

Chapter 12

Complexing of Metal Ions by Humic Substances

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The interaction of metal ions with humic substances is being studied using two different techniques. UV-scanning ultracentrifugation is being used to determine molecular weights and to investigate changes in aggregation brought about by metal ion complexation. The relationship between cation charge and conformation of the humic ligands is being investigated.

The complexation of U by humic substances from soils contaminated by natural processes is also being studied. Gel permeation chromatography has been used to show that different fractions of humic substances vary greatly in their effectiveness as ligands. These studies have also shown that uranium desorption is redistributed slowly between different fractions of humic substances following its initial adsorption.

It is well known that humic substances (humins, humic and fulvic acids) have a substantial capacity to complex dissolved species such as metal ions and cationic organic molecules and to interact with mineral surfaces [e.g. 1-5]. The complexation process may affect the solubility of both the humic ligand and the species bound. For example, a complex geochemical interrelationship has been identified between fulvic acids, poorly crystalline Fe oxides and Fe(II) and Fe(III) ions [6]. In addition, scavenging by organic coatings has been found to remove a range of metal ions efficiently from solution [e.g. 3,7]. Similarly, the humic ligands themselves are a complex mixture of colloidal macromolecular species, whose state of aggregation and solubility may be affected by concentration, by ionic strength and by metal binding to specific coordination sites [8-15].

These processes make the study of metal ion-humic interactions particularly complex since the humic ligands cannot be treated as isolated, simple molecules and changes in metal ion binding can significantly affect their state of aggregation and thus their behaviour. The humic macromolecule may be viewed as presenting a range of binding sites to a dissolved species. These may be either "specific" binding sites; that is, particular functional groups or combinations of functional groups may co-ordinate metal

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ions, or alternatively, metal binding may be "non-specific" [16-18]. This latter binding mode arises from the electrostatic interaction between the negatively charged humic macromolecule and positively charged cations. A widely used and relatively successful approach to the modelling of metal-humic complexation is to define a number of specific metal binding site groups (commonly 2, but on occasions more) with characteristic "stability constants" and then to make a correction to these "constants" to take account of progressive neutralisation of charge on the humic macromolecule (Figure 1) [e.g. 2,16]. Increasing metal loading of humic macromolecules causes increased aggregation of the individual macromolecules both through compression of the electrical double layers and also through the ability of metal ions to bridge between different parts of a macromolecule or even between different molecules. Thus, non-specific binding, for example of a Group I or II cation should have a lesser effect than binding (for example of a d-transition ion) in a specific site. In this paper we describe two techniques currently being used for the study both of metal ion binding by humics and of the physical state of humic-metal ion complexes.

Methods

Analytical Ultracentrifugation. This technique provides a direct means of measuring the molecular weights (usually, the weight-averaged molecular weights) of humic substances and of humic-metal complexes and has been described in detail elsewhere [20-22]. In principle, it is relatively straightforward to determine molecular weights, since a dynamic equilibrium (Figure 2) can be achieved between the centrifugal force exerted by the centrifuge (proportional to the square of angular velocity) and diffusion in the opposite direction of the humic macromolecules (proportional to the concentration gradient established in the cell). The distribution of humic material in the centrifuge cell is monitored during the experiment by measuring UV absorbance at 280 nm. Since the system is at equilibrium and all the variables except molecular weight are known or can be found (although a buoyancy correction is required, for which the partial specific volume of the humic substances must be known), a value for the molecular weight can be calculated (Equation 1).

$$\bar{M}_w = \frac{2RT \frac{d \ln c}{dx^2}}{(1 - \bar{v}_2 \rho) \omega^2} \quad (1)$$

where R = Gas constant, T = temperature (K), c = solute concentration, x = radial distance from the axis of rotation, \bar{v}_2 = partial specific volume of solute; ρ = solution density and ω = angular velocity. An example of a plot of $\ln(\text{absorbance})$ against x^2 for a fulvic acid is illustrated in Figure 3a. The plot is approximately linear for low values of x^2 but develops curvature at higher x^2 values due to the polydispersity of the sample. The main drawbacks with sedimentation equilibrium are the time required to attain equilibrium (several days) and the need to try and keep the majority of the sample in solution. With very polydisperse mixtures, or samples of very high molecular weight, there may be significant sedimentation of the sample out of the solution, thus biasing the results towards the lower molecular weight species.

