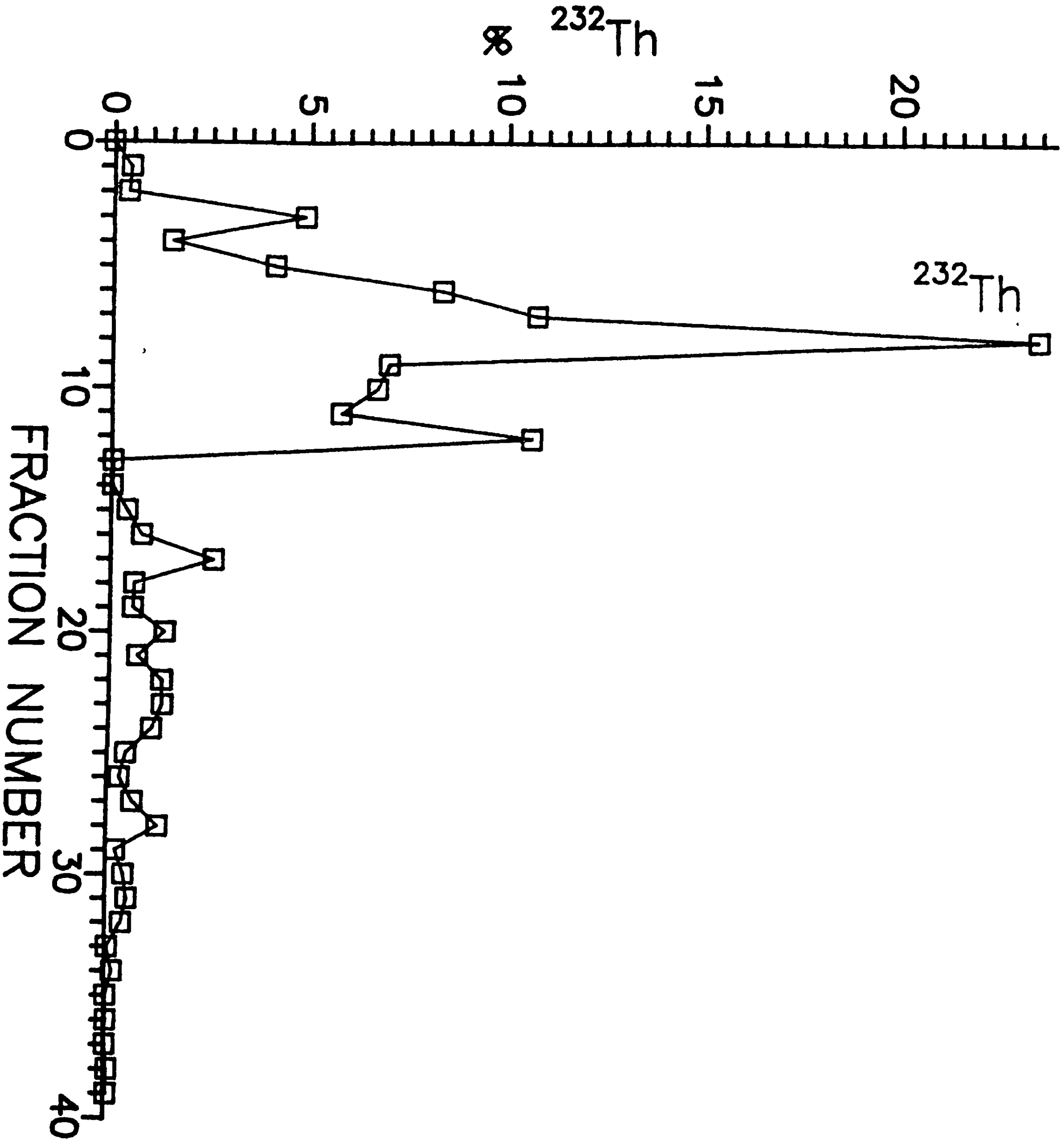


**Figure 3.33 Esk 0-5cm Humic Substances : Percentage Distribution of $^{232}$Th (G200 Sephadex)**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | low | 6 | 23 | 57 |
| 2 | medium | 17 | | |
| 3 | high | 34 | 42 | |
| 4 | low | 8 | | 41 |
| 5 | medium | 18 | 33 | |
| 6 | medium | 15 | | |
| | | | | |

**Table 3.31 Esk 0-5cm Uranium G200**

| Region | Classification | % | % | % |
|---|---|---|---|---|
| 1 | low | 6 | 79 | 83 |
| 2 | very strong | 73 | | |
| 3 | low | 5 | 10 | |
| 4 | low | 5 | | 8 |
| 5 | low | 3 | 3 | |
| 6 | - | - | | |
| | | | | |

**Table 3.32 Esk 0-5cm Thorium G200**

Southwick Merse humic material is more predominantly aliphatic in nature. The retardation of the aromatic portion of Esk humic substances is indicated from FTIR spectra of fractions obtained using G100 gel but to a lesser extent than on G25 gel (section 3.5.2.)

In order to reduce this retardation effect, the Esk humic material was fractionated using G200 Sephadex gel. The use of G200 gel involves the use of longer elution times and hence potential loss of resolution which is of importance both spectroscopically and radioanalytically. However, by the selection of a shorter column length (25cm instead of 35cm), good resolution was obtained. The U and Th distribution patterns are presented in Figure 3.32 and 3.33 and corresponding spectroscopic data in Spectrum 3.23.

### 3.7.5 U Distribution using Sephadex G200 and 0-5cm Esk Humic Substances

The U distribution pattern (Figure 3.32 and Table 3.31) was classified as follows:

low : medium : very high : low : medium : medium

The % distribution in regions 1,2 and 3 combined shows that 57% U is associated with these early fractions. Clearly this is different from both the G25 (5%) and G100 (35%) distribution patterns. This is in agreement with the proposed separation process and suggests that G200 Sephadex provides a separation process which is less strongly dominated by the aromatic character of the humic molecule. The observed distribution of U then indicates that U is not uniformly distributed throughout the humic fractions and in particular, the predominant association of U is with fractions comprising region 3. A further comparison can now be made between sampling locations following the suppression of the aromatic retardation process. A comparison is drawn between 40-45cm Merse humic material which is terrigenous in nature due to the observed degradation of the marine component (verified by CHN analysis - loss of H and N) and the0-5cm Esk humic material. The distributions of U in both cases indicate that the predominant association is with fractions comprising region 3. The more complex pattern observed for the Esk humic material can be attributed to the diversity of the input terrigenous material compared to the specific grass input at the Merse location. It would however appear that a similar and predominant interaction characteristic of U is observed with these humic materials.

