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Studies on selected organic - metal interactions of importance in the environment

by

Ian Mason

A Doctoral Thesis submitted in part fulfilment of the requirements for the award of

Doctor of Philosophy
of the Loughborough University of Technology

October 1995

Research supervisor: Dr. Peter Warwick

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ABSTRACT

This research project investigated the interaction between natural organics acids and selected metal ions. The aim of the project was to provide quantitative data on the speciation of metal ions when placed in systems containing natural organic acids. It was envisaged that such data will assist in the risk assessment of the Drigg low level waste site in Cumbria. The formation and complexing ability of these natural organic acids is discussed and the classing of these acids into high molecular weight organic acids and low molecular weight organic acids. Initial investigations used a potentiometric technique to study the interaction between nickel and europium and selected low molecular weight organic acids which were thought to occur in significant concentrations in soils and groundwaters. These experiments confirmed existing critically assessed literature values, and provided an experimental methodology for further 'in-house' measurement of such values. In addition, studies were also performed on systems containing two competing organic acids. Studies of such systems showed no synergistic effect and that they could be modelled using individual stability constants. A comprehensive investigation was performed on the interaction of nickel and europium with humic acid. High Performance Size Exclusion Chromatography (HPSEC) was assessed for its applicability to study such systems and was found to be suitable. Stability constants were determined for europium and nickel with humic acid. All data was modelled using MINTEQA2, a geochemical speciation code. Further work on these systems was carried out by a column ion exchange technique which confirmed the HPSEC data. A comparison was then carried out between batch and column exchange with the conclusion that column gave lower metal bound to the humic at high concentrations due to competition from the resin. HPSEC was used to investigate systems of low molecular weight organic acids with humic acids. In these systems no evidence for mixed complexes was found and that there was good agreement between experimental data and model predictions. The role of humic acid at alkaline pH was also investigated. It was found that humic acid showed enhanced complexation with europium and that this was attributable to phenolic groups on the humic molecule. In conclusion, results have been generated which provide data for a number of important reactions that occur in the environment.

Dedicated to my Mum and Dad for their love and kindness

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APPENDIX ONE

APPENDIX TWO

CHAPTER ONE

Introduction

1. INTRODUCTION

1.1 Background

If toxic metals breach the engineered barrier from a radioactive waste repository site, their transport through the Geosphere will primarily be determined by their concentration, the hydrogeology and the interaction of the toxic metals with the surronding media. This interaction is controlled by the type of speciation the pollutant adopts. In this particular environment, a large number of reagents are present which may complex with toxic metals forming soluble complexes which therefore may enhance the mobility of the toxic metals through the Geosphere. These reagents are inorganic, natural or anthropogenic organics.

Although in the case of the anthropogenic organics (EDTA, NTA, etc), they form strong, stable complexes with metals and are very powerful chelating agents [1], their effects are confined to localised environments.
However, natural organics produced from biological processes can occur everywhere, and hence their effects on metal migration through the Geosphere will be far more widespread.

Natural organic acids can conveniently be classed into two groups, namely high molecular weight organic acids and low molecular weight organic acids. Both groups have the ability to form stable complexes with metal ions. As a consequence of this ability it is essential that their reactions with metal ions under a variety of conditions are understood so that geochemical speciation codes can quantitatively predict the speciation, and ultimately the migration, of toxic metal ions in the Geosphere.

1.2 Aims of the project

The aims of the research are listed below:

1. To identify the type of low molecular weight organic acids which are likely to be present in the far field of a waste repository site.

2. To determine quantitatively the interactions of both low and high molecular weight organic acids with toxic metals of interest.

3. To decide whether geochemical modelling codes, as they stand, are able to predict metal speciation in the presence of natural organic acids.

4. To determine the role of naturally occurring high molecular weight organic acids in competition reactions with low molecular weight organic acids for metal complexation.

5. To evaluate the complexation of toxic metals with high molecular weight organic acids at alkaline pH.

6. Data provided from the report will be used in groundwater modelling studies of the Drigg low level disposal site

1.3 Formation and Occurrence of Low Molecular Weight Organic Acids in Soils and Groundwaters

The formation of low molecular weight organic acids in soils and groundwater can occur through a number of processes. In theory, any soil or aquifer will contain these acids, but only in minor concentrations, with high molecular weight organic acids (humic substances) making up the majority of the organic matter. However a number of low molecular weight organic acids havebeen isolated from soils which are known to form strong complexes with metal ions (eg citric, oxalic) [2]. Early work on these acids highlighted their effect on the solubility of metals.

These acids may have been derived from plant residues, rhizosphere activity or microbial metabolism.

Significant concentrations of small organic acids in groundwaters are rare due to the action of soil microflora which assimilate them. An example of this is found in leachates from Maxey Flats, where under anaerobic conditions, there was a decline in the concentration of organic acids up to six carbons in length [4].

However, the concentrations of these aliphatic acids can be increased by a number of processes. In soils, the decay of freshly produced organic matter, as a result of complete or partial oxidation will produce citric and oxalic acids [5]. Similarly the decomposition of plant residue by anaerobic proteolytic bacteria will have amongst its products acetic, propionic and butryic acids [6]. It is this important factor of microbiological activity on natural or disposed organics that yields the production of these low molecular weight organic acids. Certain other micro-organisms will produce acetic, propionic, butryic, valeric acids whilst moulds can produce the amino acids tartaric and asparatic.

Similarly degradation of organic waste will produce high concentrations of organic acids, in a waste repository site. It has been reported in the literature that organic acids generated from chemical degradation of cellulose have the capacity for metal chelation [7]. Hydrolytic degradation has been shown to give a number of important organic acid products [8]. For example cellulose, polyesters, nylons and polyurethanes could all undergo hydrolytic reactions to give a variety of products. Cellulose is particularly important due to its abundance and the ease that it undergoes hydrolysis reactions under alkaline conditions. This reaction is complex and its subsequent products are a wide mixture of acid products. One of the major products is lactic acid [9]. In addition hydrolysis reactions on polyamides (nylons) and polyesters will give a mixture of carboxylic acids.

Microbial degradation can be under aerobic and anaerobic conditions and the first steps in both will involve the hydrolysation of various polysaccharides, proteins and fats to give intermediate products, including volatile organic acids and amino acids. These intermediates are generally more soluble than the original organic material. Only if there is sufficient oxygen will these intermediate products be oxidised to give the end products of carbon dioxide and water.

In a landfill it has been shown that for fatty acids this hydrolysation can take from 6 months to 7 years [10]. In a waste repository site, aerobic metabolism will be dominant until all available oxygen is used up and then anaerobic metabolism will take over. As can be seen from Figure 1.1 and 1.2 low molecular weight organic acids will be significant products under both these environments. In waste sites hydrolytic and fermentative bacteria, over a wide range of conditions, can produce simple organic acids as well as hydrogen and carbon dioxide. Although methanogenic bacteria are able to break down the acids there is evidence to suggest that the methanogenic bacteria do not become established in this environment due to mechanical or chemical changes and that these acids may build up [7].

Thus, in summary, due to a number of processes on natural and anthropogenic organics, a number of low molecular weight organic acids in significant concentrations are likely to be present in the Geosphere to affect the speciation of toxic metal ions.

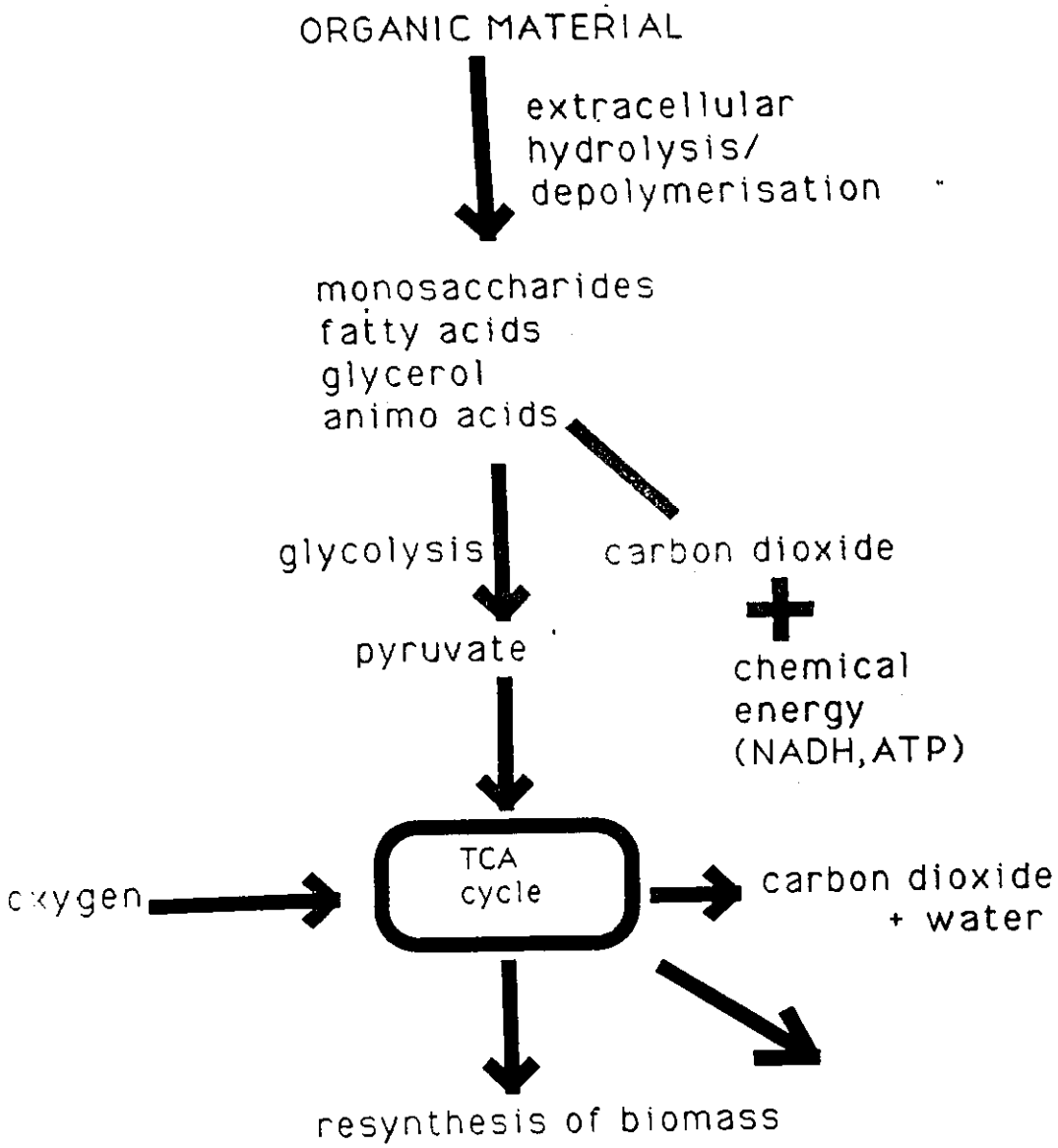


Figure 1.1: Schematic generalization of aerobic metabolism

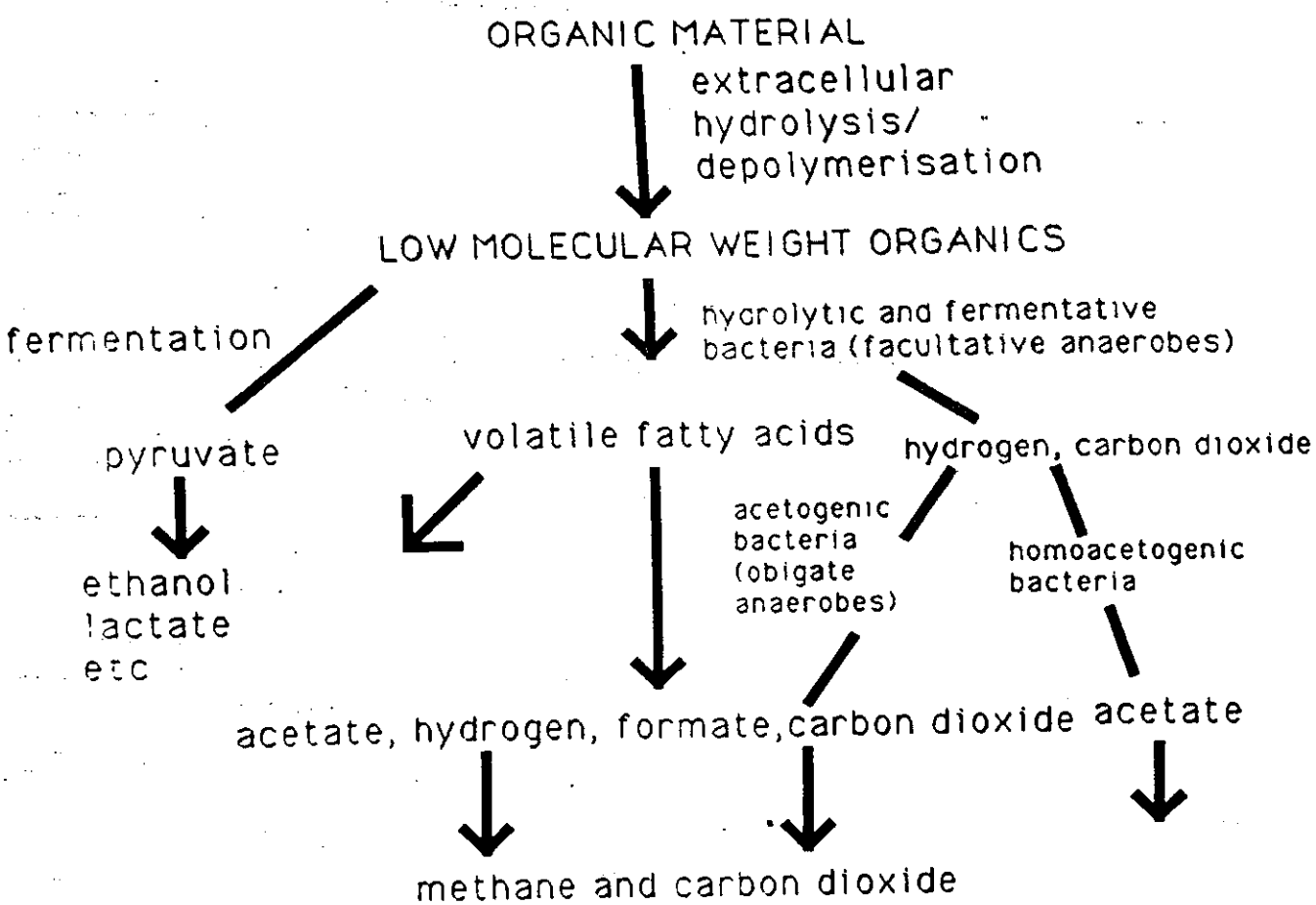


Figure 1.2: Schematic generalization of anaerobic metabolism

1.3.1 Complexation with toxic metals

Toxic metal cations released from a repository site show a strong tendency, due to a number of processes, to adsorb on mineral and soil surfaces. When these cations are complexed with organic acid ligands the charge of the complex will be less, or even reversed compared to that of the original metal cation. This change in charge may reduce the effect of the adsorption of the toxic metal onto a mineral or soil surface and hence increase the migration rate of the pollutant through the groundwater. The retention of metal ions is decreased by the formation of soluble complexes that shift metal ion exchange, physical sorption or solubility equilibria. Conversely, retention may be increased if the complexant itself sorbs onto the solid surface.

Low molecular weight organic acids, show a varying degree of complexing ability from weak (acetic acid) to strong (gluconic acid). It has been suggested that low molecular weight organics that are weaker chelating agents than high molecular weight organic acids may have a synergistic effect when these marcomolecular acids are complexing with toxic metals [11].

Previous work on the complexation of toxic metals with low molecular weight organic acids has shown that the acid ligand increases the mobility of the radionuclide through the Geosphere. Experiments carried out under anaerobic conditions using columns with different compositions of soil type show that the presence of these acids will inhibit the adsorption of metals onto mineral surfaces. Work on acetic, propionic, butryic and valeric acids has shown their ability to enhance the migration of Ni, Zn and Pb through soil and into groundwater [12].

In order to understand the behaviour of the toxic metals, consideration must be first given to the speciation that the metal cation adopts. It has been shown that under equilibrium conditions, the speciation that a large percentage of a divalent metal adopts in the presence of a fatty acid concentration of 0.3mol l^{-1}, is as mononvalent positively charged, uncharged and negatively charged complexes ie its positive charge has been reduced. In this state there is a severe reduction in the adsorption and precipitation of the metal cations [12].

Studies on acetic acid show that if it is in sufficient concentration, it will increase the migration of Am-241 [13] under near-field conditions i.e: high pH environment. Similar studies on citric acid, showed that the sorption of uranium was reduced by two orders of magnitude [14].

Using potentiometric techniques under near natural conditions, the complexes of asparatic, tartaric and citric acids with the metal ions Co^{2+}, Sr^{2+}, La^{3+} and Eu^{3+} were investigated with differing pH [15].

Using ion exchange columns, the complexes of oxalic, tartaric and citric acids with various actinides has again yielded information on the structures of the complexes with varying pH [16]. Results suggest that in general, high complexing abilities of weak acids to different metal ions are expected at pH values within or higher than the pK_a values of those acids.

Experiments carried out under near-field conditions with measurements made at pH~12 were performed at Harwell [17]. Gluconic acid was shown to have the ability to complex with plutonium and give solubilities of $>10^{-6}$M plutonium at the highest concentration (10^{-2}M) of gluconic acid. Similar results were obtained for citric acid, whose strong complexation with thorium was noted. Oxalic acid also gave significant complexation with thorium.

Hence, previous studies have indicated that such low molecular weight organic acids may play an important role in the migration of toxic metals.

1.4 High Molecular Weight Organic Acids

High molecular weight organic acids, or humic substances, play a fundamental part in the environmental chemistry of toxic metals. They are known to be strong complexing agents [18,19,20,21], able to reduce the free metal concentration and solubilize compounds, thus leading to enhanced migration through the groundwater. Alternatively, they can form insoluble complexes with metal ions, which can immobilize the toxic metal. They are also reducing agents [22] capable of affecting the oxidation state of a metal ion.

Within the general term of humic substances are three major components, defined in terms of their solubility as a function of pH [23]:

Humin - that fraction of humic substance that is not soluble in water at any pH value.

Humic acid - that fraction of humic substance that is not soluble in water under pH conditions below 2.

Fulvic acid - that fraction of humic substance that is soluble in water under all pH conditions.

For the last 200 years an extensive literature has developed on the geochemical role of these substances, and yet, there still remains uncertainity has to the precise role of humic substances in the environment.

1.4.1 Formation and Occurence of High Molecular Weight Organic Acids

The synthesis of humic and fulvic acids has been the subject of many investigations and, as such, a number of different theories have been proposed [24,25]. However, although each theory has its merits none has yet explained all aspects of humic formation.

An unification of the most realistic postulations in older theories were accumulated in the polyphenol (PP) theory of Stevenson [26]. This stated that humic formation involves the synthesis of polyphenols as a pre-requisite. However, this theory still leaves unanswered a number of questions concerning the different pathways leading to humic formation. To overcome this, a recent theory has been proposed [27].

In this study, humic formation is only considered as occuring from lignin, carbohydrates and nitrogenous compounds since other plant materials are insignificant in comparision.

The transformation of lignin is due to it being an energy source to microorganisms, which solubilise the lignin by depolymerisation, from which polyphenols are synthesised by the microorganisms. These phenols then follow two pathways. Part of the phenols may be oxidised by enzymes like laccase, tryosinase and peroxidase to quinones. Further oxidation will lead to polycondensation and to formation of humic substances. Phenols which do not undergo this pathway, undergo a series of reactions until they form aliphatic acids which then enter the Kreb's cycle [28].

Similar to lignin, carbohydrates are also solubilised by microorganisms. In this state, certain organisms will form aromatic compounds. This aromatisation leads to the formation of humic substances.

The phenols and quinones which are produced by the action of microorganisms, under the influence of oxidising enzymes, will undergo condensation between themselves and with amino acids [29]. This polymerisation may cause loss of COOH groups, and hence, the greater the degree of polymerisation the more loss of COOH groups per unit weight that will occur. This explains why fulvic acids are smallers molecules with more COOH groups per unit weight than the larger humic acids [30]. Further evidence for this observation is that fulvic acids dominate under conditions where free radical polymerisation is suppressed eg: in anaerobic conditions.

In conclusion on the formation of humic substances a number of general points can be made:

1. Any carbon source which can be transformed to phenols by the action of microorganisms can serve as a pre-cursor to humic formation.

2. Only organisms capable of transforming/producing phenols are able to assist in the formation of humic substances.

3. The humic building units condense by a free radical mechanism to form humic substances.

4. Any difference between humic and fulvic acids is due to the degree of polymerisation.

A proposed structure of a humic acid molecule is shown in figure 1.3 [31].

As a result of this formation, humic substances are ubiquitous in the environment and are the major organic fraction in soils. Humic substances also occur in all terrestrial waters.