An alternative method for molecular weight measurement is the Archibald or approach to equilibrium method [22]. In this, rather than establishing an equilibrium

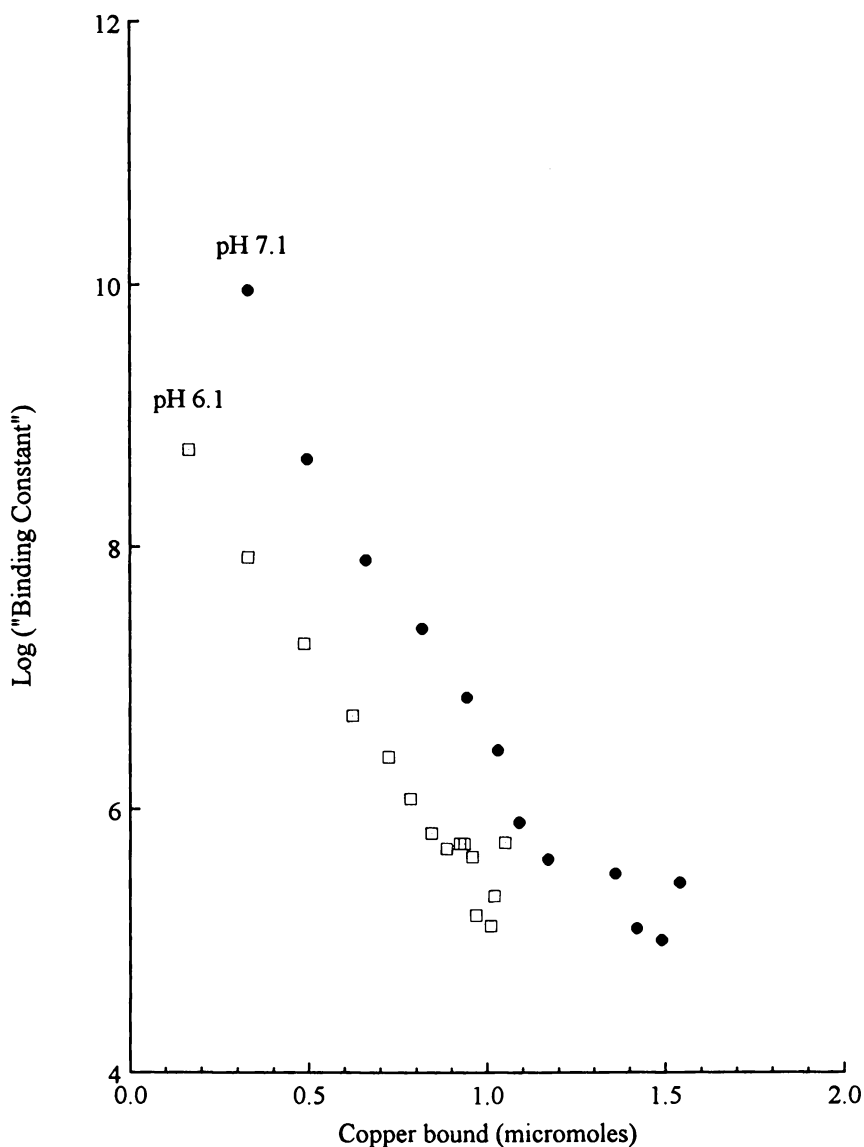


Figure 1: Effect on binding constant (K^* [19]) of neutralisation of humic charge (from [35]). Humic concentration 35 mg l^{-1} ; $I = 0.05$. Note that an approximate 10-fold increase in bound Cu^{2+} concentration leads to about 6 orders of magnitude decrease in K^* .