### 3.7.6 Th Distribution : G200 Sephadex Gel and 0-5cm Esk Humic Substances

The distribution of Th (Figure 3.33 and Table 3.32) can be classified as follows:

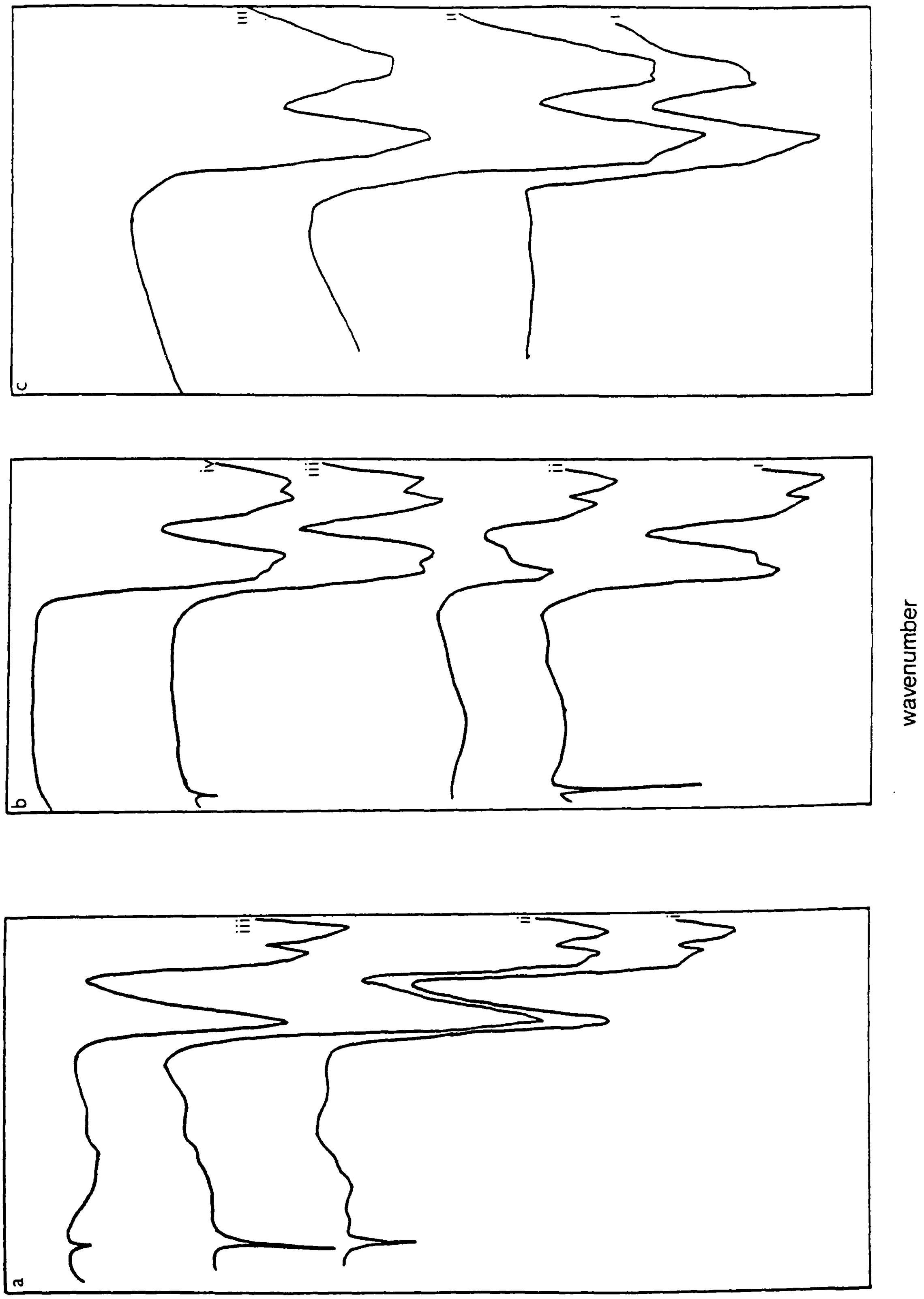
low : very high : very low : low : low : very low

and highlights a predominant association of Th with fractions eluting in region 2. 79% of Th is eluted in fractions comprising regions 1 and 2. This distribution differs markedly from that observed for U. In particular, regions 3, 4, 5 and 6 show that only 13% of Th as compared with 75% U is eluted in these fractions. The ratio of %U / %Th reflects these differences in distribution :