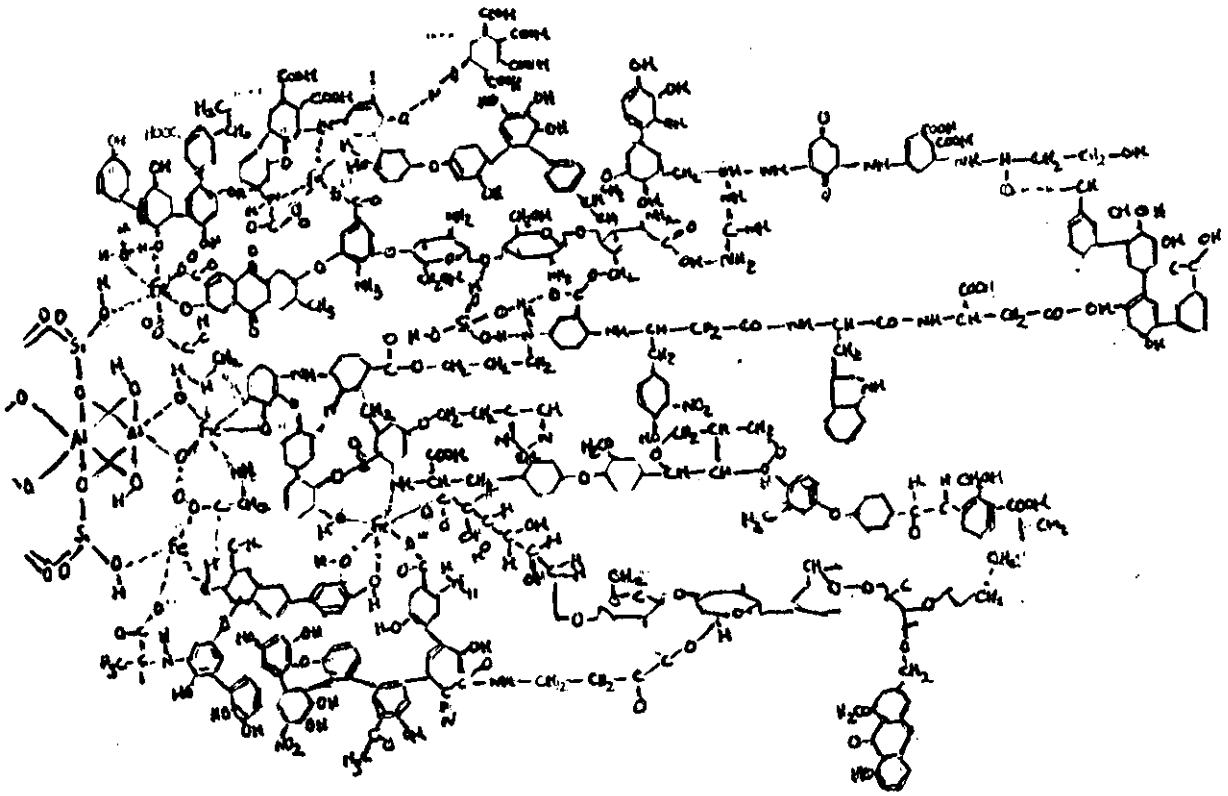


Figure 1.3: Proposed structure for a soil humic acid

1.4.2 Complexation with metals

Numerous studies have been undertaken on the binding behaviour of metal ions with high molecular weight organic acids. Due to the complex nature of humic substances a large range of analytical techniques have been used in these investigations, some which are listed in table 1.1.

Method	Value measured
Ion exchange	M or M_b
AA	M or M_b
Colorimetry	M or M_b
Equilbrium dialysis	M
Gel filtration	M_b
Centifrugation	M
Solvent extraction	M
Potentiometry	L_b
Differential Pulse Polargraphy	M
Spectrofluorimetry	L_b or L
Spectrophotometry	L
Ion - selective electrode	M
Anodic Stripping Voltammetry	M

where M = 'free' metal
M_b = 'bound metal
L = 'free' ligand
L_b = bound ligand

Table 1.1: Methods used to measure metal - humic substances interactions [32]

A number of papers have dealt with the interaction between humic substances and copper. Cabaniss and Shuman[33] investigated these interactions by the measurement of the free copper by an ion selective electrode and also the free ligand by fluorescence. By both techniques

strong complexation was observed. This was also observed, using the same techniques by Gamble et al [34]. Anodic stripping voltammetry has also been used in this system, where conditional formation constants were calculated to be 0.32 x 10^5 to 5.2 x 10^5 at pH 6.5.

Extensive work using gel chromatography [35] determined binding constants for copper with fulvic acids from lake water and peat. The obtained constants were of the order of 10^8 . In addition, the use of MnO_2 as a weak anion exchanger has been used to study copper - humic acid equilibria [36].

A large amount of data has also been published with other first row transition row metals. Saar and Weber used an ion selective electrode to derive stability constants for the reaction of cadmium with water and soil derived fulvic acids [37]. Similarly, the Schubert ion exchange method has been used to study the interaction of cadmium with humic substances from podzol B_H, producing stability constants of the order 10^5.

Choppin [22] reviewed the role of humics on actinide migration. He reported stability constants for UO_2^{2+} with soil, peat, marine and commercial humic and fulvic acids. In addition, the redox interactions of humic materials with metal ions were discussed. Soil fulvic acids reduced Pu(VI) and Pu(IV) [22]. Good correlation was found between Pu(III,IV)/(Pu(V,VI) ratio and DOC (Dissolved Organic Carbon).

Lathanide ion probe spectroscopy [38] has been used in an attempt to characterize metal binding sites on fulvic acid. A continuous multiple site ligand model was proposed from experimental results of europium with an aquatic fulvic acid from Suwannee River.

Complexation of uranyl has also been studied using time - resolved laser-induced fluorescence spectroscopy (TRLFS) [39], where no significant variation of the log K value was found in the pH range 2.7 to 6.5, and the ionic strengths of 0.1M and 1M.

In addition TRLFS has also been used on curium studies [40]. From these results a stability constant of 5.90 was evaluated, which was independent of pH and ionic strength.

Rainville and Weber [41] used a dialysis technique to measure the complexing capacity of a soil fulvic acid for the metal ions Cu^{2+}, Cd^{2+}, Mn^{2+}, Ni^{2+}, and Zn^{2+}. They concluded that at the same pH, the values obtained for Cd^{2+}, Mn^{2+}, Ni^{2+}, and Zn^{2+} were similar, but for Cu^{2+} values were higher.

A variety of electrochemical techniques have been used to study the complexation of cadmium and lead with humic acid [42]. It was concluded that lead complexes were more stable than cadmium complexes.

In summary a large number of studies have been carried out on the interaction of humic substances with metal ions. However, the majority of these studies have not investigated the effect of secondary factors, such as varying pH, ionic strength, and the influence of competing ligands. To gain further insight into the role of humic substances in the migration of toxic metals such factors need to be investigated.

1.5 Stability Constants

1.5.1 History

Stability constants are best defined as a measure of the affinity of a ligand for a metal ion in solution. The first effective measurement began with the work of J. Bjerrum on the equilibria of transition metal-ammonia complexes in aqueous solution [43]. In 1956 the work of Calvin and Smith [44] utilised an exact algebraic treatment to determine stability constants and mass balance equations. This work led to a substantial amount of published data on the equilibria of metal ions with various ligands in aqueous solution. Unfortunately, a significant proportion of the work was poor quality, due to ill-defined controls and conditions. As a result, a large amount of published data was critically assessed for their validity and collected in a series of volumes called, Critical Table of Stability Constants [45]. Consequently, these volumes have now become established as benchmarks for future work. Recently, the advent of a large range of computational methods has allowed for a more rapid and more accurate determination of stability constants.

1.5.2 Theory

Stability constants for metal complex formation provide a measure of the affinity between a ligand and a metal ion in solution.

The stability contant, K_{eq}, is a quotient involving the activities of reacting species in solution and is given by:

$$aA + bB \rightleftharpoons cC + dD \qquad (1.1)$$

$$K_{eq} = \frac{\{C\}^c\{D\}^d}{\{A\}^a\{B\}^b} \qquad (1.2)$$

This constant is directly related to the differences of Gibbs free energies of products and reactants in their standard states.

Unfortunately activities of ionic species in solutions involve intricate calculations. Therefore, since concentrations parallel activities, when the ionic strength is controlled by a non-reacting electrolyte which is present at a concentration far in excess (~ 100 times) of the reacting species, most equilibrium constants are determined at a constant ionic strength which is maintained by the supporting electrolyte. Therefore, the stability constant is now given by:

$$K = \frac{[C]^c[D]^d}{[A]^a[B]^b} \qquad (1.3)$$

In this form, the quantites can be substituted directly into mass action equations.

Any observed stability constant depends on the free energy change which is associated with a metal ion, M, during its transfer from an environment of solvent molecules and bulk electrolyte ions to one in which it is in close proximity with at least one ligand, L. Thus the stability constant gives an indication of the degree of displacement by a ligand, of an unknown number of solvent molecules, and background ions from the metal ion M.

1.5.3 Effects on stability constants

1.5.3.1 Temperature

A change in temperature will result in a change in equilibria and hence a change in the equilibrium constant value. This shift will be dependent on the sign and the value ΔH, the enthalpy change of the reaction. This can be expressed by the van't Hoff equation:

$$\frac{d\ln K}{dT} = \frac{\Delta H^0}{RT^2} \qquad (1.4)$$

If ΔH^0 is virtually constant within a temperature range of T_1 - T_2 integration of the above expression gives:

$$\log K_{T2} = \log K_{T1} + \frac{\Delta H(1/T_1 - 1/T_2)}{19.145} \qquad (1.5)$$

However, if the enthalpy change does not remain constant, a more complicated expression is needed:

$$\log K_{T2} = \log K_{T1} + \Delta H^0_{T1}(1/T_1 - 1/T_2) + \Delta C^0_p[1/19.14(T_1/T_2 - 1) - 1/8.314 \log T_1/T_2)] \qquad (1.6)$$

Thus, using these expressions, the dependence of equilibrium constants on temperature can be calculated.

1.5.3.2 Ionic strength

It is impractical to measure a thermodynamic equilibrium constant, since it requires ions to be in infinite dilution. Consequently constants are measured in finite solutions and are therefore not true constants and are multiplied by activity coefficients to obtain the true thermodynamic constant. This correction is governed by the concentrations of all the ions in the solution and their charges and the measurement of this is expressed as the ionic strength. The ionic strength, I is defined as:

$$I = 0.5\Sigma cz^2 \qquad (1.7)$$

where c and z represent the concentration and charge of an individual ion respectively. In order to minimise the deviation from an ideal infinite dilute solution, a background electrolyte is usually employed. This background electrolyte should be a strong electrolyte, have negligible association with other species in the solution, a large solubility and contribute a negligible amount to the physical or chemical property being measured.

1.5.3.3 Ionic radius

If the interaction between a metal and ligand is purely due to electrostatic interaction, then the stability constant for other metal ions of the same charge should be inveresely proportional to metal ion radii. Although for ions of similar electronic configuration this may be approximately valid, for metal ions of different groups it fails.

1.5.3.4 Ionization potential and electronegativity

When a complex is formed, electrons are lost from the central metal ion and gained by the donor ligands. Therefore, ionization potential has been used to determine the affinity for a metal ion and a ligand. This, although only a rough approximation due to electronic configurations changing during complex formation, nevertheless has produced fairly good relationships. It has been shown that for many metal ions the following relationship is valid [46]:

$$\log k_1 = p(I-q) \qquad (1.8)$$

where I is the ionization potential and p and q are constants which depend on only the ligand and experimental conditions, but not the metal ion.

1.5.3.5 Electronic configuration of the central ion

Stability orders are found for transition metal complexes of which of particular importance is the Irving-Williams order. This order was determined by considering the ionic radii and the second ionization potential of the first row of the transition metals and can be illustrated by considering the values of metal complexes with EDTA:

Metal ion	log k
V^{2+}	12.7
Cr^{2+}	13.6
Mn^{2+}	13.88
Fe^{2+}	14.30
Co^{2+}	16.45
Ni^{2+}	18.4
Cu^{2+}	18.78
Zn^{2+}	16.5

Similar relationships involving the nature of the ligand, i.e: nature of the donor atom, the basicity of the ligand can also be used.

1.6 Measurement of stability constants

A large number of analytical techniques have been used in the determination and measurement of stability constants:

Potentiometry
Spectrophotometry
Specific ion EMF measurements
NMR spectroscopy
Polarography
Ion exchange
Colorimetry
Ionic conductivity
Liquid partiton equilibria
Reaction kinetics
Partial pressure measurement
Solubility measurement

Although, there are numerous methods, in the majority of cases, it is potentiometry and spectrophotometry that are most commonly used.

1.7 Stability constants involving metals and humic substances

For a reaction involving a metal ion, M, and a 'simple' (ie: of known molecular weight and structure) ligand L, the equlibrium can be represented as shown below:

$$M + L \rightleftharpoons ML \qquad (1.9)$$

For this reaction the stability constant, K, is given by the expression:

$$K = \frac{[ML]}{[M][L]} \qquad (1.10)$$

Unfortunately this cannot be applied in the case of humic and fulvic acids. This is due to their lack of chemical and physical specifications. Humic acids are made up of complex mixtures of different functional groups which each individually have differing affinities for metal ions. In addition, they are polyelectrolytes and hence, exert an electrostatic factor in the binding with metal ions.

To account for this heterogeneous nature a number of theoretical aspects have been developed to attempt to mimic metal - humic interactions.

1.7.1 Methods to evaluate metal - humic interactions

Essentially methods to determine the strength of interaction between metal ions and humic substances can be classed into two distinct groups. These are discrete models and continuous models. To highlight the differences between these two groups, a discrete and continuous model have been adopted in this research project. The theoretical and mathematical aspects of these two approaches will be considered here.

1.7.2 Discrete model - the Scatchard Plot

To simplify the heterogenous nature of humic substances, the differing complexing sites on a humic molecule, are grouped into two types of sites. These are called 'strong sites' and 'weak sites.' This simplification is the building block for the Scatchard plot [47]. The plot attempts to bridge the gap between a single stability constant value for a metal - humic interaction and the contiuous range of stability constants which are produced by more rigorous mathematical interpretations.

Presented here is a modified version of the basic Scatchard approach which avoids the shortcomings of the basic method as discussed by Dzombak and Morel [48].

As stated, a single stability constant value is insufficient to describe the interaction between a metal ion and a humic molecule. This is due not only to the different functional groups on the humic molecule but also due to its polyelectrolytic nature which changes as the metal loading on the humic molecule varies.

Therefore if we consider that the humic molecule has two types of sites (strong site, ^{s}S and weak sites, ^{w}S) then the following equilibria will occur:

$$M + {}^{s}S \rightleftharpoons M^{s}S \qquad (1.11)$$

for which $$ {}^{s}K = \frac{[M^{s}S]}{[M][{}^{s}S]} \qquad (1.12)$$

Similarly for the weak sites:

$$M + {}^{w}S \rightleftharpoons M^{w}S \qquad (1.13)$$

for which, $$ {}^{w}K = \frac{[M^{w}S]}{[M][{}^{w}S]} \qquad (1.14)$$

In addition, the site concentrations are given by:

$$[{}^{s}S] = [M^{s}S]_{max} - [M^{s}S] \qquad (1.15)$$

$$[{}^{w}S] = [M^{w}S]_{max} - [M^{w}S] \qquad (1.16)$$

Combining of equations 1.14 and 1.15,1.16 leads to:

$$\frac{[M^{s}S]}{[M]} = {}^{s}K([M^{s}S]_{max} - [M^{s}S]) \qquad (1.17)$$

$$\frac{[M^{w}S]}{[M]} = {}^{w}K([M^{w}S]_{max} - [M^{w}S]) \qquad (1.18)$$

Since, $[MS] = [M^{s}S] + [M^{w}S]$

$$\frac{[MS]}{[M]} = {}^{s}K[M^{s}S]_{max} - {}^{s}K[M^{s}S] + {}^{w}K[M^{w}S]_{max} - {}^{w}K[M^{w}S] \qquad (1.19)$$

To derive values for ${}^{s}K$ and ${}^{w}K$, experiments are performed where the humic acid concentration is kept constant and the total metal concentration is varied over several orders of magnitude, such that for each total metal value the amount of metal bound and the amount of metal free is determined.

From such data a graph of [MS]/[M] vs [MS] is constructed. The graph is composed of two parts, an initial steep linear slope which corresponds to the strong sites of a humic molecule, and a less steep slope which corresponds to the weak sites. From the gradients of these two linear segments, stability constant values can be calculated.

Additionally, total concentrations of both strong and weak sites may be estimated by extrapolation of the two linear segments to the x - axis.

1.7.3 Continuous Model - The Differential Equilibrium Function (DEF)

A criticism against the Scatchard approach, is that it is an over simplification of metal - humic interactions and that the two derived stability constants have no real physio-chemical significance.

The differential equilibrium function [49] offers a more rigorous interpretation of metal - humic interactions since it provides a contiuous distribution of equilibrium constants as a function of metal loading, thereby taking into consideration the inherent heterogenity of the humic molecule.

If a series of experiments are considered where the humic concentration is kept constant and the metal concentration is varied, then for any point during that titration it is possible to calculate a stability constant K'.

$$K' = \frac{|M|_b}{|M|\,|L'|} \qquad (1.20)$$

In this expression K' is referred to as the average equilibrium quotient, $|M|_b$ is the total metal bound and |L'| represents all the sites on the humic molecule not combined with M. Note that |L'| also includes protonated sites as well as free sites. At a constant pH, |L'|/|L| (where |L| refers to the total concentration of free sites only) will be constant, and therefore K' is only valid for a particular pH. Correspondingly K' will vary according to the degree of metal loading on the humic molecule:

$$K' = \frac{\sum_j |M^jL|}{|M| \sum_j |^jL'|} \qquad (1.21)$$

This equation leads to:

$$K' = \frac{\sum_j {}^jK' \, {}^jL'}{\sum_j {}^jL'}$$

This equation shows that K' at any point in a metal titration is an average of the whole number of constants for all sites and is weighted by the unoccupied sites at that point. Since there is an infinite range of sites, then the ranges of ${}^jK^*$ will be contiuous. This function, ${}^jK^*$, is termed the differential equilibrium function and is related to the average equilbitrum quotient, K', by:

$$K^* = \frac{- d(K'(1 - \phi)}{d\phi} \qquad (1.22)$$

where ϕ represents the fraction of all sites occupied and is given by the expression:

$$\phi = \frac{[M]_b}{\overline{C}_L} \qquad (1.23)$$

where M_b represent the metal bound and C_L is the total site concentraion of the humic acid.

A graph of $K'(1-\phi)$ is plotted as a function of ϕ with K* being obtained from the slope of curve for each value of ϕ. A modification[31] allows calculation of K* from the humic acid concentration expressed in eg: g L^{-1}, thereby avoiding the necessity to determine the molar total concentration of sites, C_L.

1.8 Summary

In order to predict the behaviour of toxic metals and radionuclides in the Geosphere it is essential to fully understand the binding behaviour of these pollutants with the large number of complexants that are present in this environment.

One of the most important groups of complexants are the natural organic acids. Due to the abundance and complexing ability of these natural organic acids in the Geosphere it is essential to quantitatively understand their interactions with toxic metals of interest in order to provide risk assessment on the potential release of these toxic metals to the Biosphere.

This chapter has identified how these acids are formed and highlighted previous investigations that have been undertaken in studying the complexation with metals.

By an understanding of the solution chemistry of these acid risk assessments on the movement of radionuclides from sites like Drigg can be made on the migration of radiopnuclides and toxic metals.

However, there is a still a need to know, quantitatively, how these acids complex with metal ions under a variety of conditions. The aim of this work is to fill the gap in the current literature by investigating metal - natural organic acid equilibria under a variety of experimental conditions eg: changing pH, competing ligands, varying ionic strength, competing metal ions. By investigating the effects of such phenomena, the results generated offer a greater insight into how toxic metals react with important organic acids in the environment.

CHAPTER TWO

Potentiometric studies involving metal ions and low molecular weight organic acids

2 INTRODUCTION

2.1 Background

An extensive database has developed in the literature concerning the interactions with organic ligands and metals. Unfortunately the published stability constants for a particular system sometimes vary over a few orders of magnitude. This discrepancy in results is invariably due to poor experimental design and the neglecting of quoting important parameters such as ionic strength and pH. In order to successfully predict the behaviour of toxic metals it is necessary to obtain accurate stability constants for metal - ligand equilibria, such that complex environmental systems containing a multitude of species can be quantified accurately with regards to speciation.

As a consequence of these descrepancies, a methodology was developed whereby stability constants could be accurately measured by a standard technique and that values obtained could be critically compared with literature values, which had been previously assessed and whose values had been quoted under similar conditions of ionic strength and background electrolyte.