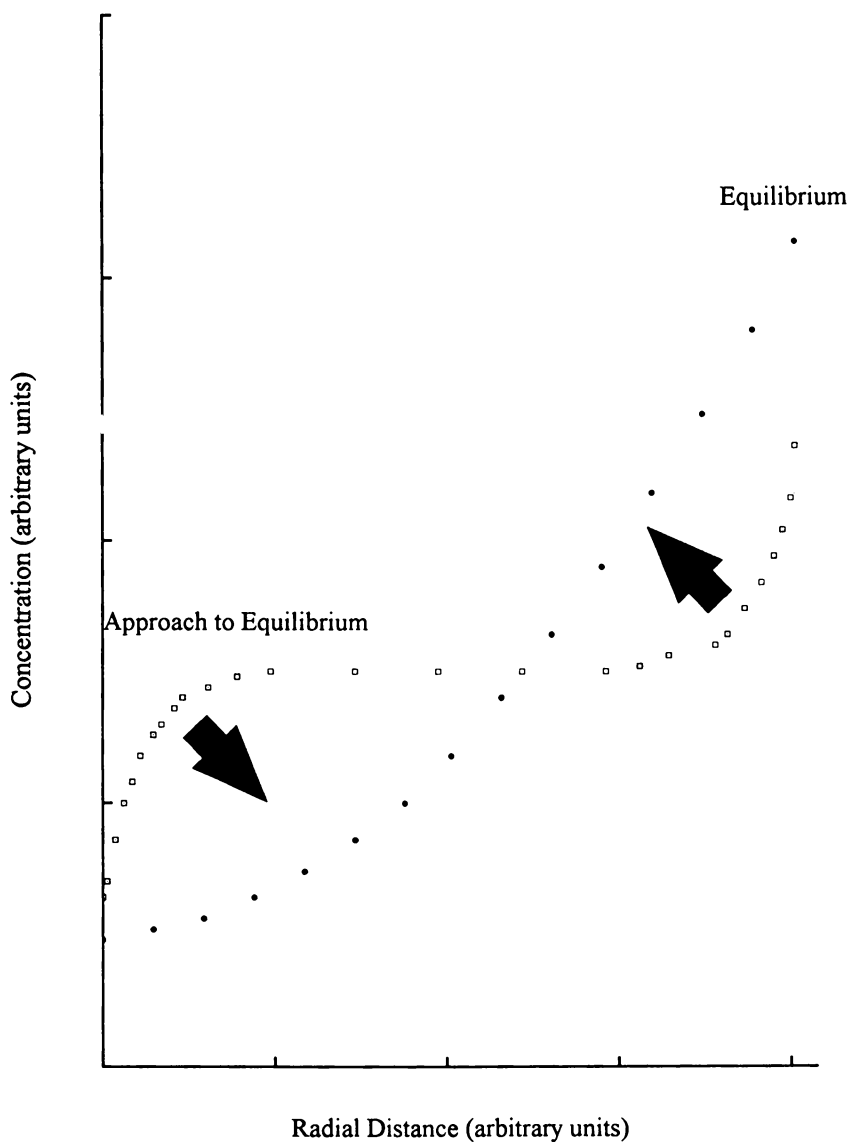


Figure 2: Concentration distributions within sample cell during an ultracentrifuge experiment. i) approach to equilibrium and ii) at equilibrium.