1 : **0.23** : **6.8** : 1.6 : **6.0** : **~10**

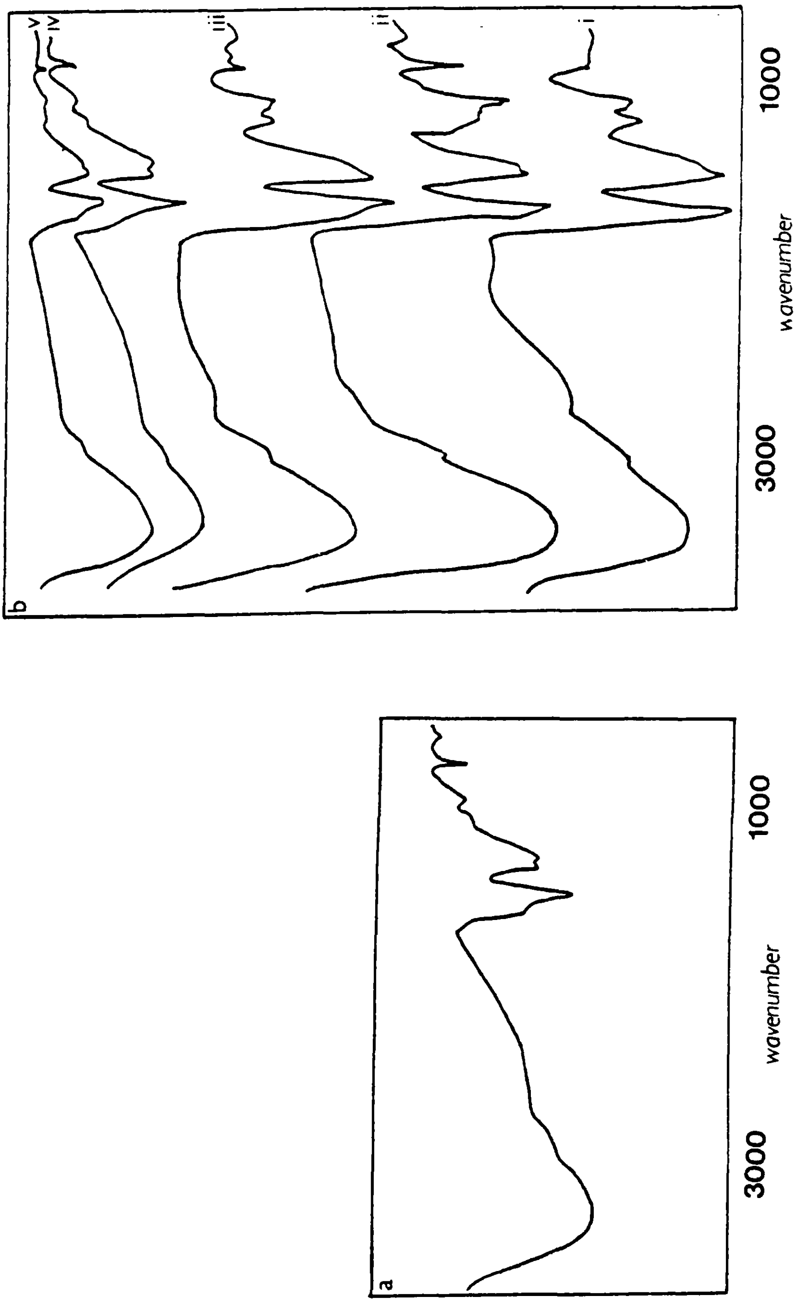
Comparison with the Th distribution pattern for 40-45cm Merse humic material shows that there are marked similarities in the distribution of Th within these two materials as was observed for U. In particular, 79% and 69% respectively of Th eluted in fractions comprising regions 1 and 2, indicating a strong and characteristic association of Th with humic molecules eluting in these fractions.

The G200 distribution pattern can again be related to certain spectroscopic differences between fractions. The spectra show that the shape of the absorptions in the region 1700-1400cm$^{-1}$ is related to the radionuclide distribution patterns of U and Th. Later fractions which contain almost no Th but small discrete associations of U also have slightly enhanced absorption relating to aromatic units although this effect is less than that observed for either G100 or G25 gel fractionation. Additionally, the pattern of UV/visible absorbance for the fractions (Spectrum 3.33) is similar to that obtained by Sequi (1971) and Guidi (1972) for bulk humic substances fractionated using G200 Sephadex gel. Specifically, small absorbances for early fractions are followed by much stronger absorbances for