The overall aim of the work was to provide a series of stability constants for a number of important low molecular weight organic acids, which would be expected to be present in groundwaters and soils, and be able to form complexes with the metal ions of interest. Two metals ion were chosen to be investigated, nickel and europium. These two metal were chosen because Ni^{2+} is a typical divalent metal ion in terms of its stability with organic ligands and Eu^{3+} is an analogue of the actinide elements. If the values produced from the work were in agreement with assessed literature values, or at least provided realistic values then the work could be further extended in the future for further 'in-house' measurement of stability constants, and to verify predictive calculations of metal-ligand interactions.

2.2 General experimental procedure

For all simple and complex metal-ligand systems studied, the experimental work consisted of the following general procedure:

(a) Determination of carbonate contamination.
(b) Protonation constant determination.
(c) Stability constant determination.
(d) Computational procedures.

2.2.1 Determination of carbonate contamination

In the potentiometric determination of acid protonation constants and metal-ligand stability constants, the presence of carbonate (from carbon dioxide in the atmosphere) can effect the values obtained. As a result, it is necessary to determine the amount of carbonate that is present in solution prior to the determinations of acid protonation and metal-ligand stability constants.

2.2.2 Theory

In the determination of carbonate contamination by Gran's method [50], a standard titration is performed. Increments of a standardised base solution are added to an acid solution up to a pH meter reading between 11 - 12. The following calculations can be used to determine the calculated p[H].

$$p[H] = X \qquad \text{(in acid region)}$$
$$p[H] = pK_w - X \qquad \text{(in basic region)}$$

where $$X = -\log_{10}[|mmol_{acid} - V_{NaOH}M_{NaOH}|/(V_0 + V_{NaOH})] \qquad (2.1)$$

$mmol_{acid}$ = initial millimoles of strong acid present.
V_{NaOH} = is the ml of standard base of molarity M_{base} added.
pK_w = $-\log_{10}([H^+][OH^-])$ at the ionic strength employed.

Differences between $p[H]_{obs}$ and $p[H]_{calc}$ tend to be greater above pH 7, and this is due to the presence of carbonate in the solution.

The underlying principle behind Gran's method for strong acids is that the free hydrogen ion concentration $[H^+]$ is reduced in direct proportion to the amount of standard base added. When the function $(V_o + V_{NaOH})10^{-MR}$ is plotted, where MR is the reading on the pH meter against moles of base added the reading is exactly proportional to $[H^+]$ and therefore the overall term $(V_o + V_{NaOH})10^{-MR}$ is proportional to the moles of hydrogen present. Therefore the intercept on the volume axis represents exact neutralization of the strong acid.

If the value obtained from the intercept shows a carbonate contamination of above 2% (%v/v), then a fresh solution should be prepared. Values above 2% will have a significant affect on the stability constant values produced.

2.2.3 Experimental

The method involved an acid-base titration. The cell is shown in figure 2.1. It consisted of a double walled glass cell through which thermostated water was pumped through to maintain a constant temperature. Inlets were provided for a burette, pH glass electrode (Ross™ electrode combination pH Model 81-02 connected to Orion Model 720A pH meter), and for the passage of nitrogen gas. The total volume of the glass cell was approximately 80ml. The following acid solution was made up in a reaction cell:

5ml of standardized volumetric HCl solution
5ml of 1.0mol l^{-1} KCl
45ml of analytical grade water

To this solution 1.0ml increments of standardised base solution were added. The system was allowed to equilibrate for a set period of time (>30sec) and pH readings were recorded.

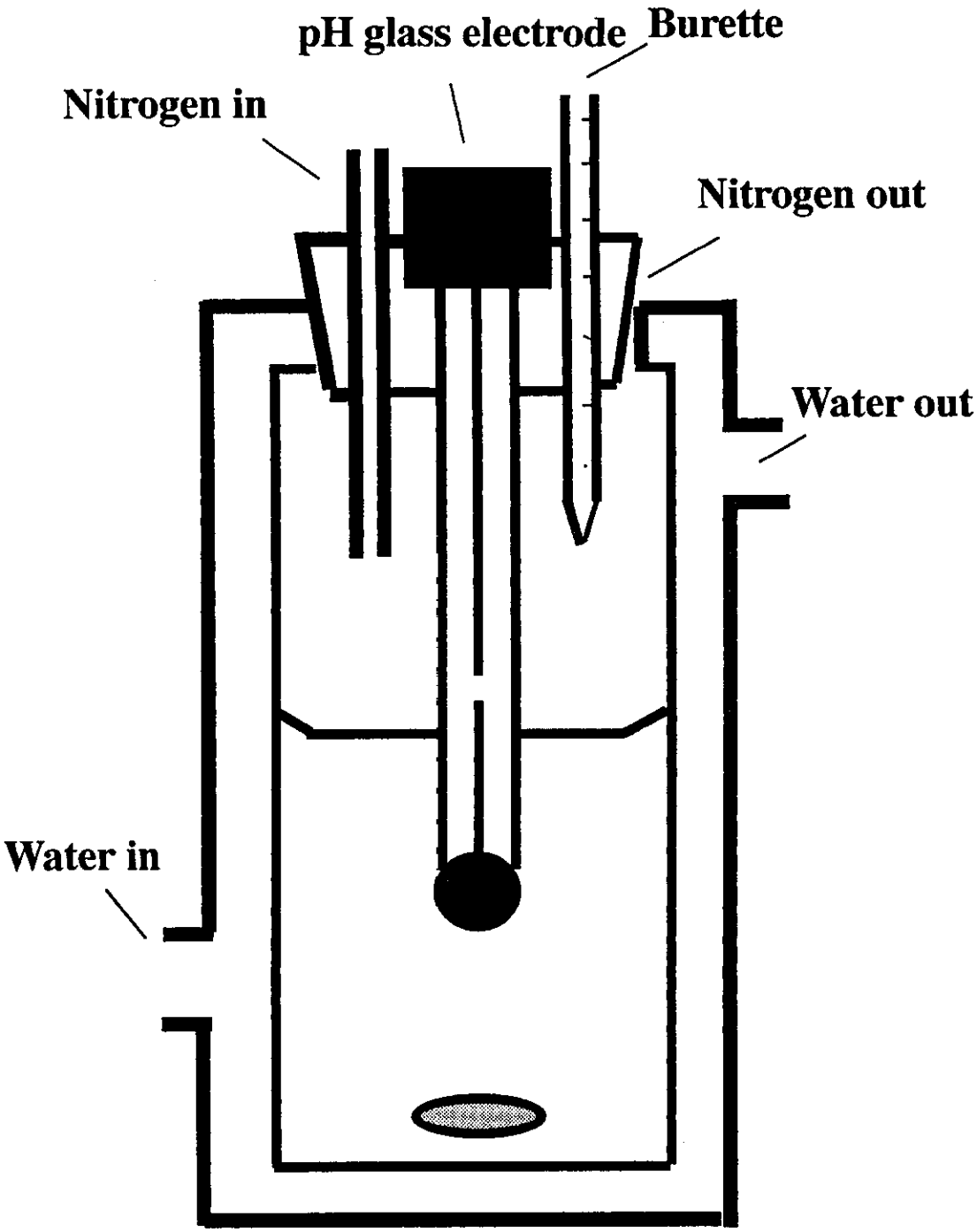


Figure 2.1: Reaction cell used for potentiometric titrations

2.3 Protonation constant determination

The protonation constant is a measure of the strength of a particular acid. The protonation constants of the acids are experimentally determined, so that the p[H] profile obtained for the acid alone can be compared with the acid-metal profile and to validate the experimental methodology.

2.3.1 Experimental

To determine the protonation constant for each acid, 0.1ml increments of standardized base were added to a well characterized solution, which contained a known concentration of acid and 5ml of 1.0mol l^{-1} KCl as the supporting electrolyte. For each increment of base added, the system was allowed to equilibrate and the pH readings were recorded.

The reaction solution was maintained at 25°C by the circulation of thermostated water through the double walled glass of the reaction cell. During the experimental determination, the cell was kept under an inert nitrogen atmosphere.

The experiment was repeated several times in order to determine reproduceability of results. The protonation constants were determined from the experimental titration data by the computer program PKAS [51].

2.4 Stability constant determination

Metal-ligand complexes may be considered as being formed by the displacement of one or more acidic protons from the ligand by the metal ion. Therefore, the addition of metal ions to a solution of a suitable ligand causes a drop in the pH of the solution. The greater the tendency for metals to combine with a given ligand , the greater the drop in pH. Thus, a titration method provides a quantitative approach for determining stability constants for reactions between metals and suitable ligands, and gives a quick, simple method of testing for complexation and, one which can be used to show the degree of complexation that has occured.

2.4.1 Experimental

In the reaction cell a known concentration of acid was placed, along with 5ml of 1.0mol l^{-1} KCl as the supporting electrolyte. A known concentration of metal salt solution was added, and the entire solution was magnetically stirred under a nitrogen atmosphere. The temperature was kept constant by using thermostated water at 25°C.

To the metal-acid solution, 0.1 ml increments of standardised base solution were added. After every increment of base added, the system was allowed to equilibrate and the pH readings were recorded.

The experiment was repeated several times in order to determine the reproduceability of the results.

For complex metal-ligand systems studied, the experimental work consisted of the following general procedure.

To a mixture of well characterized acids, containing also 5ml of 1.0mol l^{-1} KCl as the supporting electrolyte, a known concentration of metal salt solution was added and the entire solution was magnetically stirred under a nitrogen atmosphere. The temperature was kept constant by using a thermostated water bath at 25°C.

To the above reaction mixture, 0.1ml increments of standardized base solution were added. After every increment, the system was allowed to equilibrate and the pH readings recorded.

From the experimental titration data the stability constants of the various metal-acid equilibria were determined using the BEST computer program [51].

2.5 Computational method

In a potentiometric titration, the variable measured is -log[H^+] and therefore, any calculation of the data should involve the measurement of p[H] directly. This is the main feature which distinguishes BEST and PKAS computer programs from other programs available for the calculation of equilibrium constants from potentiometric data.

2.5.1 Procedure

The calculation for computing stability constants in BEST involved the following sequence:

a) A set of known and estimated overall stability constants (β's) for a particular metal-acid system were inputted and [H^+] was computed at all titration points.

b) The weighted sums of the squares of the deviations in p[H] were computed using the following relationship:

$$U = \Sigma w(p[H]_{obs} - p[H]_{calcd})^2$$

where U is the goodness of fit between the inputted stability constants and the titration curve, and w is given by:

$$w = 1/(p[H]_{i+1} - p[H]_{i-1})^2$$

where w is a weighting factor which was used to lessen the influence of the less accurate p[H] values in the steeply sloped regions of the titration curve.

c) The unknown stability constants were adjusted and the calculations repeated until no further minimization of U (the SIGFIT, i.e the goodness of fit) could be obtained, thus providing the final calculated β values. This refinement of unknown log β values proceeded as follows:

(i) enter the number of the species to be refined.
(ii) enter an increment (say .8).

The titration curve was then computed with a log β + 0.800. If U was greater than before, then the curve was recomputed with a log β value - 0.800. This process of incrementation and computation was continued up to five times until a minimum U was found. If a minimum U was not obtained in the five attempts, the best minimum fit was computed and the computation stopped.

PKAS, for protonation constant determination is a special case of the more general algorithm found in BEST. In protonation constant determination, as only two components are present, much simpler generalised equations become available and hence, the calculations are performed quicker.

The determined stability constants from the experimental titration data were inputted into the SPE computer program [51]. SPE is a FORTRAN computer program for the computation of species distribution from given equilibrium constants. SPE derives, from the given equilibrium data, species distribution of all the components in each system over the pH range studied.

Within the SPE program was the additional subroutine PHASE, which took into account any solubility products. Hence, speciation plots were obtained showing species present in both aqueous and solid phases.

From these speciation plots, tables were produced showing distribution of the metal over the pH range studied.

2.6 Metal - ligand systems

2.6.1 Nickel - acetic acid system

The carbonate concentration was calculated to be 0.8%.

Determination of the protonation constant for acetic acid

In the reaction cell the following solution was made up:

20ml of 0.009914mol l^{-1} acetic acid
(standardized against volumetric 0.10014mol l^{-1} KOH)
5ml of 1.0mol l^{-1} KCl
25ml of analytical grade water

The cell was closed and equilibrated under nitrogen. The temperature was kept constant at 25°C. Increments of 0.1ml of 0.10014mol l^{-1} KOH were added and when equilibrium was reached, the pH meter readings were recorded. The experiment was repeated a further five times for statistical analysis. The titration data was inputted into the FORTRAN 77 PKAS program[51], which evaluated the protonation constant as shown in table 2.1.

experimental run	log k_{H1}
1	4.56
2	4.54
3	4.54
4	4.56
5	4.56
6	4.58
average value	4.56 ± 0.2

Table 2.1: Acetic acid protonation constants

Stability constants of nickel-acetic acid system

In the reaction cell, the following solution was made up:

5ml of 1.0mol l^{-1} KCl
20ml of 0.009914mol l^{-1} acetic acid (standardized against volumetric 0.10014mol l^{-1} KOH)
20ml of 0.0104mol l^{-1} nickel (II) nitrate
5ml of analytical grade water

A titration was performed with 0.10014mol l^{-1} KOH base solution. After every 0.1ml increment of base, pH readings were recorded. Initial experimental runs suggested that there was insufficient acetic acid to form any significant complexation with the nickel. Hence the following solution was made up in the reaction cell:

5ml of 1.0mol l^{-1} KCl
45ml of 0.0995mol l^{-1} acetic acid (standardized against 1.0016mol l^{-1} KOH solution)
5ml of 0.0104mol l^{-1} nickel (II) nitrate

Again, a number of titrations were performed in order to achieve reproduceability of results. The results are shown in table 2.2.

experimental run	$\log k_1$	$\log k_2$
1	0.86	0.27
2	0.72	0.32
3	0.70	0.39
4	0.77	0.34
5	0.79	0.33
6	0.88	0.18
average value	0.79 ± 0.08	0.36 ± 0.1

Table 2.2: Nickel - acetic acid stability constants

The protonation constant values produced from the technique for acetic acid are in very good agreement with each other. Since acetic acid only contains one carboxylate group the titration curve of the neutralisation of the acid by base only shows one inflection point. This is shown in figure 2.2:

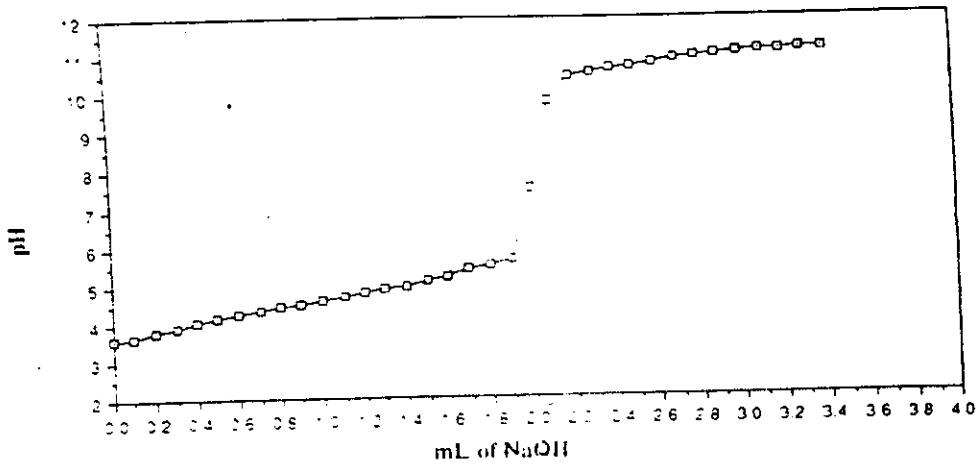


Figure 2.2: Titration curve of acetic acid

The stability constant values produced by the potentiometric technique indicate the formation of weak complexes between the nickel and the acetate ion. This would be expected for the interaction between a transition metal ion and a simple monocarboxylic acid. The second stepwise stability constant (**log k_2**) shows a large pecentage error and this is due to the small amount of this complex that was formed in the system studied. As the pH was progressively increased the nickel formed hydroxide complexes.

2.6.2 Nickel-lactic acid system

The carbonate concentration was calculated to be 0.8%.

Determination of the protonation constant for lactic acid

The solution in the reaction cell was:

20ml of 0.0093mol l^{-1} lactic acid (standardized against 0.118mol l^{-1} KOH)
5ml of 1.0mol l^{-1} KCl
25ml of analytical grade water

This was titrated against 0.118mol l^{-1} KOH solution, under a nitrogen atmosphere and a temperature kept constant at 25°C. Results are shown in table 2.3.

experimental run	log k_{H1}
1	3.64
2	3.66
3	3.60
4	3.65
5	3.60
6	3.62
average value	3.63 ± 0.3

Table 2.3: Lactic acid protonation constant

Determination of stability constants for nickel-lactic acid system

The solution in the reaction cell was:

45ml of 0.0798mol l^{-1} lactic acid
(standardized against 1.018mol l^{-1} KOH solution)
5ml of 0.016mol l^{-1} nickel (II) nitrate
5ml of 1.0mol l^{-1} KCl

This solution was titrated against 1.018mol l^{-1} KOH.

Results are shown below in table 2.4:

experimental run	log k_1	log k_2
1	1.46	0.78
2	1.49	0.71
3	1.42	0.83
4	1.63	0.96
5	1.71	0.90
6	1.55	0.93
average value	1.54 ± 0.15	0.85 ± 0.12

Table 2.4: Nickel - lactic acid stability constants

The values produced from the technique indicate the formation of weak complexes between the nickel and the lactate ion. As with the acetic acid there is a large percentage error associated with **log k_2** values. A titration curve of the nickel-lactic acid system is shown in figure 2.3.

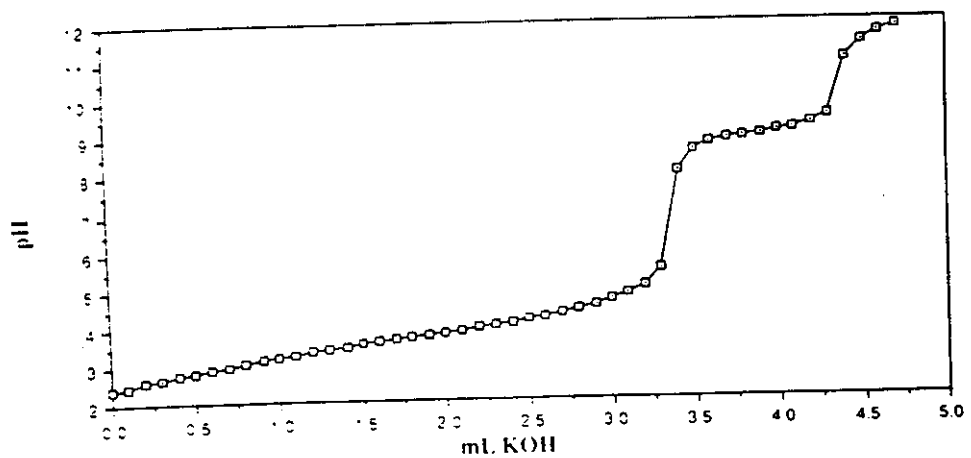


Figure 2.3: Nickel-lactic acid titration curve

From the figure it can be seen that there are two inflection points. The second inflection point is due to the formation of nickel-hydroxo complexes.

2.6.3 Nickel-citric acid system

The carbonate concentration was calculated to be 0.8%.

Determination of the protonation constants for citric acid

In the reaction cell:

20ml of 0.00945mol l^{-1} citric acid
(standardized against 0.1016mol l^{-1} NaOH solution)
5ml of 1.0mol l^{-1} KCl
25ml of analytical grade water

A titration was performed against 0.1055mol l^{-1} NaOH. Results shown in table 2.5.

experimental run	k_{H1}	k_{H2}	k_{H3}
1	5.82	4.18	2.74
2	5.81	4.18	2.82
3	5.68	4.38	3.05
4	5.72	4.19	2.89
5	5.63	4.09	2.75
6	5.62	4.32	3.09
average value	5.71 ± 0.10	4.22 ± 0.15	2.89 ± 0.17

Table 2.5: Citric acid protonation constants

Determination of the stability constants for nickel - citric acid system

The solution in the reaction cell was:

20ml of 0.00945mol l^{-1} citric acid
(standardized against 0.106mol l^{-1} KOH solution)
10ml of 0.01029mol l^{-1} nickel (II) nitrate
5ml of 1.0mol l^{-1} KCl
15ml of analytical grade water

This solution was titrated against 0.1055mol l^{-1} NaOH. Results are shown in table 2.6.

experimental run	log k_1	log k_2
1	5.09	2.72
2	5.30	2.76
3	5.25	2.90
4	5.25	2.86
5	5.00	3.15
6	5.25	2.62
average value	5.19 ± 0.15	2.84 ± 0.26

Table 2.6: Nickel - citric acid stability constants

The above stability constant values show the formation of fairly strong complexes between the nickel and the citric acid. The citrate ion has an overall charge of -3 and this high charge leads to a stronger interaction with the nickel than was the case for acetic or lactic acids. The titration curve of citric acid alone is shown in figure 2.4. Although it has three protonation constants the curve is continuous due to the close values for the constants.