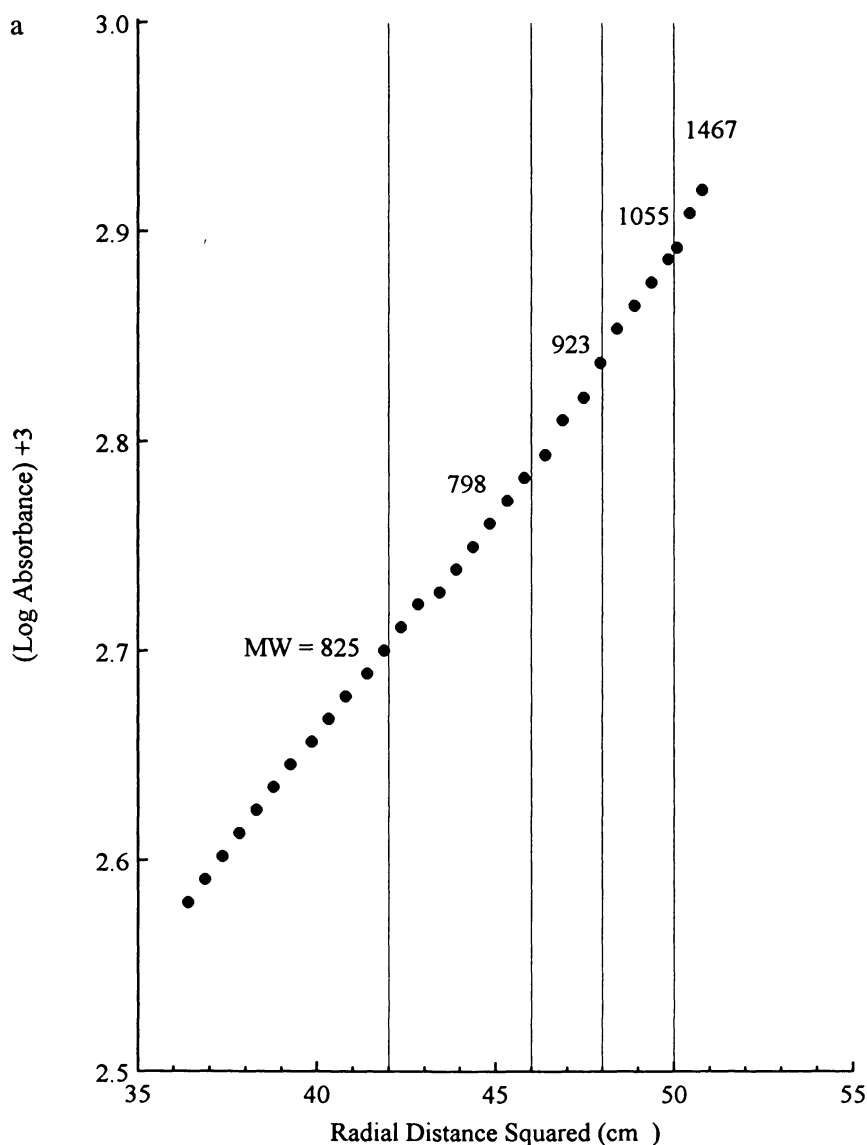
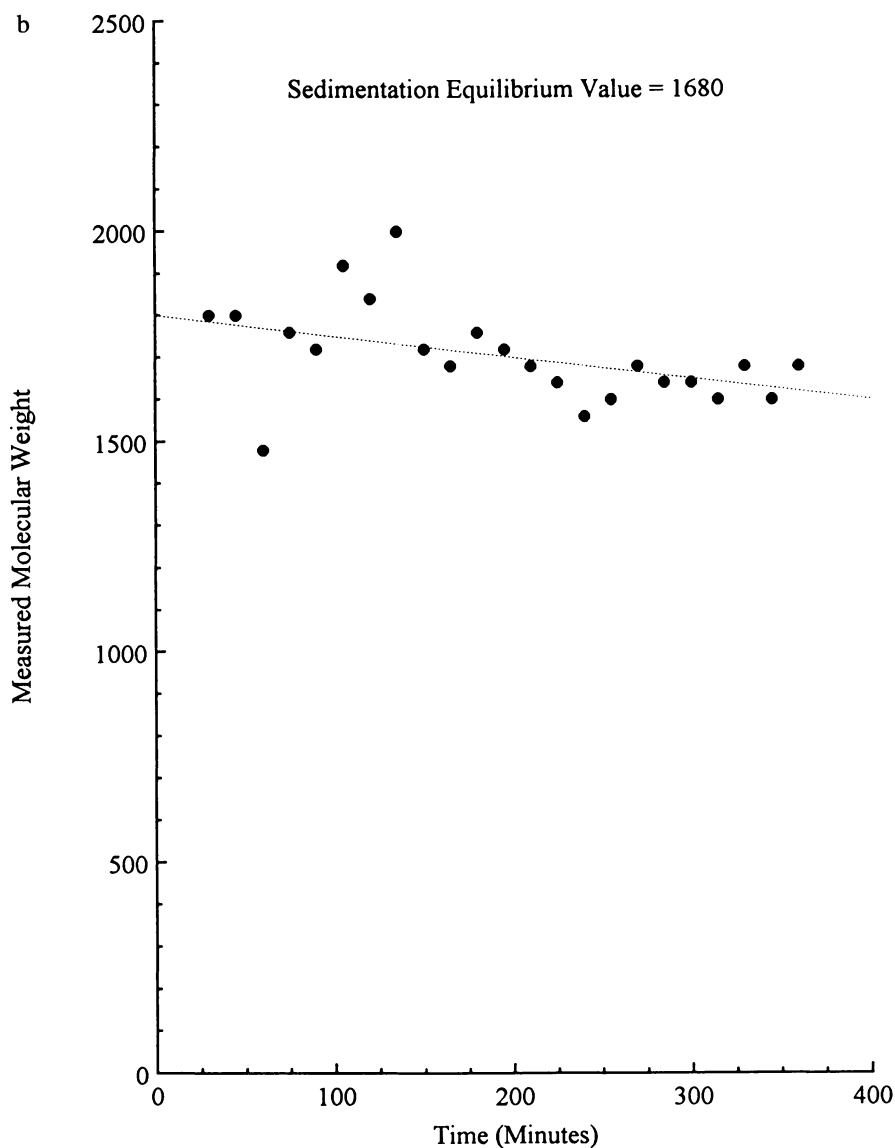