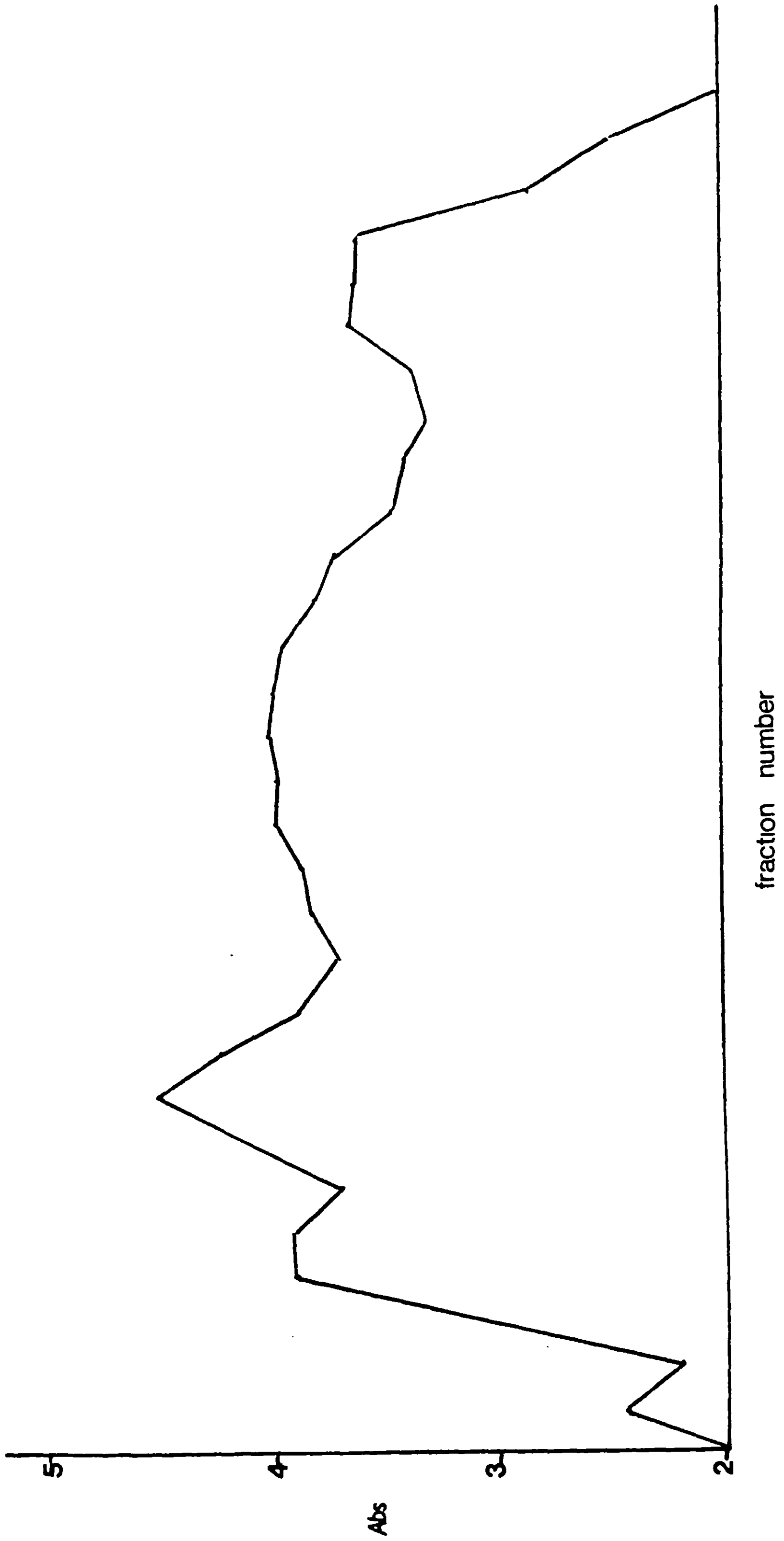


Spectrum 3.23a b c Gel Chromatographic Fractions of Esk 0-5cm Humic Substances using Sephadex G200

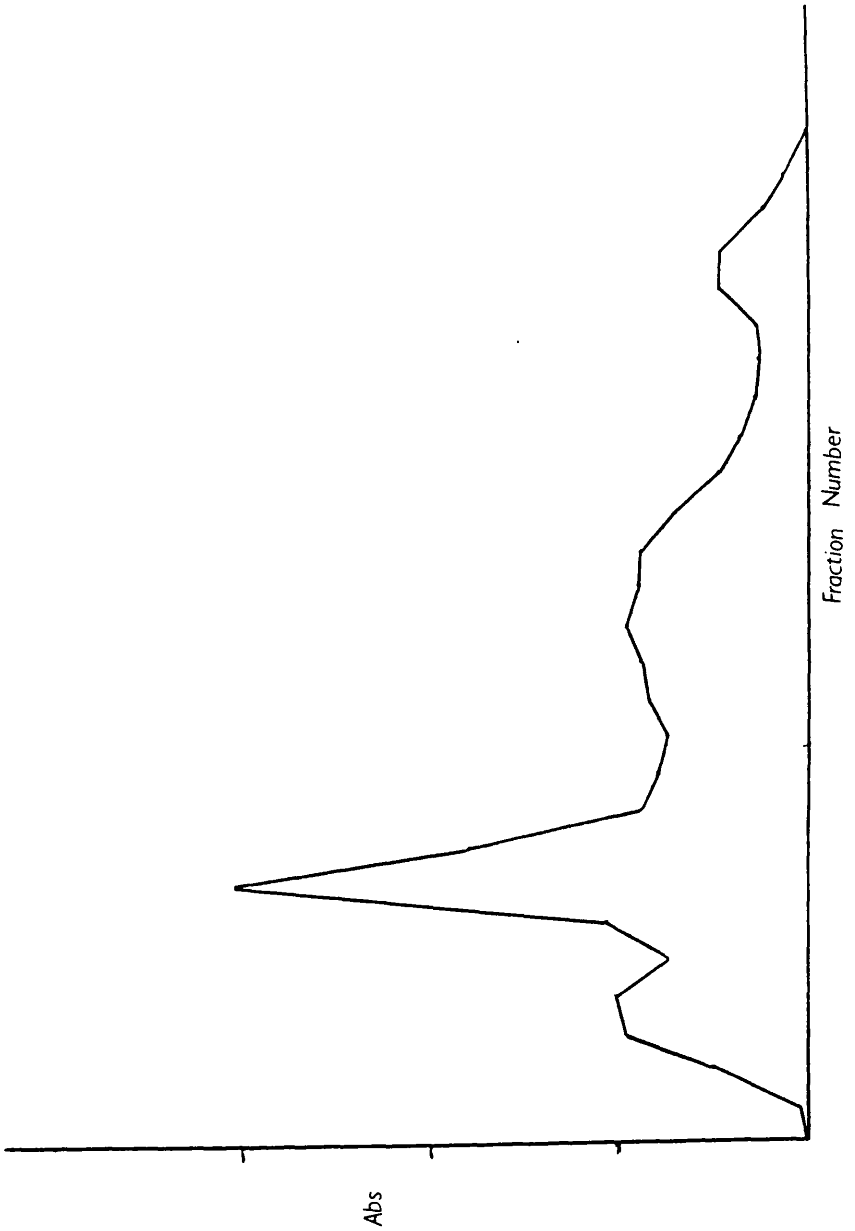
a i) Fraction 4 ii) Fraction 8 iii) Fraction 12
b i) Fraction 15 ii) Fraction 18
iii) Fraction 25 iv) Fraction 27
c i) Fraction 30 ii) Fraction 33 iii) Fraction 36