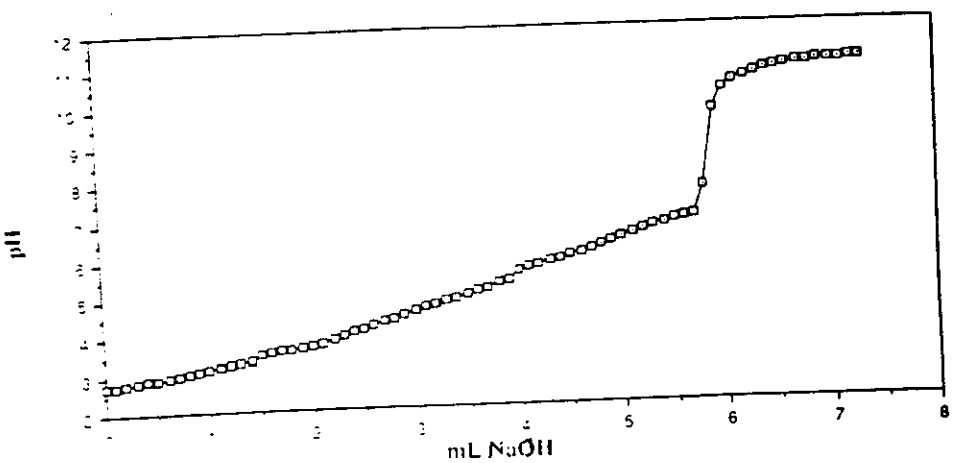


Figure 2.4: Citric acid titration curve

2.6.4 Europium - acetic acid system

The carbonate concentration was calculated as 1.5%.

Determination of the protonation constant for acetic acid

The experimental determination of the protonation constants of acetic acid has been described in section 2.6.1.

Stability constants of europium - acetic acid system

In the reaction cell the following solution was made up:

10ml of 0.01017mol l^{-1} europium (III) chloride
45ml of 0.1020mol l^{-1} acetic acid (standardized against 1.0127mol l^{-1} KOH)
5ml of 1.0mol l^{-1} KCl

A tiration was performed with 1.0127mol l^{-1} KOH solution. Results are shown in table 2.7.

experimental run	log k_1	log k_2	log k_3
1	2.04	1.27	0.42
2	2.24	1.10	0.17
3	2.14	1.30	0.16
4	2.16	1.19	0.39
5	2.22	1.09	0.33
6	1.75	1.51	0.33
average value	2.09 ± 0.25	1.24 ± 0.21	0.30 ± 0.13

Table 2.7: Europium - acetic acid stability constants

The above values again indicate the weak complexes formed by acetic acid. However, if the acetic acid is present in excess over europium in a particular system the overall formation constant, $\log \beta_3 = 3.63$. This represents the formation of the fairly stable complex, $Eu(Ace)_3$.

2.6.5 Europium - lactic acid system

The carbonate concentration was calculated as 1.7%

Determination of protonation constant for lactic acid

The protonation constant for lactic acid has been determined in section 2.6.2.

Stability constants for europium - lactic acid system

In the reaction cell the following solution was made up:

45ml of 0.08775mol l^{-1} lactic acid (standardized against 1.0127mol l^{-1} KOH)
5ml of 1.0mol l^{-1} KCl
10ml of 0.01017mol l^{-1} europium (III) chloride

A titration was performed with 1.0127mol l^{-1} KOH solution. Results are shown in table 2.8.

experimental run	log k_1	log k_2	log k_3
1	2.94	2.35	1.35
2	3.44	2.35	1.35
3	2.91	2.35	1.40
4	2.90	2.34	1.46
5	2.90	2.40	1.40
6	2.90	2.34	1.46
average value	3.00 ± 0.27	2.36 ± 0.03	1.40 ± 0.05

Table 2.8: Europium - lactic acid stability constants

From the above table, there is good consistency in the values produced from the experimental runs. As noted in the europium-acetic acid system if the lactic acid is in sufficient excess in a particular system then the europium will interact with the lactic acid to form a strong complex with an overall formation constant, $\log \beta_3 = 6.76$. Such a complex would inhibit the formation of europium carbonate and europium hydroxide complexes until higher pHs are reached, and at typical groundwater pHs would predominate the europium speciation.

2.6.6 Europium - citric acid system

The carbonate concentration was calculated as 1.1%.

Determination of the protonation constants for citric acid

The experimental determination of the protonation constants for citric acid has been described in section 2.6.3.

Stability constants of europium - citric acid system

In the reaction cell the following solution was made up:

10ml of 0.01017mol l^{-1} europium (III) chloride
20ml of 0.00945mol l^{-1} citric acid
(standardized against 0.11004mol l^{-1} KOH solution)
5ml of 1.0mol l^{-1} KCl
15ml of analytical grade water

A titration was performed with 0.1004mol l^{-1} KOH solution.

experimental run	log k_1	log k_2
1	6.68	4.68
2	7.34	4.08
3	7.42	3.70
4	7.39	3.51
5	7.50	3.67
6	7.21	4.20
average value	7.26 ± 0.15	3.97 ± 0.15

Table 2.9: Europium - citric acid stability constants

As with nickel, citric acid forms strong complexes with europium as shown in the above table. Its ability to form such complexes will inhibit formation of other europium complexes in groundwaters providing the citric acid is in sufficient concentration.

2.6.7 Nickel - salicylic acid system

The carbonate concentration was calculated has 0.5%.

Determination of protonation constants for salicylic acid

The following solution was used in this investigation:

20ml of 0.0101 mol l^{-1} salicylic acid
(standardized against 0.105 mol l^{-1} KOH solution)
5ml of 1.0 mol l^{-1} KCl
25ml of analytical grade water

The solution was titrated against 0.105mol l^{-1} KOH solution. Results for these experiments are shown below in table 2.10.

experimental run	k_{H1}	k_{H2}
1	13.58	2.64
2	13.32	2.67
3	13.56	2.66
4	13.10	2.65
5	13.38	2.67
6	13.44	2.65
average value	13.40 ± 0.24	2.66 ± 0.02

Table 2.10: Protonation constants for salicylic acid

Stability constants for nickel - salicylic acid

In the reaction cell the following solution was made up:

20ml of 0.0103mol l^{-1} salicylic acid
(standardized against 0.09948mol l^{-1} KOH solution)
5ml of 1.0mol l^{-1} KCl
10ml of 0.01021mol l^{-1} nickel (II) nitrate
15ml of analytical grade water

A titration was peformed with 0.09948mol l^{-1} KOH solution. Results are shown below in table 2.11.

experimental run	$\log k_1$	$\log k_2$
1	7.55	5.48
2	7.12	4.72
3	8.45	4.75
4	7.47	4.76
5	6.91	4.80
6	7.05	5.66
average value	7.43 ± 0.77	5.03 ± 0.47

Table 2.11: Nickel - salicylic acid stability constants

Salicylic acid was chosen for investigation as it is proposed that salicylate groups are involved in the complexation of metal ions with humic substances. From table 2.11 it can be seen that the salicylate ion forms strong complexes with nickel. Such values show the high affinity metal ions have for humic substances. Of interest in this study is the high first protonation constant for salicylic acid, which doesn't allow for the formation of the fully deprotonated salicylate ion until high pH.

2.6.8 Europium - salicylic acid system

The carbonate concentration was calculated as 1.9%.

Determination of the protonation constants for salicylic acid

See section 2.6.7.

Stability constants of europium - salicylic acid system

In the reaction cell the following solution was made up:

20ml of 0.0101mol l^{-1} salicylic acid
(standardized against 0.105mol l^{-1} KOH)
5ml of 1.0mol l^{-1} KCl
10ml of 0.01017mol l^{-1} europium (III) chloride
15ml of analytical grade water

A titration was performed with 0.105mol l^{-1} KOH solution. Results are shown below in table 2.12.

experimental run	log k_{MHL}	log k_{MH2L}
1	2.55	1.27
2	2.68	1.31
3	2.64	1.32
4	2.66	1.27
5	2.74	1.38
6	2.79	1.35
average value	2.68 ± 0.12	1.32 ± 0.05

Table 2.12: Europium - salicylic acid stability constants

Due to the high first protonation constant the formation of europium complexes with the fully dissociated salicylate ion did not occur, with europium carbonate and europium hydroxide complexes being formed instead. At nuetral and acidic pHs europium forms weak complexes with protonated salicylic acid.

2.6.9 Nickel - phthalic acid system

The carbonate concentration was calculated as 0.7%

Determination of the protonation constants for phthalic acid

The following reaction solution was used in this investigation:

20ml of 0.0173mol l^{-1} phthalic acid
(dissolved in 6ml of 0.1mol l^{-1} volumetric HCl).
5ml of 1.0mol l^{-1} KCl
25ml of analytical grade water

The solution was titrated aginst 0.106mol l^{-1} KOH solution. Initially, 1ml increments of base were added and the pH noted. Near the inflection point, 0.1ml increments of base were added. The results from these experiments are shown below in table 2.13.

experimental run	k_{H1}	k_{H2}
1	4.89	3.09
2	4.85	3.04
3	4.83	3.01
4	4.90	3.12
5	4.50	2.63
6	4.56	3.02
average value	4.75 ± 0.20	2.98 ± 0.25

Table 2.13: Phthalic acid protonation constants

Determination of stability constants for nickel - phthalic acid

The following solution was used in this investigation:

20ml of 0.01730mol l^{-1} phthalic acid
(dissolved in 6ml of 0.1mol l^{-1} volumetric HCl)
5ml of 1.0mol l^{-1} KCl
10ml of 0.0102mol l^{-1} nickel (II) nitrate
15ml of analytical grade water

Initially 1ml increments of 0.106mol l^{-1} KOH were added and pH readings were recorded. Near the inflection point, 0.1ml increments of base solution \were added. Results are shown in table 2.14.

experimental run	log k_1
1	2.44
2	2.67
3	2.59
4	2.70
5	2.72
6	2.33
average value	2.57 ± 0.20

Table 2.14: Nickel - phthalic acid stability constants

As with salicylic acid, phthalic acid was chosen as an analogue to the complexation group that occur on a humic substance. With nickel, phthalic acid forms weak complexes, as can be seen in table 2.14. With reference to chapter one and the idea of 'strong' and 'weak' sites on a humic molecule it is clear that phthalate groups represent the 'weak' sites on the humic molecule, with salicylate groups representing the 'strong' sites.

2.6.10 Europium - phthalic acid system

The carbonate concentration was calculated as 0.8%

Determination of the protonation constant for phthalic acid

See section 2.5.9

Determination of stability constants for europium - phthalic acid system

In the reaction cell the following solution was made up:

20ml of 0.01017M phthalic acid (dissolved in 6ml of 0.1M HCl)
5ml of 1.0M KCl
10ml of 0.01016M europium (III) chloride
15ml of analytical grade water

Initially, 1ml increments of 0.09948M KOH were added and pH readings were recorded. Near the inflection point, 0.1ml of increments were added. Results are shown below in table 2.15.

experimental run	$\log k_1$
1	4.52
2	4.06
3	3.93
4	4.19
5	4.26
6	3.66
average value	4.11 ± 0.43

Table 2.15: Europium - phthalic acid stability constants

As with the nickel case, europium forms relatively weak complexes with phthalic acid.

2.7 Summary and Conclusions

(1) Protonation constants

	experimental values	literature values
acetic	$\log k_{H1} = 4.56$	$\log k_{H1} = 4.56$
lactic	$\log k_{H1} = 3.63$	$\log k_{H1} = 3.66$
citric	$\log k_{H1} = 5.71$ $\log k_{H2} = 4.22$ $\log k_{H3} = 2.89$	$\log k_{H1} = 5.70$ $\log k_{H2} = 4.35$ $\log k_{H3} = 2.90$
salicylic	$\log k_{H1} = 13.40$ $\log k_{H2} = 2.66$	$\log k_{H1} = 13.35$ $\log k_{H2} = 2.81$
phthalic	$\log k_{H1} = 4.75$ $\log k_{H2} = 2.98$	$\log k_{H1} = 4.93$ $\log k_{H2} = 2.75$

Table 2.15: Protonation constants for organic acids

The experimentally derived protonation constants are in good agreement with critically assessed literature values. All literature values are at the same ionic strength as the experimental values.

The acids represent a number of low molecular weight organic acids. Acetic acid with its one protonation constant, is representative of monocarboxylic acids such as formic and butanoic acids. In contrast, citric acid has three proton-bearing groups and is a hydroxocarboxylic acid.

Saliclylic and phthalic acids were studied to mimic functional groups on humic acids. The large second protonation constant of salicylic acid indicates that the acid only becomes fully dissociated at high pH.

(2) **Stability constants - nickel**

	experimental values	literature values
acetic	$\log k_1 = 0.79$ $\log k_2 = 0.36$	$\log k_1 = 0.74$ $\log k_2 = 0.36$
lactic	$\log k_1 = 1.54$ $\log k_2 = 0.85$	$\log k_1 = 1.79$ $\log k_2 = 0.99$
citric	$\log k_1 = 5.19$ $\log k_2 = 2.84$	$\log k_1 = 5.25$ $\log k_2 = 2.84$
phthalic	$\log k_1 = 2.57$	$\log k_1 = 2.17$
salicylic	$\log k_1 = 7.43$ $\log k_2 = 5.03$	$\log k_1 = 6.95$ $\log k_2 = 4.75$

Overall, experimental derived results show agreement with literature values. All literature values are at the same ionic strength as the experimental values.

The weak complexing ability of acetic acid is shown by its stability constants with nickel. In order for any appreciable amount of complexation to be observed the acetic acid concentration had to be far in excess of the nickel concentration. In contrast, citric acid due to its greater electrostatic attraction forms a strong complex with nickel.

Salicylic acid forms far stronger complexes than phthalic acid and this is due to the hydroxyl group on the aromatic ring as opposed to the carboxylate group.

The good agreement between experimental and literature values was taken as validation of the experimental design and methodology

(2) **Stability constants - europium**

	experimental values	literature values
acetic	$\log k_1 = 2.09$ $\log k_2 = 1.24$ $\log k_3 = 0.30$	$\log k_1 = 1.94$ $\log k_2 = 1.53$ $\log k_3 = 0.60$
lactic	$\log k_1 = 3.00$ $\log k_2 = 2.36$ $\log k_3 = 1.40$	$\log k_1 = 3.0$ $\log k_2 = 2.3$ $\log k_3 = 1.4$
citric	$\log k_1 = 7.26$ $\log k_2 = 3.97$	$\log k_1 = 7.80$ $\log k_2 = 4.05$
phthalic	$\log k_1 = 4.11$	$\log k_1 = 3.6$
salicylic	$\log k_{MHiL} = 2.68$	$\log k_{MHiL} = 2.59$

There is good agreement between experimental and literature values. All literature values are at the same ionic strength as the experimental values. The results again highlight the strong complexation of citric acid, in contrast to the weak complexes formed by acetic acid.

Salicylic acid due to its high second protonation constant formed complexes of MH_xSal type in the system studied. At higher pH where the salicylic acid is fully dissociated, hydrolysis and carbonate reactions predominate for europium.

Such equilibrium constants provide data for modelling of radionuclide migration through groundwaters, and in particular Drigg leachate where a number of volatile organic acids have been identified.

2.8 Competing systems

The work involved investigating the complex equilibria involving two low molecular weight organic acids and selected metal ions, using potentiometric pH titrations. Experimental data obtained are used to calculate metal - ligand stability constants, and to determine metal speciation over a wide pH range, for the various complex equilibria studied. The systems that have been studied:

1. nickel/citric acid/lactic acid
2. nickel/acetic acid/lactic acid
3. nickel/salicylic acid/lactic acid
4. europium/citric acid/lactic acid
5. europium/citric acid/acetic acid

For competing ligand systems the following mass equations can be used to describe the overall equilibria of the system:

(1) Metal added to a mixture of ligands

If a metal, M is added to two ligands, L and L*, then:

$$M + L \rightleftharpoons ML \qquad K_L = \frac{[ML]}{[M]\,[L]}$$

and

$$M + L^* \rightleftharpoons ML^* \qquad K_L^* = \frac{[ML^*]}{[M]\,[L^*]}$$

Therefore, $2M + L + L^* \rightleftharpoons ML + ML^*$

Overall, $$K = \frac{[ML]\,[ML^*]}{[M]^2[L][L^*]}$$

Then, $$K = K_L \times K_L^*$$

(2) Ligand replacement

$$M + L \rightleftharpoons ML$$

and $$ML + L^* \rightleftharpoons ML^* + L$$

Therefore, $$K = \frac{[ML^*]\,[L]}{[ML]\,[L^*]}$$

Overall, $$K = \frac{K_L^*}{K_L}$$

(3) Mixed ligands

$$M + L \rightleftharpoons ML$$

and $$ML + L^* \rightleftharpoons MLL^*$$

Therefore, $$K = \frac{[MLL^*]}{[ML]\,[L^*]}$$

Overall, $$K = K^* \times K_L$$

Application of the above formula will derive overall equilibrium constants for a competing ligand system and which may subsequently be incorporated into geochemical speciation codes. For the competing ligand systems that follow, overall equilibrium constants are calculated.

2.8.1 Nickel/citric acid/lactic acid system

The carbonate concentration was calculated as 1.2%.

Determination of the stability constants for metal acid equilibria

In the reaction cell the following solution was made up:

10ml of 0.00989 mol l^{-1} citric acid
(standardised against 0.1021 mol l^{-1} KOH solution)
30ml of 0.00925 mol l^{-1} lactic acid
(standardised against 0.1021 mol l^{-1} KOH solution)
5ml of 1.0 mol l^{-1} KCl
10ml of 0.01022 mol l^{-1} nickel (II) nitrate

A titration was performed with 0.1031 mol l^{-1} KOH solution.

Results

		log k
1.	$M + L \rightleftharpoons ML$	5.17
2.	$M + L^* \rightleftharpoons ML^*$	1.58
3.	$ML^* + L^* \rightleftharpoons ML_2^*$	1.31

where: M - nickel (II) ion
L - citrate ion
L* - lactate ion

The above stability constant values are similar to values obtained from the simple system containing one metal ion and one acid ligand [52], and also with reported literature values [53]. A speciation plot and table of the system are shown in figure 2.5 and table 2.18.

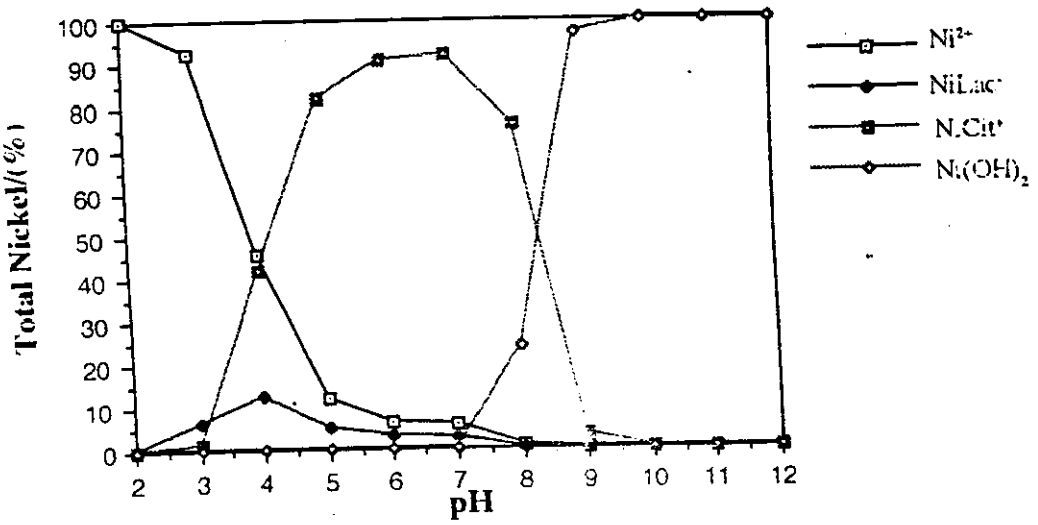


Figure 2.5: Speciation plot of nickel/citric acid/lactic acid system

pH	Ni^{2+}	$NiCit^-$	$NiLac^+$	$Ni(OH)_2$
2.0	100.0	0.0	0.0	0.0
3.0	95.8	1.0	3.2	0.0
4.0	52.0	40.5	7.2	0.0
5.0	15.0	81.7	3.1	0.0
6.0	7.5	90.8	1.6	0.0
7.0	6.3	92.2	1.4	0.0
8.0	3.1	82.0	0.0	14.9
9.0	0.3	45.0	0.0	54.7
10.0	0.0	10.2	0.0	89.8
11.0	0.0	0.3	0.0	99.7

Table 2.18: Species table of nickel/(%) in nickel/citric acid/lactic acid system

At the starting pH, the majority of the nickel was present as the free aqua ion. With increasing pH more of the citric acid became deprotonated, and hence more citrate ion was formed, to complex with the nickel. Due to the far greater stability of the nickel - citrate complex relative to the lactate complex, virtually all of the nickel was bound to the citrate rather than the lactate ion. From the pH range 5.0 - 8.0, over 80% of the metal

was bound to the citrate ion. This complex inhibited the formation of hydroxo complexes. Little $NiLac^+$ species were formed.