Figure 3: a) Molecular weight determination by equilibrium ultracentrifugation; b) Comparison of molecular weights determined by the Archibald method and by sedimentation equilibrium.

Figure 3. *Continued*

between settling and resuspending forces, advantage is taken of the fact that there can be no mass flux across the ends of the sample cell. Changes in the humic concentration at one end of the solution column are then monitored and a molecular weight value derived from the rate of change of concentration. In practice, since the UV detector is effective only over a limited absorbance range, it is usual to follow the decrease in concentration at the meniscus, rather than the increase in concentration at the base of the column. Data are then derived from Equation 2, a specific case of Equation 1.

$$\bar{M}_w = \frac{RT \left(\frac{dc}{dx} \right)_m}{(1 - \bar{v}_2 \rho) \omega^2 c_m x_m} \quad (2)$$

where the subscript m denotes a value at the meniscus. The concentration gradient $(dc/dx)_m$ is measured during approach to equilibrium and used to obtain \bar{M}_w as a function of time. Extrapolation of \bar{M}_w to zero time gives the weight average molecular weight for the whole sample. In many cases, both sedimentation equilibrium and approach to equilibrium give comparable results (Figure 3b), although for high molecular weight humics, this is not the case if, during sedimentation equilibrium, material is lost from the concentration profile. Clearly, both equilibrium ultracentrifugation and the Archibald method can be applied to metal-humic complexes as readily as to isolated humics.

Gel Permeation Chromatography. This is again a potentially valuable technique since it can, in principle, separate complex mixtures of macromolecules directly on the basis of molecular size. Indeed, in cases such as proteins, with molecules of very different sizes, and where appropriate calibration standards are available, it is possible to obtain well resolved, chemically pure fractions and to determine molecular weights directly. There is, however, the possibility of artefacts arising due to interaction with the gel [23,24]. This may be irreversible; that is sample material may simply be retained on the top of the gel column, or it may be more subtle, in which case the retarded fractions are eluted, but later than would be expected solely on the basis of molecular size.

There are a number of disadvantages in the application of gel permeation chromatography to studies of humics. There are no usable molecular weight standards, so it is difficult to make credible molecular weight measurements directly [25-28]. The polydispersity of humics can often lead to broad, overlapping bands, so that individual fractions may well not be cleanly separated [e.g. 23; see later]. There is also often extensive interaction with the gel, and whilst sample losses may be minimised by careful control of experimental conditions, it is impossible to prevent interaction with the gel entirely. Thus, the separation achieved is probably best viewed as arising from both size effects and interaction effects. In spite of these difficulties, gel chromatography of humic substances has been widely used, both to fractionate and characterise humic substances and also to study the reactions of humic substances with species such as metal ions [e.g. 29-32]. Both ultracentrifugation and gel permeation chromatography are presently being applied to the characterisation of metal ion-humic interactions.

Results and Discussion

Ultracentrifugation. Recently, ultracentrifugation has been used in conjunction with metal binding measurements, microcalorimetry and modelling studies. A range of well-characterised humic and fulvic acid samples are being used in these experiments and the effects on measured molecular weight of metal binding are presently being investigated. In particular, whilst it is well known that, at high concentrations of metal ions, humic substances precipitate [33,34], very little is known about the processes of metal-humate colloid formation and precipitation [35]. Initial investigations into metal-humic aggregation have been carried out using the Archibald method to follow changes in molecular weight in response to metal binding. In general, a significant increase in weight averaged molecular weight (\bar{M}_w) occurs as metal ion concentration increases. An example is given in Figure 4, which shows changes in \bar{M}_w with total copper and lanthanum concentrations, $[\text{Cu}]_T$ and $[\text{La}]_T$.