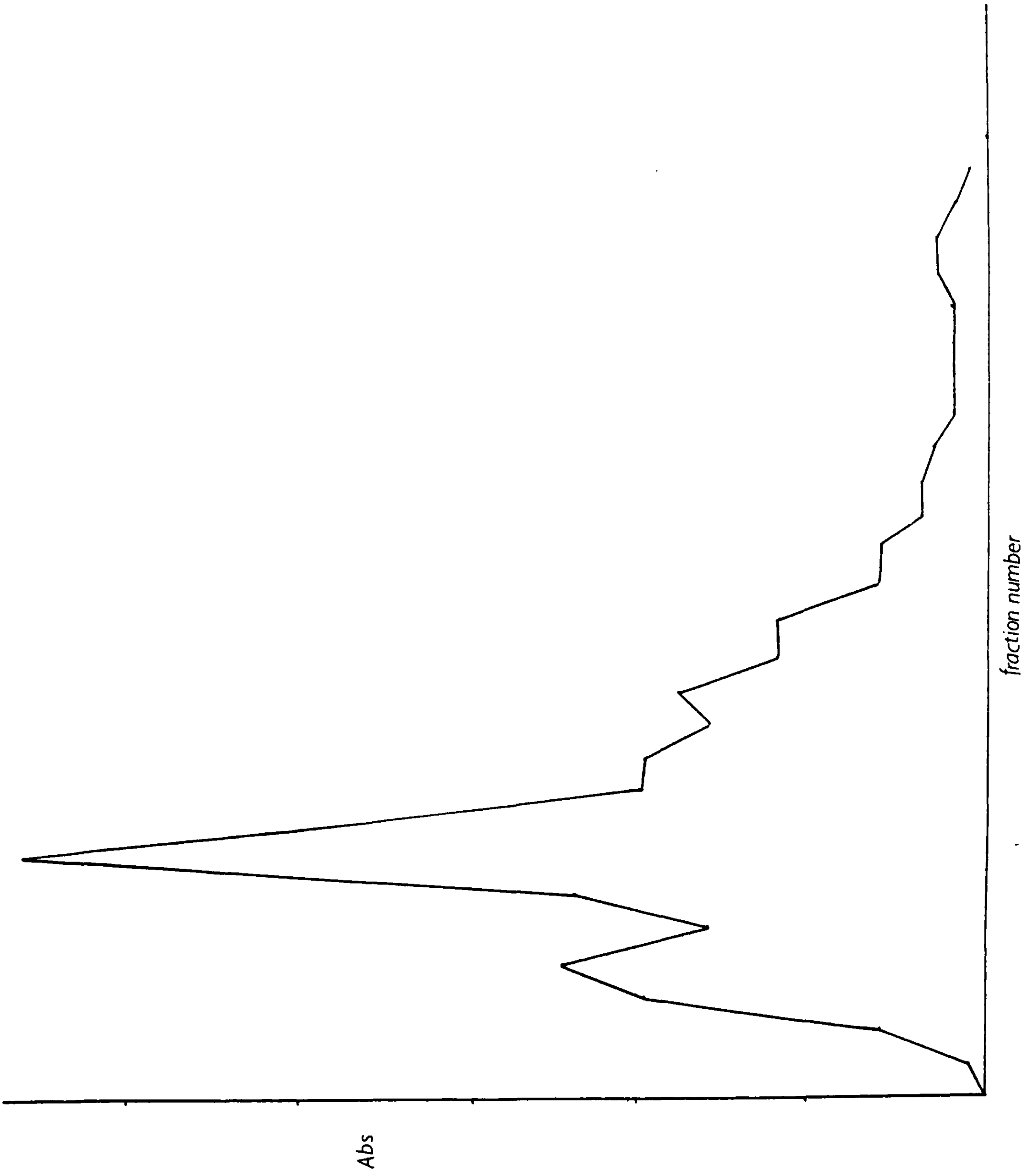
**Spectrum 3.24a b A Gel Chromatographic Fraction of Muncaster 10-15cm Humic Substances : Spectra Obtained using FTIR Microscopy**
**a A Spectrum of the Most Common Absorption Pattern Obtained for this Fraction**
**b Individual Spectra Obtained for this Fraction**



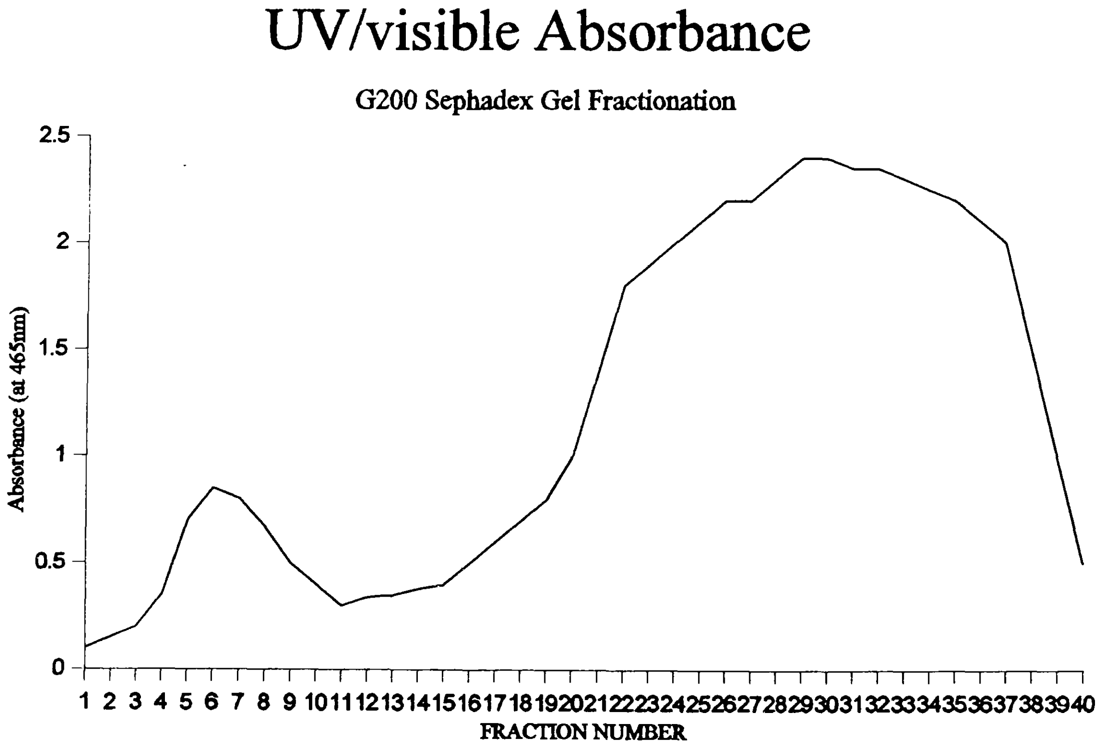
Spectrum 3.30 UV/Visible Absorbance for Humic Substances : Muncaster 10-15cm



Spectrum 3.3 | Absorbance at 465nm : Muncaster 10-15cm



Spectrum 3.32 Absorbance at 665nm : Muncaster 10-15cm



**Spectrum 3.33 UV/Visible Absorbance for Humic Substances Fractionated using Spehadex G200 Gel : Esk 0-5cm**

later fractions.