As the pH became progressively more alkaline, the nickel precipitated out as the hydroxide species. At pH 11 virtually all the total nickel concentration precipitated out of solution.

Addition of the nickel to the two organic acids can be speciated by an overall equilibrium constant, **K**, as outlined in section 2.8.

Therefore the overall **K** for the addition of a metal ion to a mixture of two ligands:

experimental values:

$$\mathbf{K} = K_1 \times K_2$$

$$\log \mathbf{K} = 5.17 + 1.58$$

$$\log \mathbf{K} = 6.75$$

Thus the above overall constant can be used to speciate the equilibria between the metal ion and the two organic acids.

2.8.2 Nickel/acetic acid/lactic acid system

The carbonate concentration was calculated as 1.2%.

Determination of the stability constants for metal acid equilibria

In the reaction cell the following solution was made up:

20ml of 0.01025mol l^{-1} acetic acid
(standardised against 0.103mol l^{-1} KOH solution)
20ml of 0.00925mol l^{-1} lactic acid
(standardized against 0.103mol l^{-1} KOH solution)
5ml of 1.0mol l^{-1} KCl
10ml of 0.01022mol l^{-1} nickel (II) nitrate

A titration was performed with 0.103mol l^{-1} KOH solution.

Results

		$\log \mathbf{k}_1$
1.	$M + L \rightleftharpoons ML$	0.68
2.	$M + L^* \rightleftharpoons ML^*$	1.50

where M - nickel (II) ion.
L - acetate ion.
L* - lactate ion.

There is good agreemnt between stability constants calculated from the above system and the single metal ligand systems. This suggests the lack of any synergistic effect in the above system, and that such systems can be speciated using individual metal ligand stability constants.

A speciation plot and table of the system are shown in figure 2.6 and table 2.19.

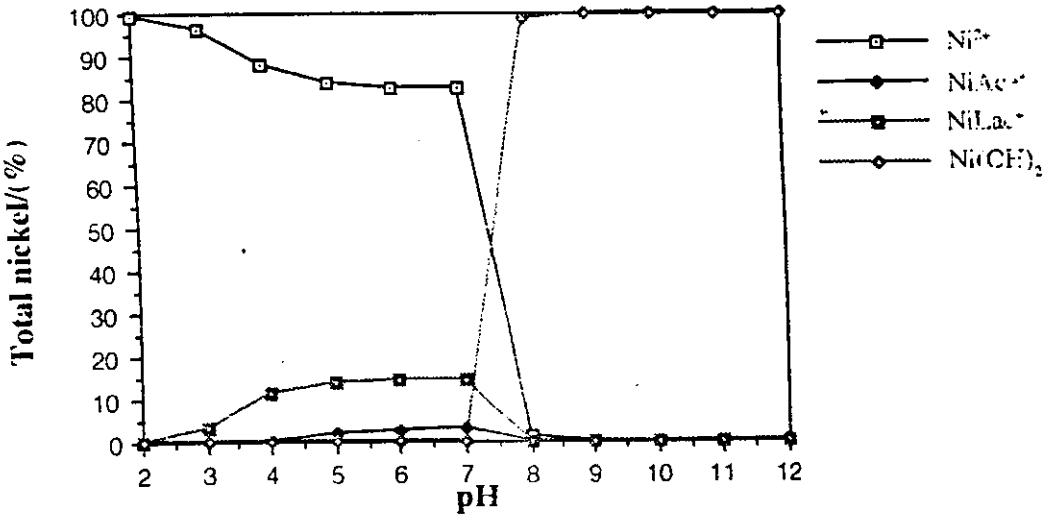


Figure 2.6: Speciation plot of nickel/acetic acid/lactic acid system

pH	Ni^{2+}	$NiAce^{+}$	$NiLac^{+}$	$Ni(OH)_2$
2,0	100.0	0.0	0.0	0.0
3.0	97.2	0.4	2.6	0.0
4.0	89.9	1.3	9.3	0.0
5.0	85.8	1.7	12.1	0.0
6.0	85.0	0.4	12.6	0.0
7.0	19.0	0.0	3.0	76.8
8.0	1.9	0.0	1.9	97.8
9.0	0.2	0.0	0.0	99.8
10.0	0.0	0.0	0.0	100.0
11.0	0.0	0.0	0.0	100.0

Table 2.19: Species table of nickel/(%) in nickel/acetic acid/lactic acid system

Due to the low stability constant of nickel acetate, a negligible amount of the complex was formed in the system. Lactic acid complexed with approximately 10% of the free nickel within the pH range 4.0 - 6.0. However, the majority of the total nickel remained as the free aqua ion until pH 7.0, when it formed the nickel hydroxide, and precipitated out of solution.

Again, for the addition of a metal to the two ligands an overall constant for the equilibria can be calculated.

experimental values: $\mathbf{K} = K_1 \times K_2$

$\log \mathbf{K} = 0.68 + 1.50$

$\log \mathbf{K} = 2.18$

2.8.3 Nickel/salicylic acid/lactic acid system

The carbonate concetration was calculated as 1.1%.

Determination of the stability constants for metal acid equilibria

In the reaction cell the following solution was made up:

10ml of 0.0106mol l^{-1} salicylic acid
(standardised against 0.1022mol l^{-1} KOH solution)
20ml of 0.00925mol l^{-1} lactic acid
(standardised against 0.1022mol l^{-1} KOH solution)
5ml of 1.0mol l^{-1} KCl
10ml of 0.01022mol l^{-1} nickel (II) nitrate

A titration was performed with 0.1022mol l^{-1} KOH solution.

Results

		log k
1.	$M + L \rightleftharpoons ML$	7.00
2.	$M + L^* \rightleftharpoons ML^*$	1.69
3.	$ML^* + L^* \rightleftharpoons ML_2^*$	1.12

where M - nickel (II) ion
L - salicylate ion
L*- lactate ion

As in the previous mixed systems, there is good agreement between the above stability contants and those determined in the single metal ligand systems, and with those quoted in the literature.

A speciation plot and table of the system is shown in figure 2.7 and table 2.20.

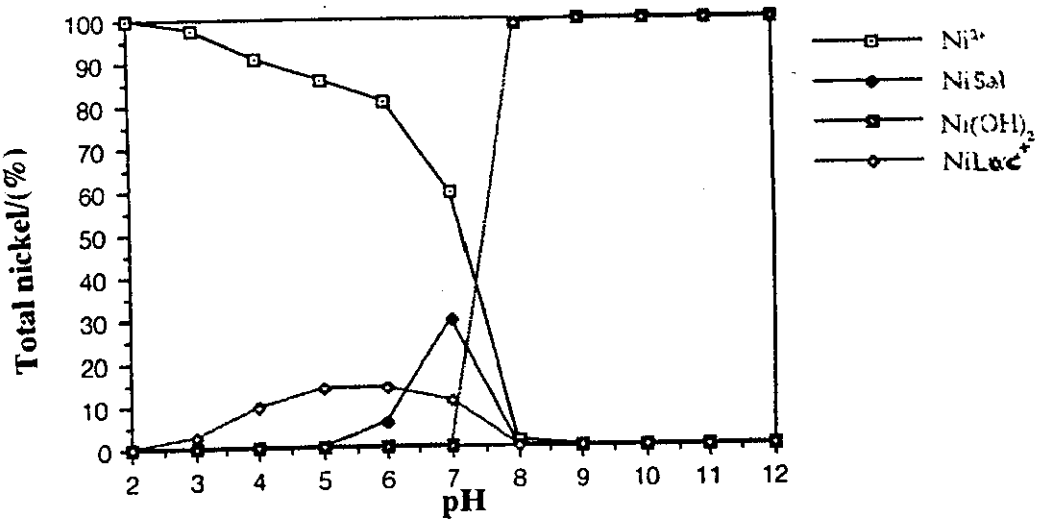


Figure 2.7: Speciation plot of nickel/salicylic acid/lactic acid system

pH	Ni^{2+}	NiSal	$NiLac^{+}$	$Ni(OH)_2$
2.0	100.0	0.0	0.0	0.0
3.0	97.5	0.0	2.5	0.0
4.0	90.6	0.0	9.4	0.0
5.0	87.1	0.8	10.1	0.0
6.0	86.5	4.5	9.0	0.0
7.0	57.9	33.5	8.6	0.0
8.0	2.1	0.3	0.0	97.6
9.0	0.0	0.0	0.0	100.0
10.0	0.0	0.0	0.0	100.0
11.0	0.0	0.0	0.0	100.0

Table 2.20: Species table of nickel/(%) in nickel/salicylic acid/lactic acid system

Dissociation of the second proton of salicylic acid did not occur until high pH's. Hence, complexation of nickel to the salicylate ion did not occur until above pH 7. At acid pH's, the majority of the nickel remained as the free aqua ion. As seen in the previous system with a similar concentration of lactic acid, approximately 10% of the total nickel concentration was bound to the lactate ion. The presence of lactic acid in the mixture inhibited the formation of any complexes of the form MH_xSal.

For the system the overall constant was calculated:

experimental values: $\mathbf{K} = K_1 \times K_2$

$\log \mathbf{K} = 7.00 + 1.69$

$\log \mathbf{K} = 8.69$

As with the previous systems no synergistic effect was observed between the two organic acids.

2.8.4 Europium/citric acid/lactic acid system

The carbonate concentration was calculated as 0.5%.

Determination of the stability constants for metal acid equilibria

In the reaction cell the following solution was made up:

10ml of 0.00989mol l^{-1} citric acid
(standardised against 0.1201mol l^{-1} KOH solution)
40ml of 0.0820mol l^{-1} lactic acid
(standardised against 1.0257mol l^{-1} KOH solution)
5ml of 1.0mol l^{-1} KCl
10ml of 0.01011mol l^{-1} europium (III) chloride

A titration was performed with 1.0257mol l^{-1} KOH solution.

Results

		log
1.	$M + L \rightleftharpoons ML$	7.42
2.	$M + L^* \rightleftharpoons ML^*$	3.12
3.	$ML^* + L^* \rightleftharpoons ML_2^*$	2.36
4.	$ML_2^* + L^* \rightleftharpoons ML_3^*$	1.45

where: M - europium (III) ion
L - citrate ion
L*- lactate ion

A speciation plot and table of the system is shown in figure 2.8 and table 2.21.

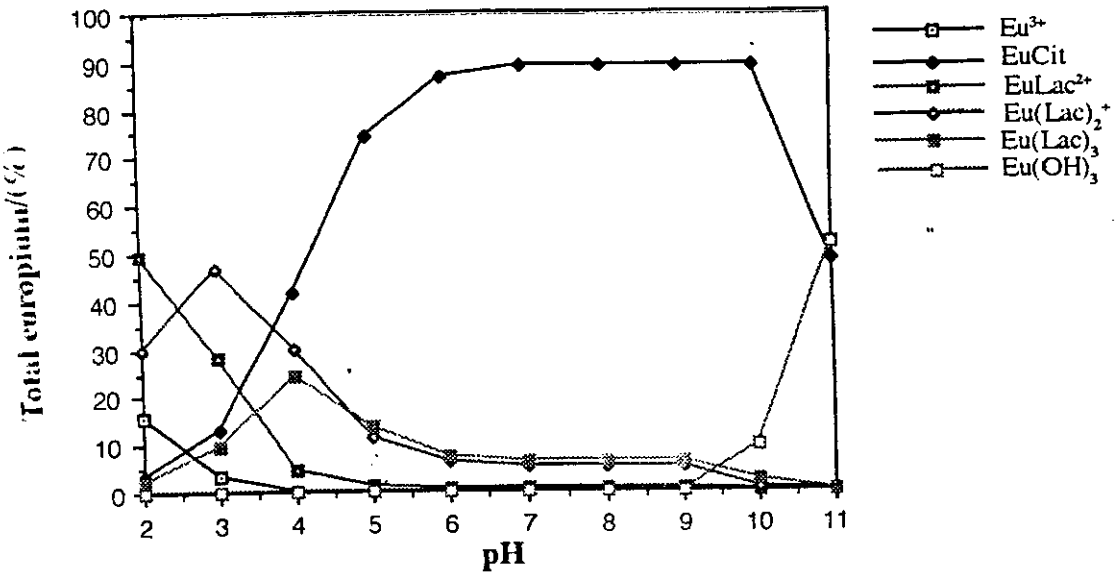


Figure 2.8: Speciation plot of europium/citric acid/lactic acid system

pH	Eu^{3+}	EuCit	$EuLac^{2+}$	$Eu(Lac)_2^+$	$Eu(Lac)_3$	$Eu(OH)_3$
2.0	15.5	3.6	49.2	29.6	2.2	0.0
3.0	3.1	12.9	28.0	46.4	9.6	0.0
4.0	0.0	41.4	4.5	29.7	24.3	0.0
5.0	0.0	74.2	1.2	11.3	13.4	0.0
6.0	0.0	86.4	0.6	5.9	7.4	0.0
7.0	0.0	88.5	0.5	4.9	6.1	0.0
8.0	0.0	88.7	0.4	4.8	6.1	0.0
9.0	0.0	88.8	0.0	4.8	6.1	0.0
10.0	0.0	88.6	0.0	0.5	2.1	9.8
11.0	0.0	48.4	0.0	0.0	0.1	51.1

Table 2.21: Species table of europium/(%) in europium/citric acid/lactic acid system

Even at low pH's , the majority of the euorpium was complexed. Due to the excess of lactic acid, the species EuLac, $Eu(Lac)_2$, $Eu(Lac)_3$ were all present in siginificant concentrations up to pH 5.0. From pH range 5.0 - 10.0, virtually all the europium was bound to the citrate ion. This EuCit complex inhibited the formation of any hydroxo complexes. This agrees with previous work done on the speciation of europium with citric acid [54].

With the excess lactate present, upon the addition of citrate a ligand displacement reaction occurs. The equilibria for this reaction can be calculated using an overall constant:

experimental values: $\mathbf{K} = K_1/K_2$

$\log \mathbf{K} = 7.42 - 3.12$

$\log \mathbf{K} = 4.30$

2.8.5 Europium/citric acid/acetic acid system

The carbonate concentration was calculated as 0.5%.

Determination of the stability constants for metal acid equilibria

In the reaction cell the following solution was made up:

10ml of 0.00989mo l^{-1} citric acid
(standardised against 0.1201mol l^{-1} KOH solution)
30ml of 0.09557mol l^{-1} acetic acid
(standardised against 1.0257mol l^{-1} KOH solution)
5ml of 1.0mol l^{-1} KCl
10ml of 0.01011mol l^{-1} europium (III) chloride

A titration was performed with 1.0257mol l^{-1} KOH solution.

Results

		log k
1.	$M + L \rightleftharpoons ML$	8.10
2.	$M + L^* \rightleftharpoons ML^*$	2.24
3.	$ML^* + L^* \rightleftharpoons ML_2^*$	0.82
4.	$ML_2^* + L^* \rightleftharpoons ML_3^*$	0.74

where: M - europium (III) ion
L - citrate ion
L*- acetate ion

A speciation plot and table of the system is shown in figure 2.9 and table 2.22.

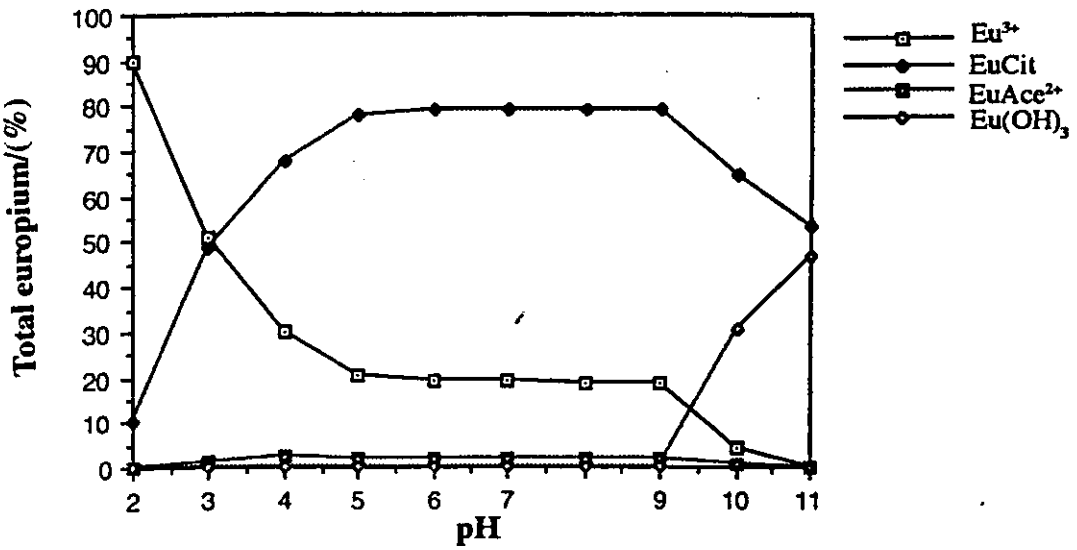


Figure 2.9: Speciation plot of europium/citric acid/acetic acid system

pH	Eu^{3+}	EuCit	$EuAce^{2+}$	$Eu(OH)_3$
2.0	89.7	10.1	0.1	0.0
3.0	50.6	48.4	0.9	0.0
4.0	30.2	67.6	2.2	0.0
5.0	20.2	77.8	1.9	0.0
6.0	19.0	79.1	1.9	0.0
7.0	18.9	79.2	1.9	0.0
8.0	18.8	79.2	1.9	0.0
9.0	18.8	79.2	1.9	0.0
10.0	4.0	64.8	0.4	30.8
11.0	0.2	53.2	0.0	46.6

Table 2.22: Species table of europium/(%) in europium/citric acid/lactic acid system

Over a large pH range, in excess of two thirds of the europium is bound to the citric acid. Since the stability constant of the europium citrate is far greater than europium acetate, very little of the europium acetate is formed. At higher pH's , hydrolysis takes place.

For the system an overall constant can be calculated.

experimental values:

$$\mathbf{K} = K_1 \times K_2$$

$$\log \mathbf{K} = 8.10 + 2.24$$

$$\log \mathbf{K} = 10.34$$

2.9 Summary

The experimental methodology and computational procedures have provided stability constant data that shows good agreement with literature values. The work has provided a collection of data which can provide a basis for subsequent studies involving low molecular weight organic acids and their role in toxic metal migration through the geosphere.

For the various complex equilibria that have been studied, the following conclusions can be reached:

1. Protonation constant values for acid and stability constant values for various metal - acid equilibria are in good agreement with critically assessed literature values at the same ionic strengths and temperatures.

2. These systems can be modelled using simple mass action equations. From these individual stability constants, an overall stability constant for each complex system can be calculated.

3. If the complexation between the metal and the organic acid is sufficiently stable, or if the concentration of the acid is sufficiently in excess then metal hydrolysis is suppressed.

4. No synergistic effect was observed in the competing ligand systems and the stability constants calculated were in agreement with constants from the individual metal ligand systems.

5. Overall equilibrium constants and individual constants can be incorporated into groundwtaer speciation models and assist in determining the speciation and likely migration of toxic metals.

2.10 Errors in the determination of stability constants

There are many errors associated in the determination of stability constants. Measurment errors include incorrect calibration of the p[H] meter-electrode system, faulty or drifting measurements, impure ligand and incorrect preparation, standardization of acid, base and/or metal salt solutions. Also electronically faulty meter and recording errors.

Calibration and electrode care is important. Calibration of the p[H] meter should be done on a regular basis. Manufacturers' recommendation for storage of the reference electrode should be practised. Careful observation should be made to ensure no crystallization of KCl in the opening that provides the liquid junction to the experimental solution, thus preventing normal conductivities between the electrodes performance and cause increasing drift.

A source of considerable errors results from inaccurate standardization of the reagents. For example, a small error in the concentration of a triprotonated ligand (eg: citric acid) would produce a three - fold error in the available concentration of the hydrogen ion. This may or may not dramatically effect the equilibrium constants from the major species present, but it could result in disproportionate errors in the constants calculated for minor solution species.

Several others precautions which should be noted are maintenance of an inert atmosphere, careful temperature control, ionic strength and that the supporting electrolyte should be inert. Temperature control is necessary since electrode response for a given concentration of $[H^+]$ is a function of temperature. Also the chemical equilibrium is a function of temperature, and so the temperature should remain constant and be frequently monitored.

CHAPTER THREE

Investigations into metal - humic acid binding

3 INTRODUCTION

As stated in chapter one, numerous studies have been conducted on the interactions of metals with humic substances. Unfortunately, constants produced vary by several orders of magnitude for a particular metal ion. This can be due to a number of factors, including choice of analytical technique, incorrect interpretation of the data and over-extrapolation of measured parameters.