At copper concentrations greater than 2×10^{-4} M, complete precipitation occurs. In these experiments, the aggregation processes have been found to occur over a relatively narrow range of metal concentrations, about an order of magnitude in the case of copper. Moreover, the humics can tolerate addition of a certain amount of copper ($[\text{Cu}]_T < 1.0 \times 10^{-5}$ M) before the onset of aggregation in spite of the fact that there is very significant metal binding in this concentration range since the strongest metal binding sites are being occupied [36].

Since the humic macromolecules have large negative charges in solution [37], it is to be expected that cation charge will have a substantial effect on the interactions between metal ions and humics [38]. This can be illustrated by comparing the binding of lanthanum and that of copper. Complete humic precipitation occurs at $[\text{La}]_T = 2.2 \times 10^{-5}$ M, almost an order of magnitude lower than the copper concentration required for precipitation. Moreover, whereas aggregate sizes increase only relatively slowly on addition of copper, they increase much more rapidly with lanthanum. Should complete flocculation leading to precipitation follow the Schulze-Hardy rule for a lyophobic colloid, the ratio of flocculation concentrations for Cu^{2+} and La^{3+} ions would be $(3/2)^6 = 11.4$, which compares with the experimental value of approximately 9.

Gel permeation. For several years, gel permeation has been used to study the association of a number of actinide elements with humic substances [39,40]. This work has concentrated on a number of sites in the vicinity of the Sellafield nuclear fuel reprocessing plant in north west England. More recently, other work has shown that humic substances may play a dominant role in determining actinide migration. This is exemplified by some studies carried out at the Needle's Eye natural analogue site in south-west Scotland. At this site, uranium migration from a mineralised vein has been extensively studied over a number of years [e.g. 41-43]. In a small, highly organic area at the foot of the cliff in which the vein outcrops, uranium has been found to be strongly complexed by humic substances; indeed over 90% of the uranium in the soil is organically bound [42]. In addition, recent work [44] has shown that the desorption of uranium from this soil is kinetically hindered, thus substantially reducing uranium mobility [43]. This suggests that the kinetics of sorption-desorption reactions are a fundamental control on element migration. Although many studies exist in which metal-humic complexation has been treated as an equilibrium process (some examples include [16-18,45-48]), there are fewer in which reaction rates are considered (though see [12,49-54]).