To summarise, the use of different gel types to fractionate humic materials which vary in their degree of aromatic or aliphatic character can provide additional information relating to the processes occurring during separation of components of humic materials :

i) humic substances with a high aromatic content interact strongly with gels which have a high matrix content eg. G25;

ii) the G100 gel theoretically separates on the basis of size but this study has shown that a chemical separation occurs to a certain extent during the fractionation of humic substances;

iii) the G200 gel can be used to minimise interactions between highly aromatic humic substances and the gel matrix during the separation procedure. Hence, it is possible to compare the actinide distributions for humic materials which have varying aromatic character.

### 3.8 Amino Acid Analyses : G25 Sephadex Gel and Esk 0-5cm Humic Substances

Gel chromatographic fractions were also analysed for their amino acid content (Figure 3.34). The total amount of amino acids (nmole/fraction) was typical of a terrigenous humic material and was therefore significantly lower than that expected for a marine humic material. The relative amounts of different amino acids differs significantly from that observed in previous studies of humic acids (Stevenson, 1982). Additionally, the distribution of amino acids between fractions was not uniform, in that early fractions contained significantly more amino acids than later fractions. The small number of samples analysed, however, made further interpretation of the data difficult although it is possible that the relative amounts

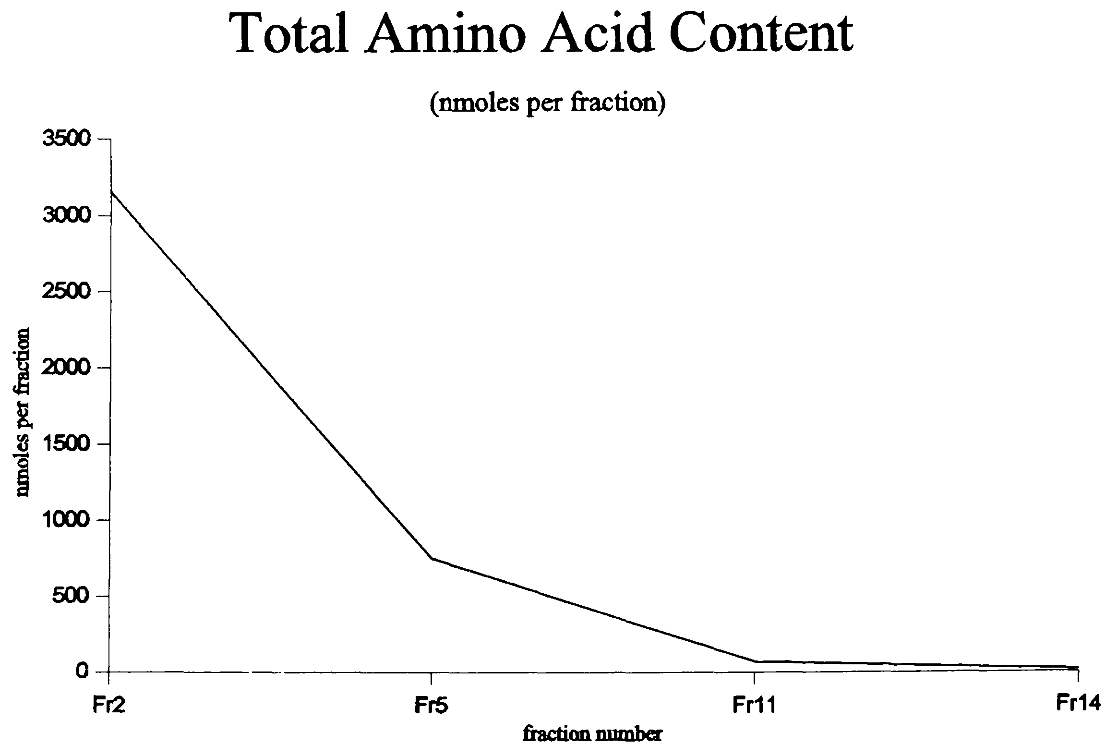


**Figure 3.34 Amino Acid Analyses of Gel Chromatographic Fractions of Humic Substances (G25 Sephadex)**

of different amino acids in the fractions may also vary.