As a consequence, the aim of this study was to investigate a number of important factors:

1. A quantitative determination of two experimental techniques, namely high pressure size exclusion chromatography and ion exchange. To determine the applicability of the techniques for the studying of metal humic substance systems.

2. To determine the speciation of europium and nickel with humic acid, as a function of the metal concentration.

3. To study the binding behaviour of such complexes and to experimentally investigate the theories of non-localised and localised binding of metals to humic substances.

4. To critically evaluate the data and the results that the techniques produce and to offer future recommendations for their use.

Initial studies were concerned with the application of a high pressure size exclusion chromatography study of metals with humic acids. From these studies ion exchange techniques were then applied to investigate factors such as pH and dissociation.

3.1 High Performance Size Exclusion Chromatography (HPSEC)

An investigation was undertaken to study metal - humic acid interactions using a high pressure size exclusion chromatographic technique (HPSEC). Previous work had shown that the technique can easily separate the bound (eg europium humate) and free metal (eg europium) [55]. However, results from the technique sometimes tend to under estimate the amount of bound metal compared to other techniques (eg ion exchange) and more importantly, the amount of bound metal predicted by computer models [56].

Two potential problems of the HPSEC method could be dissociation of the metal humate complex and sorption within the HPSEC system. As the bound and free metal are separated down the chromatographic column, in order to restore the equilibrium dissociation of the metal - humate complex will occur according to Le Chatelier's principle. To investigate dissociation, identical samples were injected at two different flow rates and the amounts of bound and free metal eluted from the columns compared. To investigate the possibility of sorption, the activities of injected samples were measured in the absence and presence of the column.

The complexation of both europium and nickel with Aldrich sodium humate was investigated in the study.

The instrumentation used for high pressure size exclusion chromatography is shown schematically in figure 3.1. The instrumentation consists of a Philips PU 4000 Series Liquid Chromatograph, fitted with a PU 4100 Gradient Pumping System, a PU 4021 Diode Array Detector (DAD), a Rheodyne injection valve with a 100µl sample injection loop. The DAD and Radiometric series A-140 detector are controlled by PU 6003 and PU 6000 integration and control software. All injected samples were filtered through 0.45µm Acro discs prior to injection. All mobile phases were filtered through 0.45µm filter discs. The columns used were a TSK SWX3000L guard column and a TSK GW3000 analytical columns.

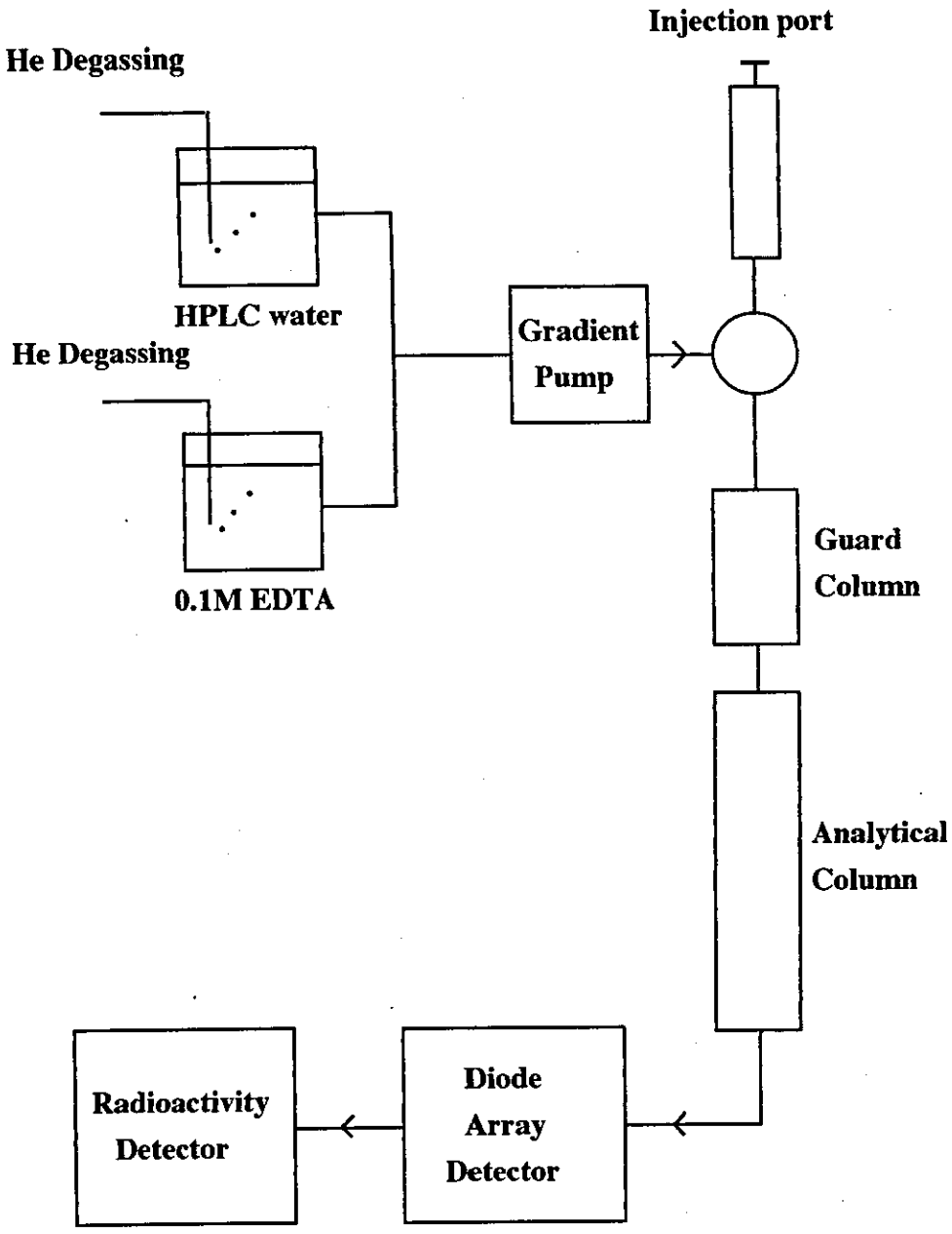


Figure 3.1: Schematic diagram of the High Pressure Size Exclusion Chromatographic apparatus used in europium - Aldrich sodium humate experiments.

3.2 Preliminary europium - Aldrich sodium humate experimental work

Stock solutions - preparation

A solution of 100ppm (weight by volume, w.v) Aldrich sodium humate was prepared as a stock solution. To this solution 0.0585g analar sodium chloride was added to make the ionic strength 0.01M.

20µl of ^{152}Eu (from Amersham stock solution containing 33.4 MBq ml^{-1} and 0.07 mg ml^{-1}) was placed into 5ml of HPLC water to make a stock solution of approximate activity of 1MBq/ml. From this stock solution, 1ml was added to a 10ml sample of the humic solution. From this solution 1ml was added to 10ml HPLC water and 100µl samples of this solution were injected onto the HPSEC.All europium - humic acid solutions were left for five days, since this was sufficient for the solutions to reach equilibrium [57].

Investigation of dissociation of metal-humate complexes

To investigate dissociation, the same sample was injected six times at two different flow rates of .50ml/min and .25ml/min. The pH of the sample was 6.0. The conditions of the column and detector for the .50ml/min set of experiments are summarised below:

Flow rate	- .50ml/min
Pressure	~ 38 bars
Mobile phase	- HPLC water/0.1mol l^{-1} EDTA
UV absorption wavelength	- 254nm

Concentration of sample:

aldrich sodium humate	10ppm (w.v)
europium	1.390 x 10^{-7} mol l^{-1}

The following stepped elution and injection scheme was used to achieve separation of the humic bound europium and the 'free' europium through the HPSEC system.

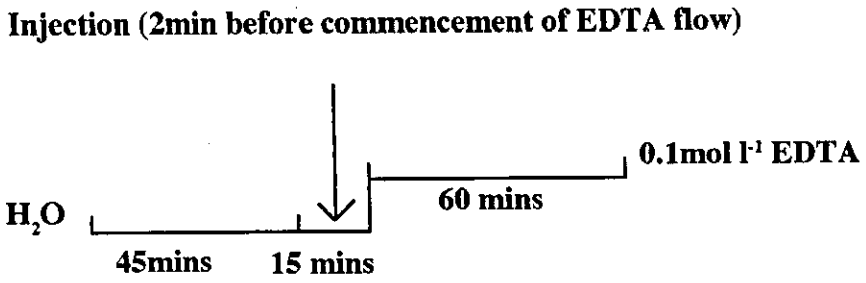


Figure 3.2: Stepped elution profile used in europium complexation study at flow rate .500ml/min

The scheme employed in this europium complexation study has been successfully used in a previous investigation [58]. Water was chosen as the initial mobile phase to specifically enhance sorption, thereby allowing separation of the humic bound europium from the 'free' europium. Since water is used as the mobile phase the gyration radius of the humic acid will increase and hence will elute earlier [23]. A mobile phase of 0.1mol l^{-1} EDTA was used as a second mobile phase to elute the 'free' europium.

Figure 3.3 shows a typical DAD chromatogram and illustrates how the EDTA front reaches the UV detector immediately after the europium - humate complex.

Figure 3.4 shows a typical radiometric chromatogram highlighting the bound and free europium.

To determine the amount of europium bound the classical cut and weigh technique, as shown in figure 3.5 was used. Since the peaks are non-Gaussian (due to the heterogenity and molecular size fractions of the humic acid) and a 'tailing effect' is observed, integrated peak areas will overestimate the amount of free europium.

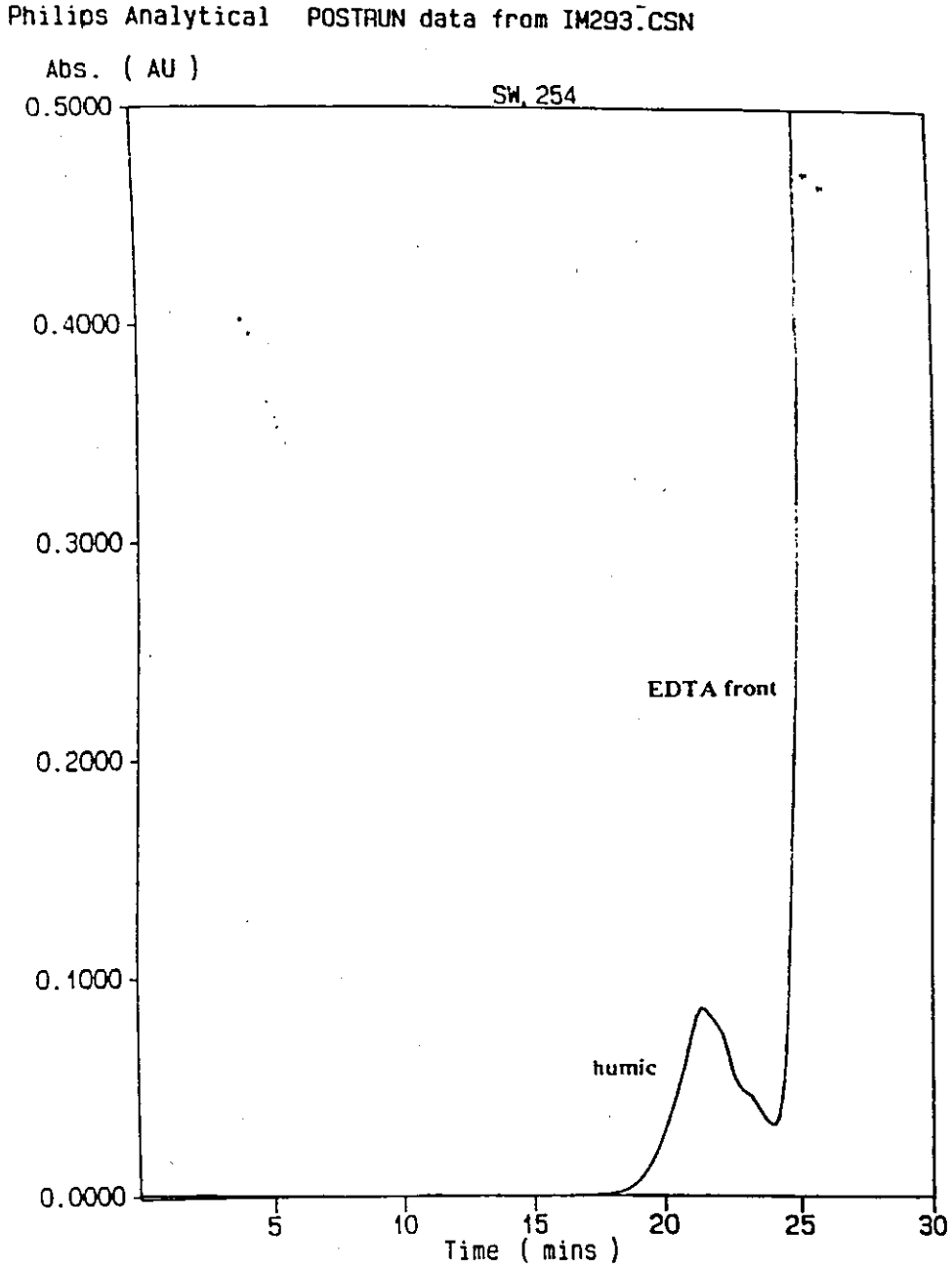


Figure 3.3: DAD chromatogram at 254nm

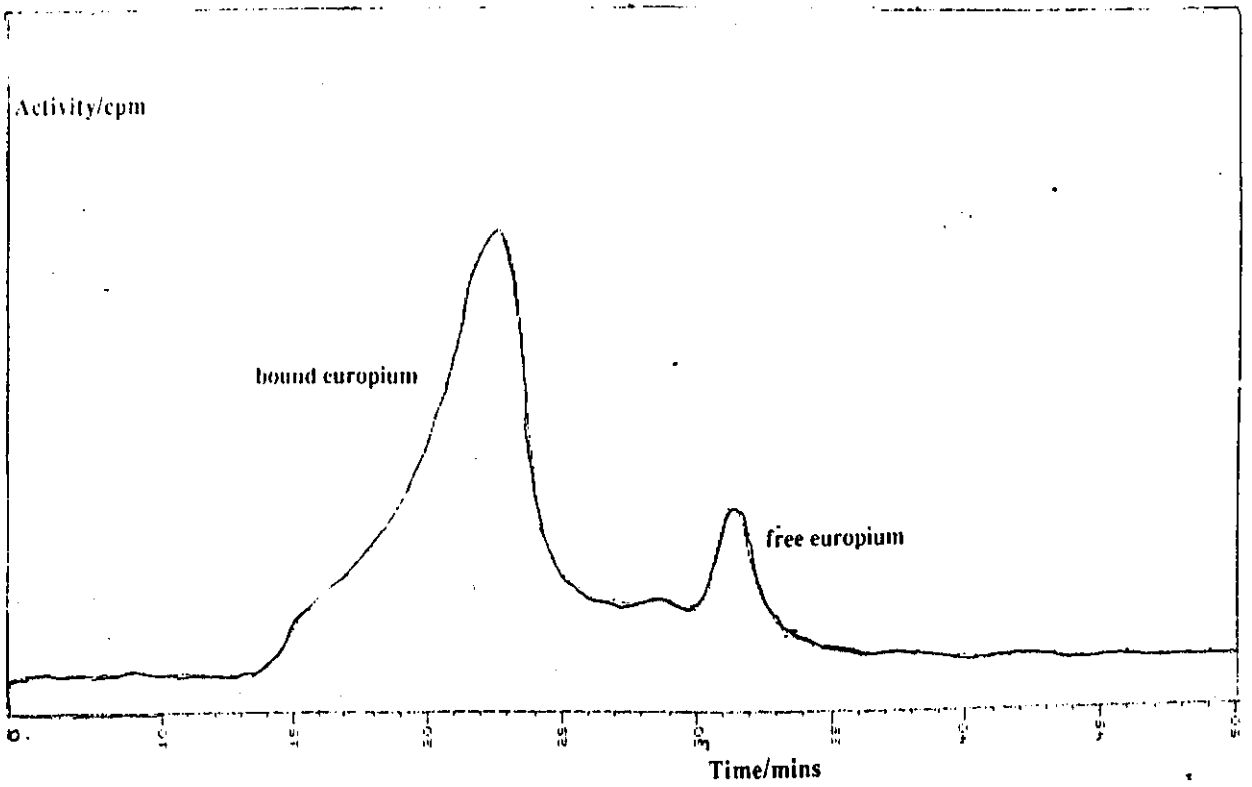


Figure 3.4: Radiochromatogram of europium

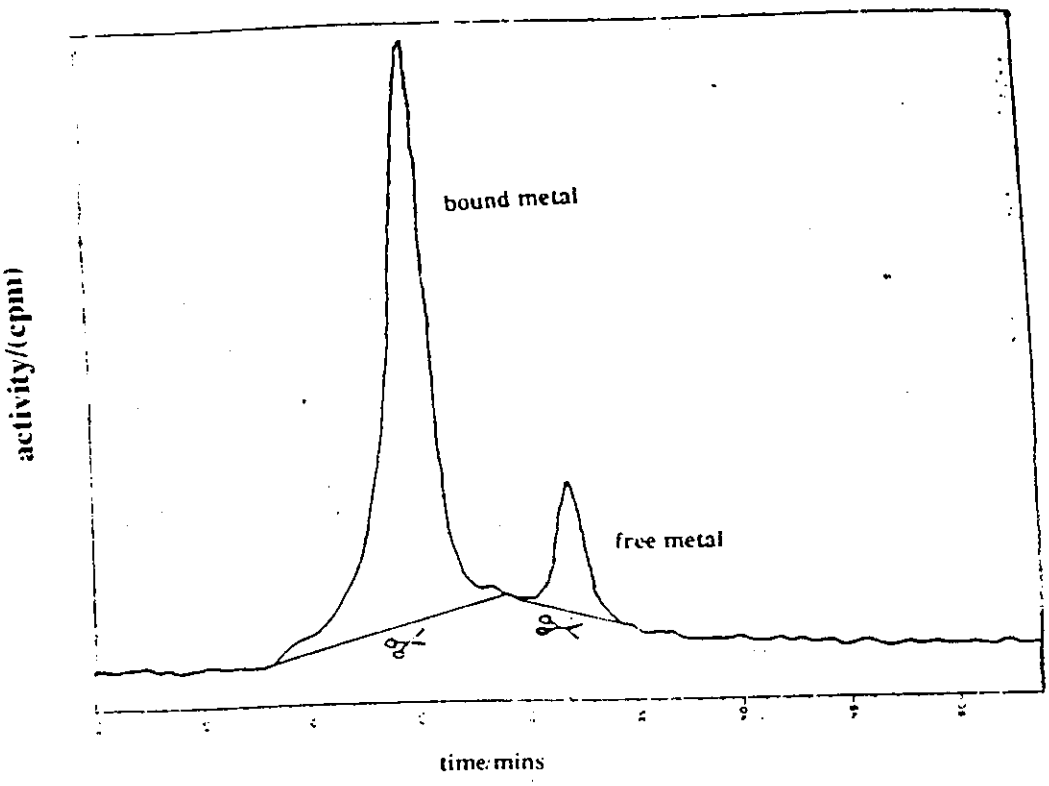


Figure 3.5: The cut and weigh technique

The results from the two different flow rates are shown in tables 3.1 and 3.2. The results were obtained by employing the previously mentioned classical cut and weigh technique. It is generally accepted that such a technique as an associated error of 2% on the measured parameters.

Run number	Area of Eu-AHA complex peak/%	Eu-free peak/%
1	89.9	10.1
2	93.3	6.7
3	85.2	14.8
4	89.3	10.7
5	88.0	12.0
6	94.3	5.7
Average of Eu-AHA complex = 90.0 ± 4.5		

Table 3.1: Europium - Aldrich sodium humate results at .50ml/min flow rate

Run number	Area of Eu-AHA complex peak/%	Eu-free peak/%
1	93.3	6.7
2	86.9	13.1
3	83.3	16.7
4	84.2	15.8
5	87.2	12.8
6	92.2	7.8
Average of Eu-AHA complex = 87.9 ± 5.0		

Table 3.2: Europium - Aldrich sodium humate results at .25ml/min flow rate

From the above tables it was concluded that the change in flow rate had no effect on the europium - Aldrich sodium humate equilibria within experimental error.

Investigation of sorption of europium-humate and europium

Sorption experiments were initially carried out in the absence and presence of the chromatographic size exclusion columns and with HPLC grade water as the mobile phase. Consequentally, a significant amount of sorption of free europium occurred within the columns. This highlights the need for the use of EDTA to desorb the free europium.

To further investigate any sorption effects, the activities of samples were measured using the stepped elution method shown in figure 3.2, both in the absence and presence of the columns. The activity was collected by a Pharmarcia LKB Redifrac fraction collector and measured on a Philips PW4800 automatic gamma counter. For error analysis both sets of experiments were repeated in sextuplet. The results are shown in table 3.3 and table 3.4.