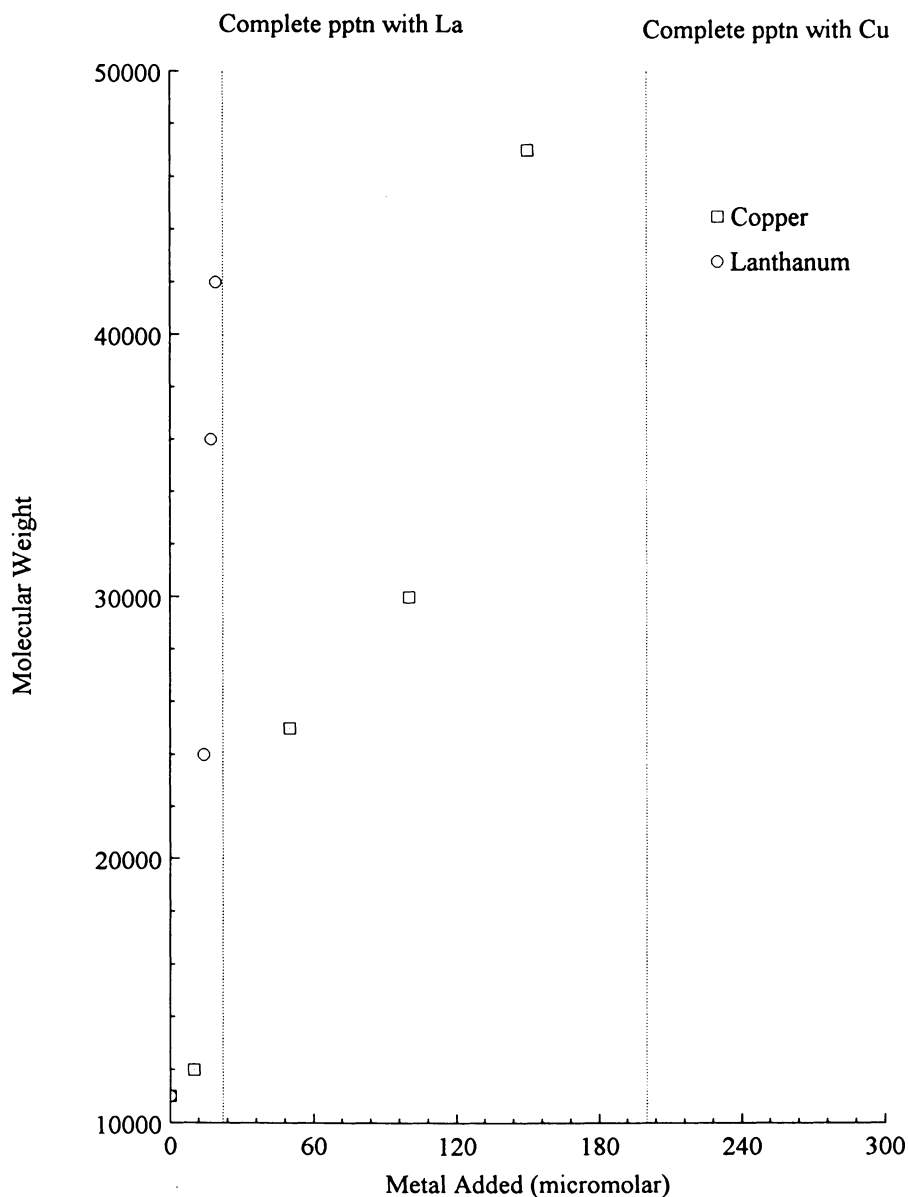


Figure 4: Molecular weight change in response to addition of copper and lanthanum. Humic concentration 21 mg l^{-1} ; pH 6-7; $I = 0.05$.

The desorption of uranium from these soils can be described by a number of rate constants of the order of 10^{-4} to 10^{-6} hr^{-1} [44]. Gel permeation fractionation experiments using the humic substances from the Needle's Eye site suggest that they comprise three poorly-resolved fractions (Figure 5). This behaviour is quite different to that observed with humic substances from a range of other sites, where a larger number of better-resolved fractions are usually found. In the Needle's Eye humic substances, the majority of the uranium is held in the late-eluting fraction, with relatively little in the earlier ones. This U-binding fraction has a molecular weight (measured by ultracentrifugation) of 10000 or below, compared with about 70000 for the more rapidly eluted fraction. The dominance of U binding by this relatively low molecular weight material suggests that it is the chemistry of this fraction which, to a large extent, controls U behaviour.

Radiotracer experiments, using ^{235}U and Needle's Eye humic substances have also shed light upon changes in U binding which take place following an initial, fairly rapid complexing step. The ^{238}U pattern represents the end result of a long equilibration time (hundreds of years) whilst the ^{235}U patterns represent much shorter equilibration times (Figure 5). It appears that the ^{235}U is proportionately less strongly associated with the high molecular weight fraction than ^{238}U . However, as equilibration time increases, the distributions of the two isotopes resemble one another more closely. This suggests that a slow change in U speciation occurs following sorption. This may represent a change in the state of aggregation of the humic macromolecules, brought about by the presence of the U cations, or it may arise from the gradual transfer of uranium from a non-specific "surface" association to specific metal binding sites [54,55]. In any event, the observed combination of complex sorption-desorption kinetics and slow, post-sorption speciation changes will complicate predictive modelling of such systems.