## 3.9 Advances in FTIR Spectroscopic Studies of Humic Substances by the use of FTIR Microscopy

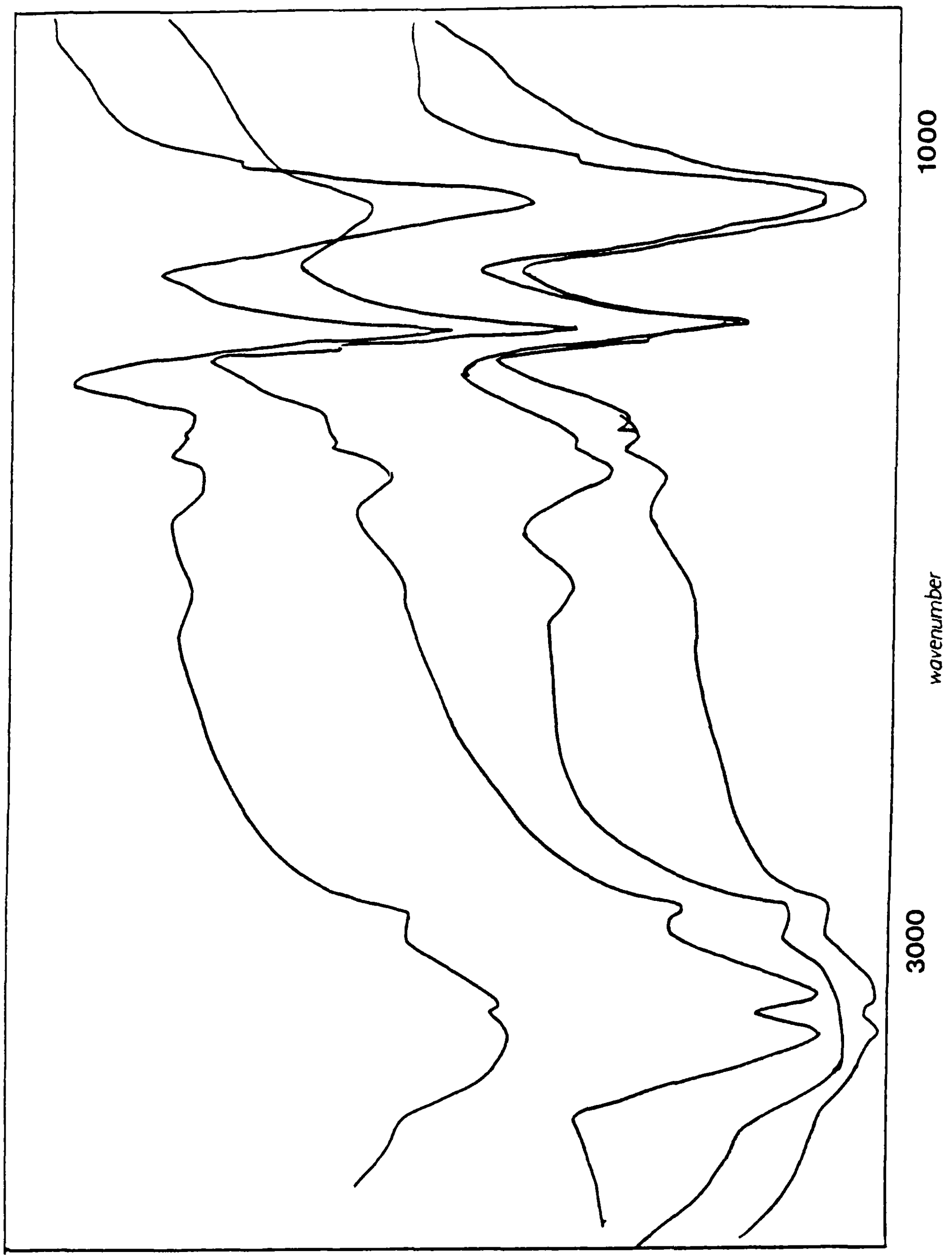
FTIR microscopy requires only a minute amount of sample to be placed on a reflective gold surface and a single drop of humic substances in dilute ammonia solution is sufficient to give excellent spectra following the evaporation of the solvent. The air-drying of the sample spot results in a progressive fractionation of the humic material based on solubility of the macromolecules. Therefore, scanning of the slide results in the observation of groups of macromolecules with differing composition. Within a specific gel chromatographic fraction, although the spectroscopic features (including the shapes of absorptions) observed are characteristic of the bulk fraction, macromolecules with varying amounts of these functional groups can be observed (Spectrum 3.24). In addition, minor components of bulk humic substances can be observed and resulting spectra are of higher resolution than previously observed (Spectrum 3.25). An example of a spectrum of a simple organic compound (Spectrum 3.26) is included for comparison and highlights the advances made in the spectroscopic studies of humic substances by the use of FTIR microscopy on its own and also the combination of gel chromatography and FTIR microscopy.

Furthermore, these spectroscopic studies indicate that:

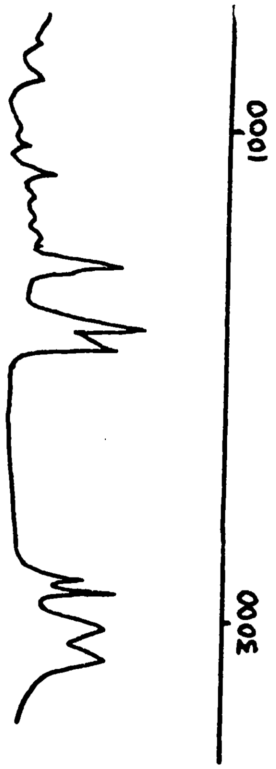
i) humic fractions obtained using G100 Sephadex gel fractionation do not contain macromolecules of uniform composition although they do display certain common characteristics

ii) it is possible that U, Th, Pu and Np may interact highly specifically with macromolecules with different proportions of functional groups and which co-elute due to certain common characteristics of the macromolecules.

The observed differing solubilities displayed by molecules within these fractions would therefore be of relevance in determining the environmental behaviour of these actinides once bound to humic substances.



**Spectrum 3.25 Spectra Obtained using FTIR Microscopy for Southwick 65-70cm Humic Substances**



Spectrum 3.26 FTIR Spectrum of a Simple Organic Compound containing Amino and Carboxyl Functional Groups

# CHAPTER 4
# CONCLUSIONS

## 4.1 Introduction

This study has attempted to characterise the environmental behaviour of natural and anthropogenic actinides in their interactions with humic substances from soils and sediments. A number of conclusions can now be drawn relating to :
i) the isolation of humic materials from soils and sediments;
ii) the use of chromatographic and spectroscopic techniques in the characterisation of humic substances;
iii) the use of radioanalytical techniques in conjunction with i) and ii) to characterise actinide interactions with humic substances.
These will be discussed in turn in the following sections.

## 4.2 Traditional Methodology for the Separation of Humic and Fulvic Acids

At an early stage in this study, it was concluded that humic and fulvic acids were not representative of natural humic material either with respect to composition or their interactions with actinides. This conclusion resulted from the following:

i) the molecular size distribution of the humic fraction must be altered due to the condensation reactions occurring during precipitation of humic acid (the lowest $E_4/E_6$ ratios coincided with the position of the two main bands observed during fractionation of humic acid);

ii) the two bands observed on the gel column are experimental artefacts resulting from the chemical separation of humic and fulvic acids and this was shown in

FTIR spectra of humic and fulvic acids separated after elution of humic substances from the gel column.

iii) the actinide distribution patterns obtained for humic substances could not be accounted for by the summation of corresponding humic and fulvic acid patterns for the same material.

iv) the strong mineral associations identified for humic acids in other studies (up to 30%) again result from the separation procedure by the co-precipitation and subsequent chemical reaction of fine clay and humic components.

The results of this study have shown altered size distribution and actinide distributions within the humic fraction which occur during the acid precipitation step in the isolation of humic and fulvic acids. Therefore an important conclusion is that interpretations of actinide mobility in the environmnent following complexation by humic compounds based on interactions with humic or fulvic acids would not be valid.

### 4.3 Alternative Methodology for the Isolation of Humic Substances

The investigation of factors resulting in the alteration of the humic fractions of soils and sediments led to the following criteria for the isolation of humic substances :

i) the minimal treatment required for isolation of a humic substance involves an extraction using a dilute aqueous alkaline solution;

ii) the use of dilute alkali and control of pH are essential in the extraction of a representative and unaltered humic fraction;

iii) the exhaustive extraction of humic substances can be achieved using aliquots of alkali and short extraction times followed by immediate dialysis and freeze drying.