Run number	measured activity/cpm
1	131646
2	133569
3	129226
4	127485
5	125441
6	130171
average	129590 ± 4064

Table 3.3: Sorption study of europium -Aldrich sodium humate (without columns)

Run number	measured activity/cpm
1	118122
2	124150
3	109331
4	117474
5	128935
6	126134
average	120691 ± 5731

Table 3.4: Sorption study of europium - Aldrich sodium humate (with columns)

Results suggest that there is only slight sorption being observed and analysis of the data indicates this to be the free europium. This small loss in activity is negligible compared to the overall counts and is insignificant in the determination of metal speciation. Therefore the stepped elution scheme shown in figure 3.2 provides an excellent method for the separation of europium humate and free europium.

Conclusion

From the preliminary work on the europium - Aldrich sodium humate equilibria the following conclusions can be reached:

1. The amount of europium bound is approximately 90% of the total metal concentration. This agrees with ion exchange experiments performed on the same sample[56].

2. Varying the flow rate from .50ml/min to .25ml/min had no effect, within experimental error, on the amount of complex formed. Thus, dissociation of the europium - humate complex during the time spent in the chromatographic columns is insignificant.

3. Sorption effects are insignificant, as the major sorbing species is 'free' europium, which is subsequentally desorbed by the EDTA mobile phase. However, it is essential that elution schemes are sufficiently long to remove previous mobile phases.

4. HPSEC is a useful analytical technique for speciation of metal - humic equilibria, providing that the metal - humate complexes are of sufficient stability to pass through the column with minimal dissociation.

From the above conclusions it was decided that further investigations into the europium humic acid system could be carried out using HPSEC.

3.3 Nickel - Aldrich sodium humate experimental work

Table 3.5 shows samples covering a range of metal concentrations that had been prepared and used during a nickel - Aldrich sodium humate investigation using an ion exchange resin [56]. These samples were used on the HPSEC investigation in order to obtain a direct comparison between the two techniques. The apparatus was the same as that used in the europium complexation experiments, except SynChroPak GPC-60 guard (50 x 4.6 mm i.d.) and analytical (250 x 4.5 mm i.d.) size exclusion columns were used. The conditions of the column and the detector are summarised below:

Flow rate	- .23 ml/min
Pressure	~ 36 bars
Mobile phase	- 0.05mol l^{-1} NaCl/0.5mol l^{-1} NaCl
UV absorption wavelength	- 254nm

Each sample contained 60ppm (w.v) Aldrich sodium humate and 0.001mol l^{-1} 4-morpholineethanesulfonic acid monohydrate (MES) buffer to maintain a pH 6.9.

Sample	Volume AHA (ml)	Amount of $Ni(NO_3)_2$ solution added	Amount of Ni^{63} solution added (µl)
1	5.00	50µl of 1 x 10^{-1} mol l^{-1}	25
2	5.00	25µl of 1 x 10^{-1} mol l^{-1}	25
3	5.00	50µl of 1 x 10^{-2} mol l^{-1}	25
4	5.00	25µl of 1 x 10^{-2} mol l^{-1}	25
5	5.00	50µl of 1 x 10^{-3} mol l^{-1}	25
6	5.00	25µl of 1 x 10^{-3} mol l^{-1}	25
7	5.00	50µl of 1 x 10^{-4} mol l^{-1}	25
8	5.00	25µl of 1 x 10^{-4} mol l^{-1}	25
9	5.00	50µl of 1 x 10^{-5} mol l^{-1}	25
10	5.00	25µl of 1 x 10^{-5} mol l^{-1}	25

Table 3.5 - Nickel solutions used during the investigation

The following salt-gradient elution profile was used to achieve separation of the humic bound nickel and the 'free' nickel. This scheme was based on a previous study [58].

A = 0.05 M NaCl

B = 0.50 M NaCl

Flow rate = 0.230 ml min^{-1}

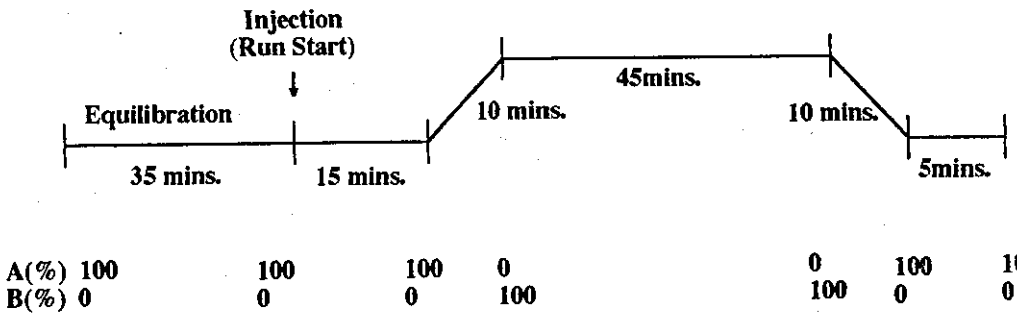


Figure 3.6: Salt-gradient profile used in Ni-AHA complexation study

Figure 3.7 shows a typical chromatogram. Results for the nickel - Aldrich sodium humate study are shown in table 3.6.

$[M]_t$ Total Ni concentration (mol l^{-1})	[ML] Ni-AHA complex (mol l^{-1})	[M] Ni-free (mol l^{-1})	$\frac{[ML]}{[M]}$	log [ML]	log [M]
9.805×10^{-4}	4.510×10^{-5}	9.354×10^{-4}	0.0482	-4.346	-3.029
4.927×10^{-4}	2.464×10^{-5}	4.681×10^{-4}	0.0526	-4.608	-3.330
9.811×10^{-5}	5.298×10^{-6}	9.282×10^{-5}	0.0571	-5.726	-4.032
4.955×10^{-5}	2.973×10^{-6}	4.658×10^{-5}	0.0638	-5.527	-4.332
9.889×10^{-6}	6.329×10^{-7}	9.256×10^{-6}	0.0684	-6.199	-5.034
4.988×10^{-6}	3.492×10^{-7}	4.639×10^{-6}	0.0753	-6.437	-5.334
1.022×10^{-6}	7.358×10^{-8}	9.484×10^{-7}	0.0776	-7.133	-6.023
5.325×10^{-7}	3.994×10^{-8}	4.926×10^{-7}	0.0811	-7.399	-6.308
1.357×10^{-7}	1.111×10^{-8}	1.246×10^{-7}	0.0892	-7.954	-6.904
8.692×10^{-8}	8.692×10^{-9}	7.823×10^{-8}	0.1111	-8.601	-7.106

Table 3.6: Nickel - Aldrich sodium humate complexation study results

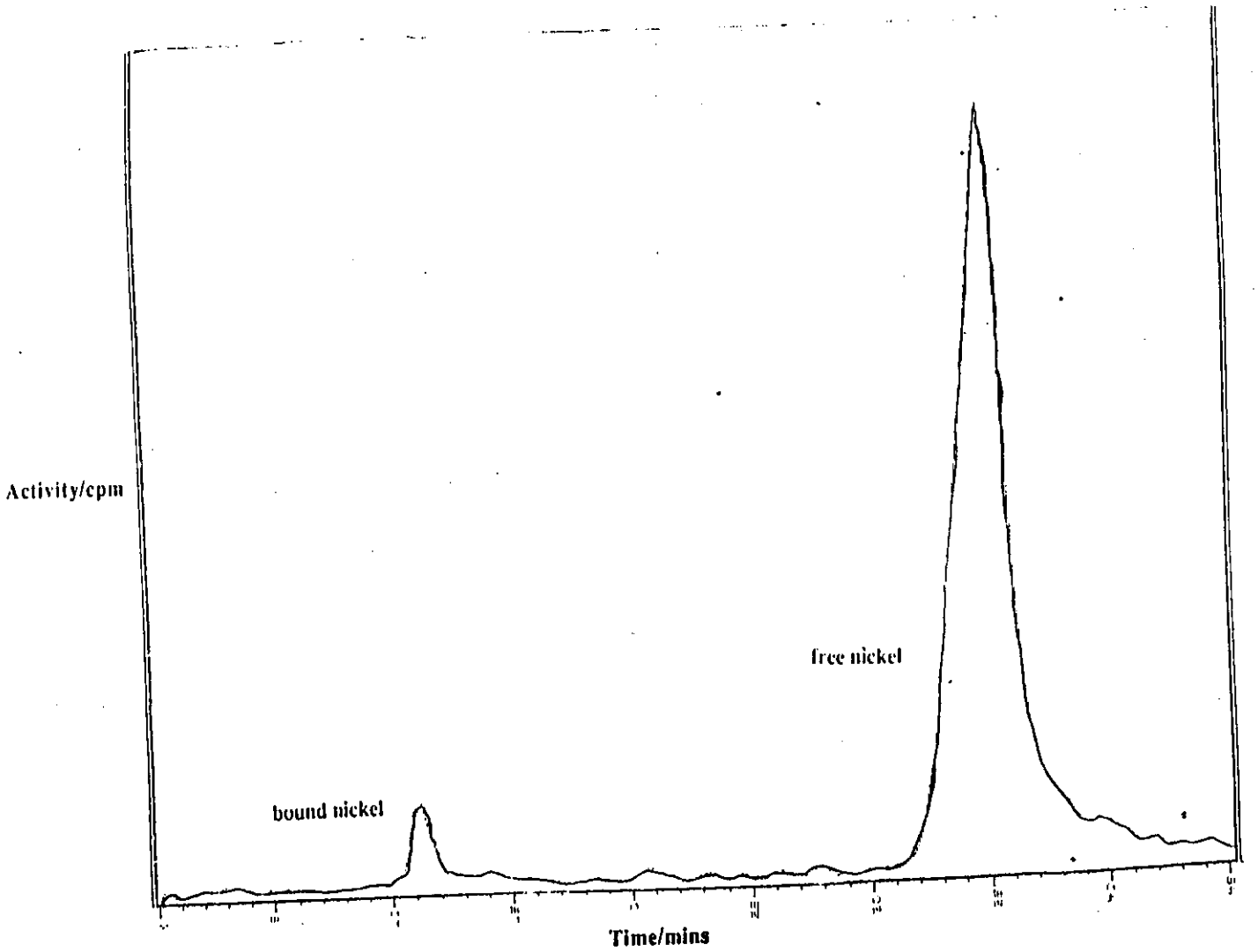


Figure 3.7: Radiochromatogram of nickel - aldrich sodium humate

From the HPSEC data stability constants for the nickel humic acid system were calculated utilising three approaches:

1. The strong and weak site Scatchard plot.
2. A Scatchard plot with an equilibrium constant for each order of metal concentration.
3. The differential equilibrium function (DEF).

Figures 3.8 and 3.9 show plots utilising the Scatchard and DEF methods.

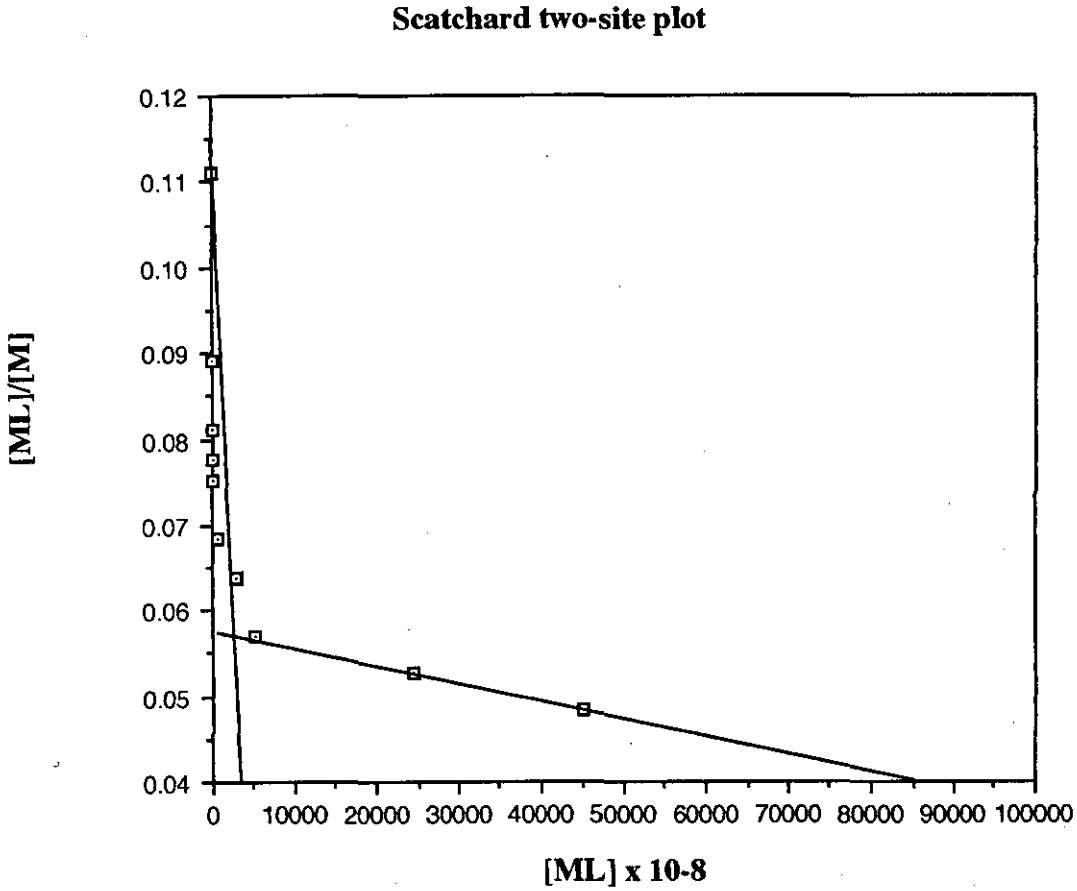


Figure 3.8: Scatchard plot of nickel - Aldrich sodium humate

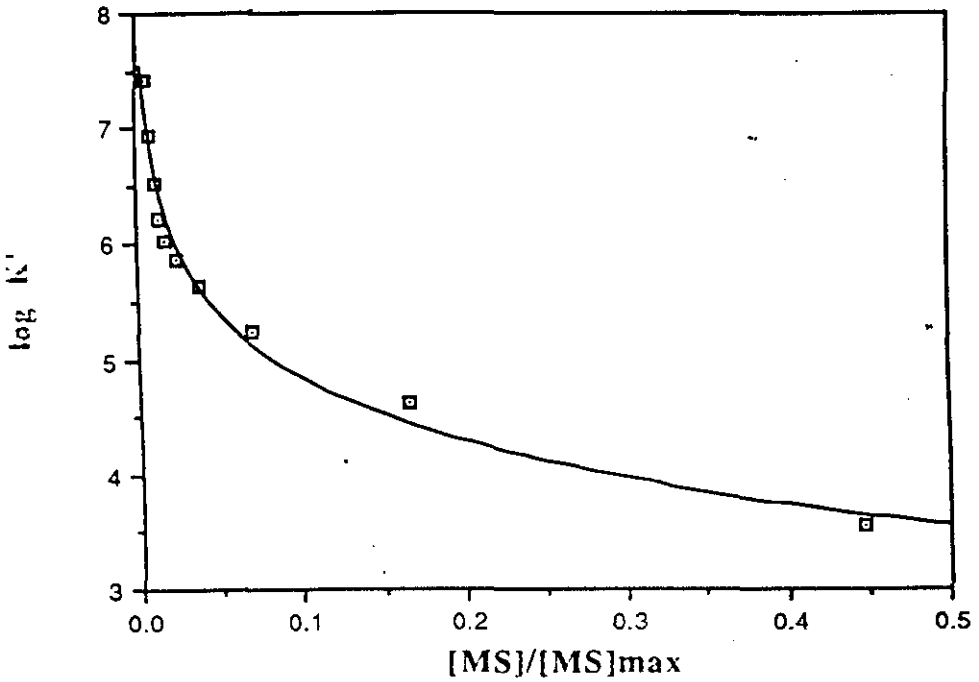


Figure 3.9: DEF plot of nickel - Aldrich sodium humate

Modelling approaches for determining metal - humic equilbrium constants

Three mathematical approaches have been used in this study of nickel with Aldrich sodium humate. The Scatchard plot [47] has often been used in biochemistry and aquatic chemistry as a means of evaluating stability constants. With metal - humic interactions the plot of [ML]/[M] vs [ML] yields a curve which can be convienently divided into two linear segments. From the slopes of these two linear segments stability constants can be calculated which correspond to metal interactions with humic sites that fall into two distinct categories, namely strong and weak. Criticism against this approach is that the above intrepretion of metal - humic interactions is over - simplified and that the two derived stability constants have no real physio-chemical significance. A modified Scatchard approach, whereby an equilibrium constant is calculated for each order of metal concentration attempts to overcome this criticism whilst still supplying data which can be easily incorporated into geochemical speciation codes. The differential equilibrium function [49] is a more rigorous interpretation of metal - humic interactions since it provides a contiuous distibution of equilibrium constants as a function of metal loading, thereby accounting for the inherent heterogenity of humic molecules.

Table 3.7 shows a comparision of the three mathematical approaches for the evaluation of the nickel - Aldrich sodium humate equilibrium constants.

order of metal concentration	Scatchard two site approach	Scatchard plot metal concentration	DEF
10^{-8}		6.96	7.40
10^{-7}	$\log {}^{s}K = 4.28$	5.49	6.51
10^{-6}		4.22	5.85
10^{-5}	$\log {}^{w}k = 1.64$	3.46	4.25
10^{-4}		2.33	3.42

Table 3.7: Comparison of approaches used to evaluate equilibrium constants

From the above table important conclusions can be drawn. The choice of interpretation of experimental data has a profound effect on the resulting stability constant. For a particular order of metal concentration in the nickel humic acid system the derived stability constant varies by at least a couple of orders of magnitude. For example at 10^{-7}, the two site approach produces a value of 4.28 as opposed to the continuous model which gives a value of 6.51. Clearly, with regards to geochemical speciation this will have siginificant consequences when attempting to model such systems, since for accurate prediction of species in solution the stability constants should be known within 0.1 log unit.

For reference, the above equilibrium constants are quoted with values from the literature in Appendix 1.

To attempt to overcome the problem of stability constant determination, it was decided to interpret the data in terms of the amount of the metal bound to the humic acid and of the free metal, and not to produce stability constants from experimental data. Using this approach the problems of mathematical interpretation and its significance are avoided. Where stability constants are calculated, then they are done so by the Scatchard plot. This allows for incorporation into modelling codes.

The amount of nickel bound determined by the HPSEC and ion exchange techniques are compared in table 3.8:

$[M]_t$ **Total Nickel concentration**	**[ML] Nickel bound/% HPSEC**	**[ML] Nickel bound /% Ion exchange**
9.805×10^{-4}	4.6	13.8
4.927×10^{-4}	5.0	11.1
9.811×10^{-5}	5.4	14.2
4.955×10^{-5}	6.0	21.2
9.889×10^{-6}	6.4	30.4
4.988×10^{-6}	7.0	44.0
1.022×10^{-6}	7.2	60.0
5.325×10^{-7}	7.5	79.2
1.357×10^{-7}	8.2	89.8
8.692×10^{-8}	10.0	90.0

Table 3.8: Comparison of nickel bound by HPSEC and Ion exchange

The amount of bound nickel determined by the HPSEC technique was considerably less than the amount determined by ion exchange. This possible under estimation of the amount of metal bound again may be due to sorption and/or dissociation of nickel - humate during the passage of the sample through the chromatographic column. The effect of sorption has been investigated [58] and was shown to be negligible.

To investigate dissociation, experiments were peformed at three flow rates on one Ni-AHA sample. The results are shown in table 3.9:

Flow rate ml/min	**% Nickel bound**	**% Nickel free**
.230	7.0	93.0
.500	12.4	87.6
.750	15.3	84.7

Table 3.9: Ni-AHA dissociation study experiments
($M_t = 1.357 \times 10^{-7}$ mol l^{-1})

The above results suggest that the nickel - humate complex is dissociating down the column, since at higher flow rates there is more nickel bound to the humic acid. This dissociation is far more signifcant in the nickel studies than in the europium experiments due to the Ni^{2+} being more labile than the Eu^{3+}.

Conclusions

From the experiments performed the following conclusions can be reached:

1. The high pressure size exclusion chromatographic technique provides a measure of the amount of metal bound to humic acid.

2. Results with europium are in agreement with ion exchange results and the effects of dissociation and sorption are negligible.

3. However, results with nickel are not in agreement and operating at different flow rates has shown that the nickel - humate complex may well undergo dissociation.

4. The data was interpretated by three techniques to evaluate stability constants. The interpretation of the data produces a wide range of values.