Conclusions

Whilst it is well known that complexation by humic substances is an important control on the environmental behaviour of metal ions in many contexts, the precise nature of the metal-humic reaction remains poorly understood. Analytical ultracentrifugation and gel permeation chromatography both have the potential to provide information on these reactions and such studies illustrate fully the complexity of metal-humic systems. Ultracentrifugation allows identification of changes in the state of aggregation of humic substances in response to metal binding, and the data illustrate the ready formation of high molecular weight aggregates. Gel permeation allows the fractionation of metal-humic complexes and shows clearly that metal ion-humic interaction is selective. This suggests that both the chemistry of the metal ions and of the humic ligands will play a part. Moreover, the rates of sorption and desorption reactions have an important effect on metal ion migration. Slow, post-adsorption speciation changes are also identified which may also have an influence. As a result of such complex processes and our relatively poor understanding of the underlying chemistry, the incorporation of metal-humic interactions into speciation and migration models remains a major challenge.

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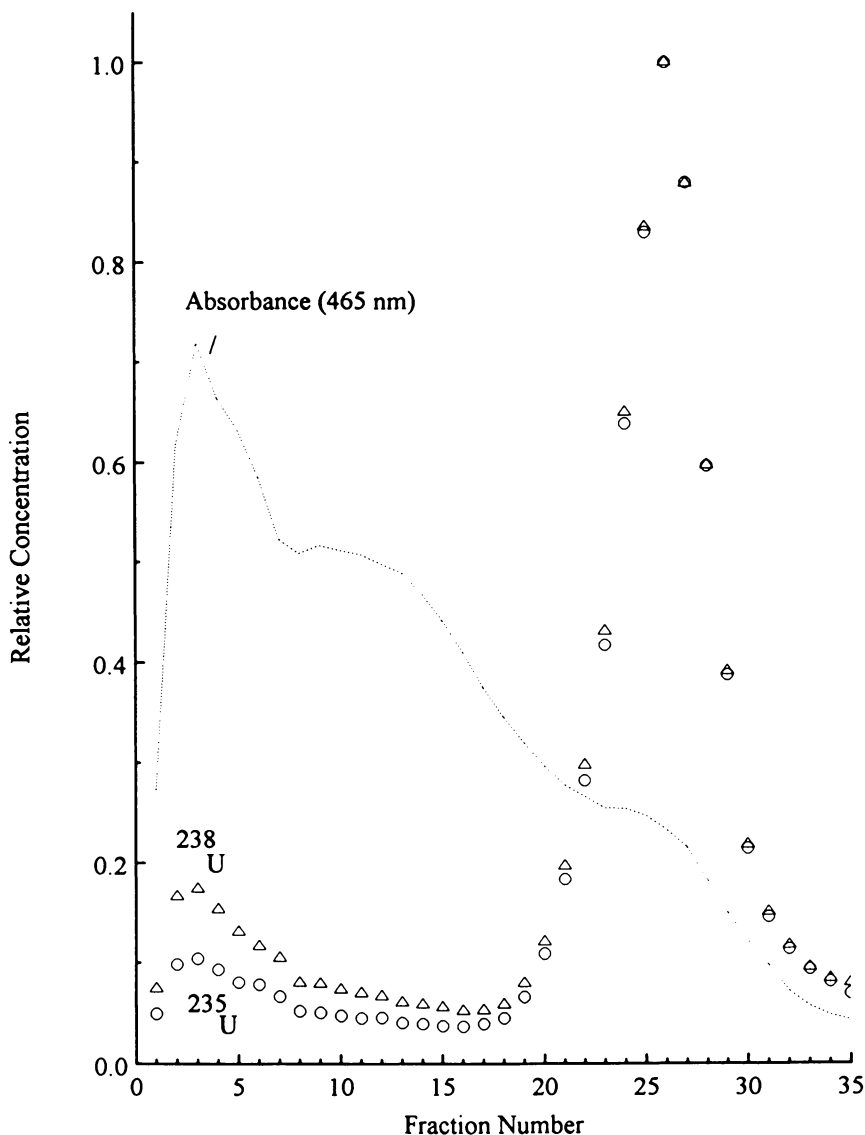


Figure 5: Gel permeation separation of Needle's Eye humic substances, showing distribution of ^{235}U radiotracer (circles) and ^{238}U (triangles) between fractions. ^{238}U concentration 1500 mg kg^{-1} ; ^{235}U concentration 500 mg kg^{-1} ; pH 8-9; $I = 0.1$. UV absorbance at 465 nm is used as an indicator of organic matter concentration.

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