For the remainder of the study, humic substances, prepared as defined in section 2.3.1, were characterised using radioanalytical and spectroscopic techniques. The general characteristics of humic substances are, however, similar to those attributed to humic and fulvic acids in that they :

i) are high molecular weight natural organic molecules;

ii) display a broad range of molecular weights;

iii) are highly heterogenous;

iv) have a high capacity for binding metals, and in particular actinides, due to their charge and size characteristics.

### 4.4 Gel Chromatography in the Study of Humic Substances

Traditionally, humic substances were separated into humic and fulvic acids which were reported to be less heterogeneous than the unfractionated humic material and therefore better starting materials for subsequent studies of humic characteristics. However, the chemical separation was considered too severe and so in this study, in order to characterise humic substances, gel chromatography was used as an alternative fractionation technique to provide fractions which contained humic macromolecules which had one or more features in common.
Gel chromatographic fractionation of humic substances does not occur purely on the basis of size and is influenced by a number of factors :

i) the high proportion of functional groups incorporated into humic macromolecules

ii) the presence of aromatic groups incorporated into humic macromolecules

A number of further conclusions can be drawn from this study :

i) the composition and hence the origin of the humic material influences its separation on the gel column;

ii) where interactions between the gel and the humic material occur, this technique is of little use in the determination of average molecular weight or molecular weight distribution of such a humic material;

iii) gel chromatography is a useful fractionation technique where there is an understanding of the processes occurring during the passage of the humic material through the gel.

### 4.5 Spectroscopic Studies of Humic Substances

Although spectroscopic studies have often been discounted for the structural characterisation of humic substances due to the simplicity and broad nature of spectra, this study has shown that FTIR, UV and fluorescence spectroscopic information is of great importance in identifying alterations in humic materials resulting from chemical treatments during isolation. The use of the modified methodology employed in this study primarily identified that humic materials from diverse origins were compositionally different. In addition, fractionation of humic substances using gel chromatography prior to spectroscopic characterisation produced fractions which gave spectra with greater fine structure highlighting the application of such techniques to the characterisation of complex mixtures of biological molecules. The improved interpretation on the basis of the greater fine structure achieved for fractions of humic substances using particularly FTIR spectroscopy and FTIR microscopy have been invaluable in :

i) elucidating the processes occurring on different gel columns (ie. G25, G100 and G200 Sephadex and Superdex 75) using FTIR spectroscopy;
ii) identifying functional groupings and associations between functional groupings which are common to macromolecules in particular fractions using both FTIR spectroscopy and microscopy;

iii) identifying spectral differences between fractions which correlate with changes in actinide distribution.

Additional and complementary information is provided in the UV/visible spectra of humic fractions. These spectra provide not only confirmation of interpretations made on the basis of FTIR spectra (eg. the aromatic character of a humic material) but the visible part of the spectrum also provides a measure of the concentration of humic material in fractions and is therefore used as a monitor of the separation procedure. The intensity of the absorptions at 465 and 665nm also provides useful information relating to the predominance of specific but unidentified components in humic fractions and this is tentatively related to the actinide binding capacity of macromolecules in a particular fraction.

Similar conclusions can be drawn from the preliminary fluorescence studies presented in section 3.5.2. Again, this technique has a capacity for determining the validity of interpretations made on the basis of FTIR spectroscopic data. Although the fluorescence spectra of humic fractions are broad, the changes in the position of the fluorescence maximum correlate well with the actinide pattern obtained for the particular humic material and tentatively indicate that each discrete association represents an association of the actinides with different components of humic substances. Clearly certain of these discrete bands are more important for individual actinides. Further, quantitative information from this type of study would be of great value in modelling humic-actinide interactions.

## 4.6 Actinide Interactions with Humic Substances

A comparison with sequential leaching data showed that an alkaline extract comprising humic substances did indeed account for a high proportion of the binding capacity of the organic fraction of soils and sediments. More importantly, the humic fraction which may comprise as little as 1-2% of the total soil mass accounts for >60% of the actinide binding capacity of the soil.

From this study it is apparent that actinide interactions with humic substances differ markedly from those observed for humic and fulvic acids. Whereas interactions with the latter presented a simple picture of actinide behaviour, the studies of humic substances show that these interactions are extremely complex. Certain difficulties have been encountered in studying actinide associations with humic substances, namely that gel fractionation is influenced by the composition of humic substances and therefore comparable fractions of humic materials from diverse origins may vary compositionally. These differences are, however, vitally important in characterising the behaviour of individual actinides in their associations with humic substances. It can be concluded that :

i) actinide interactions are highly specific;

ii) a number of discrete associations for each actinide have been observed;

iii) it is highly probable that there is a correlation between the nature of the oxygen containing functional groups prevailing in a humic fraction and the binding capacity for a particular actinide element;

iv) a further correlation is observed for highly terrigenous humic materials between the presence of aromatic units and U and Th distributions;

v) definitive information regarding differences in assocations between actinides can only be obtained where interactions between the gel and the humic material are minimised.

## 4.7 Overview

To summarise the conclusions of this study, it has been shown that the complexity of humic substances and indeed actinide interactions with these large biomolecules requires an approach involving wide-ranging techniques and extensive analyses with the requirement that the technique doesn't influence the result. Following the implementation of the methodology developed during this study it has been possible to :

i) elucidate the separation processes occurring during gel fractionation of humic material;

ii) advance the structural characterisation of humic substances;

iii) further the understanding of actinide interactions with humic substances.

## 4.8 Further Work

In the context of determining the mobility of actinides following complexation by humic substances, further work is required to quantify the solubility of the macromolecules in fractions which contain high proportions of actinides. In addition, further refinement of fractionation procedures would be of value in determining the similarities or differences in binding characteristics where fractions are important for more than one actinide element. An additional spectroscopic technique, NMR spectroscopy, could provide valuable quantitative information relating to the proportions of certain functional groups. In combination with advanced mass spectrometric measurements and the determination of concentration of humic material this could lead to information required to model actinide-humic complexes and hence the prediction of their long-term mobility.

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