3.4 Europium - Aldrich sodium humate work

Using the HPSEC method described in section 3.2.1 a range of concentrations of analar grade europium (III) chloride solutions, spiked with radioactive Eu-152 (from Amersham stock solution containing 33.4 MBq ml^{-1} and 0.07mg ml^{-1}), were prepared in a background electrolyte of 0.01mol l^{-1} analar sodium chloride. To each of these solutions 10ppm (w.v) Aldrich sodium humate was added. The pH of the samples were adjusted to 6.0 by the dropwise addition of sodium hydroxide and/or hydrochloric acid. Conditions of the column and DAD detector are summarised below:

Flow rate	- .75 ml/min
Pressure	~ 48 bars
Mobile phase	- HPLC water/0.1mol l^{-1} EDTA
UV absorption wavelength	- 254nm

The stepped and injection scheme used to achieve separation of the organically bound europium and 'free' europium was as in the previous dissociation/sorption experiments. The eleunt was fraction collected. A radiochromatogram is shown in figure 3.7.

$[M]_t$ Total Eu concentration (mol l^{-1})	[ML] Eu-AHA complex (mol l^{-1})	[M] Eu-free (mol l^{-1})	$\frac{[ML]}{[M]}$	log [ML]	log [M]
1.41×10^{-8}	1.38×10^{-8}	0.30×10^{-9}	46.000	-7.860	-9.523
6.41×10^{-8}	6.09×10^{-8}	3.21×10^{-9}	18.972	-7.215	-8.494
1.14×10^{-7}	1.03×10^{-7}	1.10×10^{-8}	9.364	-6.987	-7.959
5.14×10^{-7}	4.38×10^{-7}	7.61×10^{-8}	5.756	-6.359	-7.118
1.00×10^{-6}	6.79×10^{-7}	3.21×10^{-7}	2.115	-6.168	-6.493
5.00×10^{-6}	2.03×10^{-6}	2.98×10^{-6}	0.681	-5.693	-5.526
1.00×10^{-5}	2.32×10^{-6}	7.68×10^{-6}	0.302	-5.634	-5.115
5.00×10^{-5}	ppt				
1.00×10^{-4}	ppt				

Table 3.10: Results of europium - Aldrich sodium humate systems

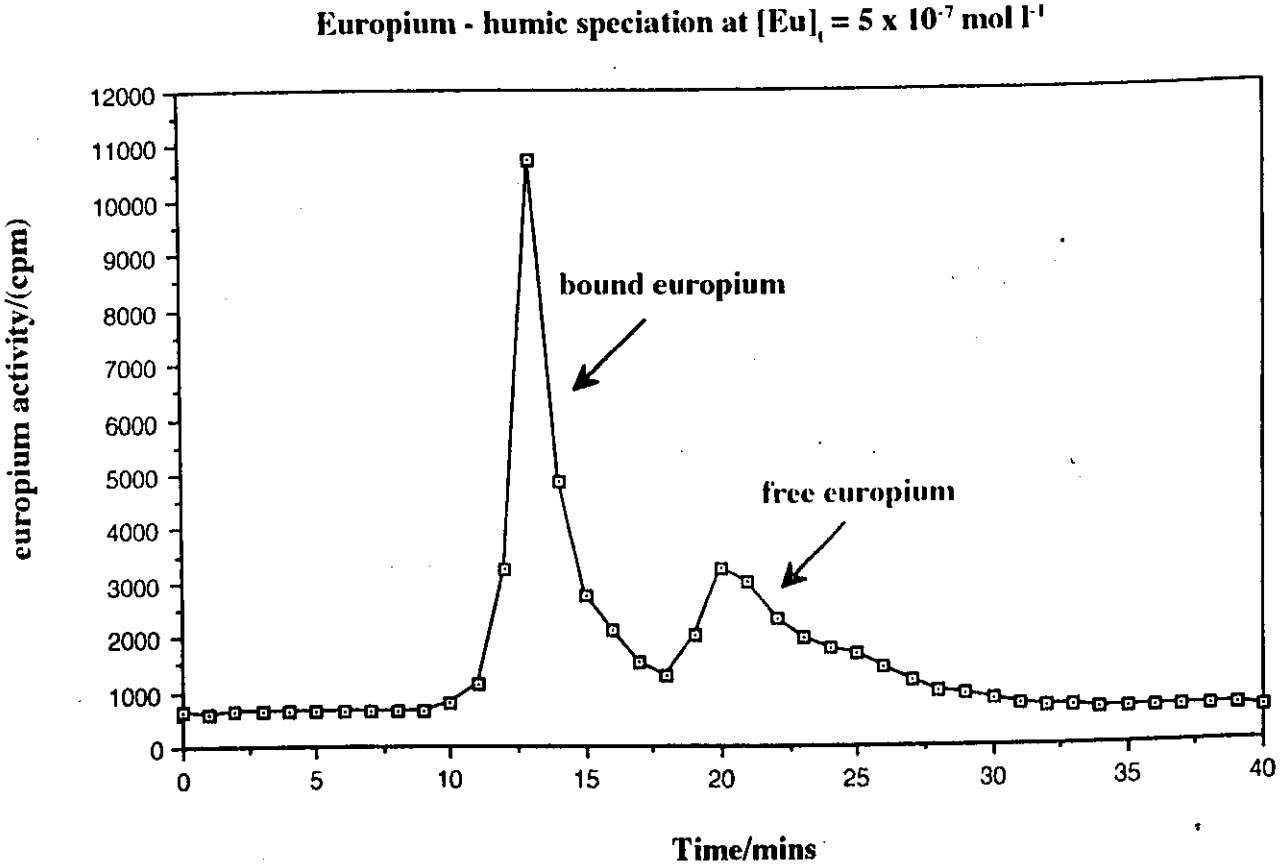


Figure 3.7: Radiochromatogram of europium - Aldrich sodium humate

Figure 3.9 shows a Scatchard plot of the data and the derived stability constants for europium - Aldrich sodium humate.

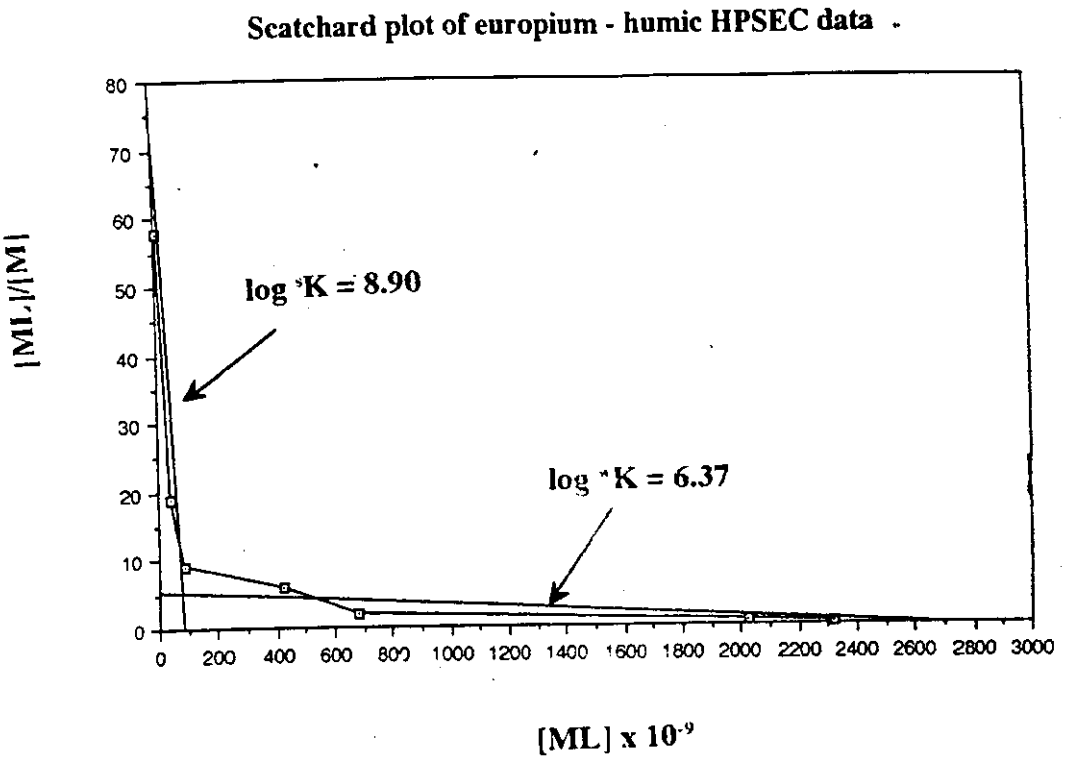


Figure 3.9: Scatchard plot of europium - aldrich sodium humate

The above derived stability constants are compared with literatue values shown in Appendix 1.The experimental data was modelled using MINTEQA2 [59]. MINTEQA2 is a geochemical speciation code, which has in its database thermodynamic values for DOM (dissolve organic matter) with selected metal ions.

The experimentally determined stability constant values indicate the formation of a strong complex between humic acid and europium. Extrapolation of the above data to trace levels of metal (eg: 10^{-12} mol l^{-1}), as would be found in groundwaters, would yield even higher stability constants values. The comparison between experimental and model data is shown in figure 3.10.

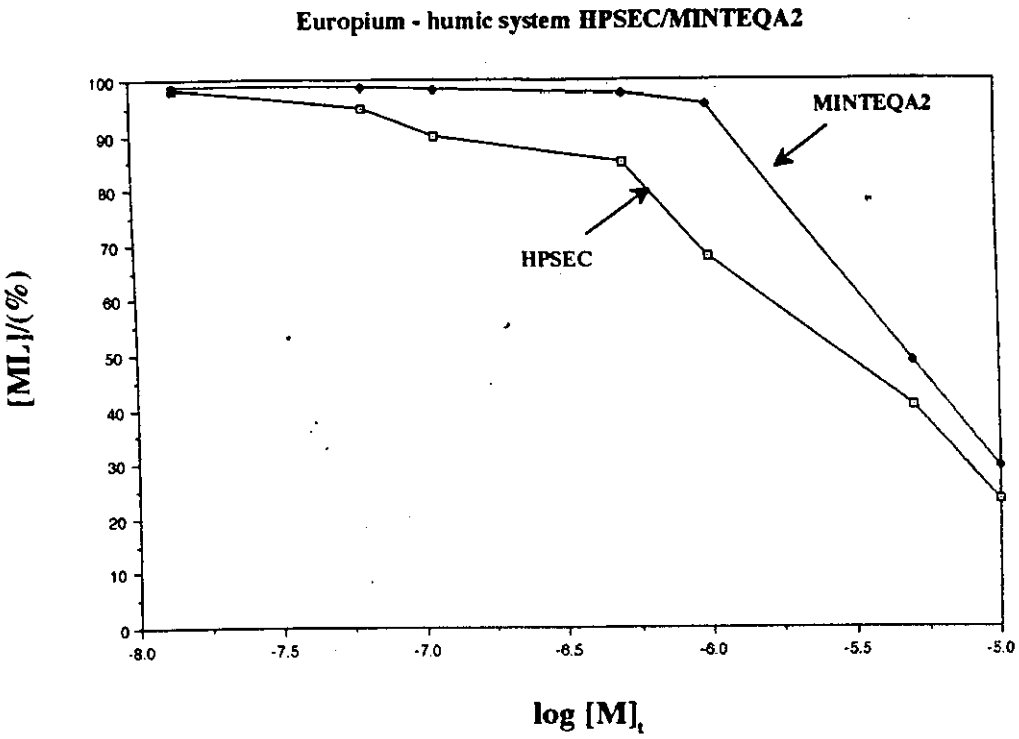


Figure 3.10: Speciation of europium - Aldrich sodium humate study

Conclusion

Analysis of the HPSEC data and the derived stability constants are in good agreement with previous reported literature values. The experimental data is also consistent with an ion exchange study on this system. Comparison of the experimental data with the computer speciation code MINTEQA2 shows reasonable agreement over the europium concentration range. Any discrepancies between model and experimental could be due to differing sites capacities between the Aldrich sodium humate used experimentally and Suwannee fulvic acid used in the MINTEQA2 database. MINTEQA2 also employs a Gaussian distribution for its metal-DOM (dissolved organic matter) interaction with a stability constant log K value of 6.4 and a standard deviation of 1.7 to account for the continuous range of log K's associated with humic material. However, over the entire metal concentration range the experimental results are consistently lower than the model predictions.

3.5 Ion exchange studies

Theory/Background

The exchange equilibria that are fundamental in ion exchange involves the following:

(i) electrical neutrality is always maintained.

(ii) ion exchangers behave as ionised salts.

(iii) the active ions in the ion exchanger are mobile.

The mechanism of ion exchange can be viewed in terms of the Law of Mass Action. An ion exchange reaction proceeds as follows:

$$RX^+ + Y^+ \rightleftharpoons RY^+ + X^+ \qquad (3.1)$$

where R stands for the non-exchangeable part of the resin, and X^+ and Y^+ for the mutually exchangeable ion. Since the reaction is reversible the Law of Mass Action may be applied.

$$[Y^+]_r [X^+]_s \rightleftharpoons K [X^+]_r [Y^+]_s \qquad (3.2)$$

where the subscripts r and s refer to resin and solution phases respectively.

In a solution containing an ion exchanger, complexing ligand and a metal ion, the metal will partition itself between the resin phase and the solution phase and will do so to satisfy the equilibrium constant for the resin and the stability constant of the complex in solution. The ion exchanger can operate in both batch and column modes. In column ion exchange the cation exchanger will remove any cationic species from the passing solution. This removal of

species will effect the equilibrium of the complexes in solution.

Most studies of metal - humic acid using ion exchangers have been carried out using the batch method, and particularly the Schubert method [60,61]. The Schubert method, involves the distribution of trace metal ion between the ion exchange resin and the complexing ligand in solution.

In the absence of complexing ligand L the distribution coefficient, D_o, of the metal is given by:

$$D_o = \frac{[M^{x+}]_{res}}{[M^{x+}]_{soln}} \qquad (3.3)$$

where $[M^{x+}]_{res}$ and $[M^{x+}]_{soln}$ represent the amount of metal on the resin and in solution respectively.

When a ligand is added to the system, the metal is redistributed.

$$D = \frac{[M^{x+}]_{res}}{\Sigma [M]} \qquad (3.4)$$

where $\Sigma [M] = M_f + M_b$ i.e: the free and bound metal respectively in solution. Combining equations 3.3 and 3.4 leads to:

$$\frac{D_o}{D} - 1 = \frac{M_b}{M_f} = \Sigma \beta_i [L]^i \qquad (3.5)$$

Hence when n = 1 (ie: a stoichiometry of 1:1):

$$\log \frac{D_o}{D} - 1 = \log \beta + \log [L] \qquad (3.6)$$

By applying this equation the stability constant, β, can be calculated.

Unfortunately, in the case of humic acids it is difficult to calculate the free ligand concentration accurately. To overcome this problem, and to compare results by cation column ion exchange as well as HPSEC results, the Schubert method was modified by allowing a range of metal concentrations to be investigated.

In the Schubert method, trace metal concentrations are used relative to the amount of exchangeable sites on the resin and hence equation 3.3 is valid. However as the metal concentration is increased more exchangeable ions are displaced and the free resin concentration no longer remains constant. Consequently, a more rigorous approach is needed to define the equilibria taking place. The cation exchange reaction can be represented as:

$$M^{x+} + xNaRes \rightleftharpoons M(Res)x + xNa^{+} \qquad (3.7)$$

with the equilibrium constant given by

$$K = \frac{[M(Res)x][Na^{+}]^{x}}{[M^{x+}][NaRes]^{x}} \qquad (3.8)$$

And for the reaction with the complexing ligand:

$$M^{x+} + L^{y-} \rightleftharpoons ML^{x-y} \qquad (3.9)$$

$$\beta = \frac{[ML^{x-y}]}{[M^{x+}][L^{y-}]} \qquad (3.10)$$

Rearrangement of equation 3.8:

$$[M^{x+}] = \frac{[M(Res)x]}{K([NaRes]/[Na])^{x}} \qquad (3.11)$$

For low metal concentrations:

$$[M^{x+}] = \frac{[M(Res)x]}{D_o} \qquad (3.12)$$

With increasing metal concentrations:

$$[M^{x+}] = [MRes]/D_{MRes} \qquad (3.13)$$

where D_{MRes} represents the distribution of the metal between the resin and the solution at higher metal concentrations.

Thus having experimentally measured $[M]_{solution}$ and determined $[M^{x+}]$, [ML] can be calculated:

$$[ML] = [M]_{solution} - [M^{x+}] \qquad (3.14)$$

Hence, by using the above approach a series of [ML] can be calculated for each metal concentration.

The aim of the ion exchange studies was to investigate the following effects:

(i) To compare cation column ion exchange with both HPSEC results and MINTEQA2 model predictions.

(ii) To investigate the effect of pH on europium humate interaction.

(iii) To compare batch and column ion exchange since one is a dynamic process (column) and the other an equilibrium process (batch).

(iv) To apply the column and batch techniques for nickel humate equilibria and to compare with the europium systems.

3.5.1 Experimental set-up for column cation exchange experiments

The ion exchange procedure used to investigate metal - ligand interactions was based on a technique described by Choppin [57]. The Dowex 50x4 100-200 mesh cation exchange resin used in the experiments was prepared by loading the resin (~50g) on to a chromatographic column and successively washing with numerous bed volumes of concentrated HCl, distilled water, 0.1mol l^{-1} EDTA, distilled water again and finally 0.1mol l^{-1} NaOH solution. Finally the column of ion exchange resin was washed with the appropriate buffer solution used to prepare the metal - humic samples. Each 500µl sample of solution under investigation was rapidly separated on a short column of freshly prepared resin (12mm i.d x 38mm length) which had been placed to a depth of 4cm in a plastic column fitted with a clean glass wool plug at the bottom of the column. The samples were eluted with 5ml of the same buffer. The eluates were monitored for γ and/or β activity as appropriate. From a knowledge of the initial activity of each sample, and the eluted activities, the percentage of bound metal in each solution was calculated.

3.5.2 Column ion exchange control experiments

The following set of experiments were performed to show that all the activity that was injected onto the column was removed by the buffer solution. A sample was prepared, containing 50ppm humic acid and 1 x10^{-5} mol l^{-1} analar grade europium (III) chloride + radiolabelled Eu-152 (from Amersham 251MBq ml^{-1} with 0.538mg ml^{-1} inactive Eu carrier) in a background electrolyte of 0.01mol l^{-1} $NaClO_4$ held at pH 6.5 by 0.01mol l^{-1} MES buffer solution.

A 500µl sample of the above solution was injected onto an ion exchange column. The column was eluted with 5ml of 0.01mol l^{-1} MES buffer solution at pH 6.5 to remove the bound europium and then 5ml of 0.1mol l^{-1} EDTA to remove the free europium from the column. The experiment was repeated several times and the results (corrected for background) are shown in table 3.11.

Eu total activity /cpm	Eu bound activity /cpm	Eu free activity /cpm	bound + free /cpm	(bound + free)/total /%
112352	68574	42783	111357	99.1
112352	66783	43075	109858	97.8
112352	66049	43992	110041	97.9
112352	66054	44002	110056	98.0
112352	67121	43321	110442	98.3
112352	68079	43088	111167	98.9

Table 3.11: Cation exchange control experiments

The result show that over 98% of the europium that is injected onto the column is eluted using MES buffer and EDTA eluants.

Another control experiment was performed to prove that no free europium is removed by the MES buffer. To investigate this a solution containing 1 $x10^{-5}$ mol l^{-1} $EuCl_3.6H_2O$ + Eu-152 were prepared in a 0.1mol l^{-1} $NaClO_4$ with the pH of the solution being maintained at pH 6.5 by 0.01mol l^{-1} MES buffer solution.

A 500µl sample was injected onto a column of ion exchange and the column was eluted with 2 x 5ml 0.01mol l^{-1} MES buffer solution at pH 6.5. No activity was eluted from the column. The column was then eluted with 5ml of 0.01mol l^{-1} EDTA which removed all the activity.

3.5.3 Europium - humic acid system

The europium - humic acid solutions under investigation were as in section 3.4. The results for the europium - humic acid system are shown below in table 3.12:

$[M]_t$ Total Eu concentration (mol l^{-1})	[ML] Eu-AHA complex (mol l^{-1})	[M] Eu-free (mol l^{-1})	$\frac{[ML]}{[M]}$	log [ML]	log [M]
1.41×10^{-8}	1.39×10^{-8}	2.00×10^{-10}	69.500	-7.857	-9.699
6.41×10^{-8}	6.13×10^{-8}	2.82×10^{-9}	21.738	-7.213	-8.550
1.14×10^{-7}	1.01×10^{-7}	1.30×10^{-8}	7.769	-6.996	-7.886
5.14×10^{-7}	4.25×10^{-7}	8.94×10^{-8}	4.754	-6.372	-7.049
1.00×10^{-6}	7.37×10^{-7}	2.63×10^{-7}	2.802	-6.133	-6.580
5.00×10^{-6}	2.28×10^{-6}	2.72×10^{-6}	0.838	-5.642	-5.565
1.00×10^{-5}	2.81×10^{-6}	7.19×10^{-6}	0.391	-5.551	-5.143
5.00×10^{-5}	ppt				
1.00×10^{-4}	ppt				

Table 3.12: Ion exchange results on europium - humic acid system

The above results were plotted with the HPSEC results and the MINTEQA2 model predictions as shown in figure 3.11.

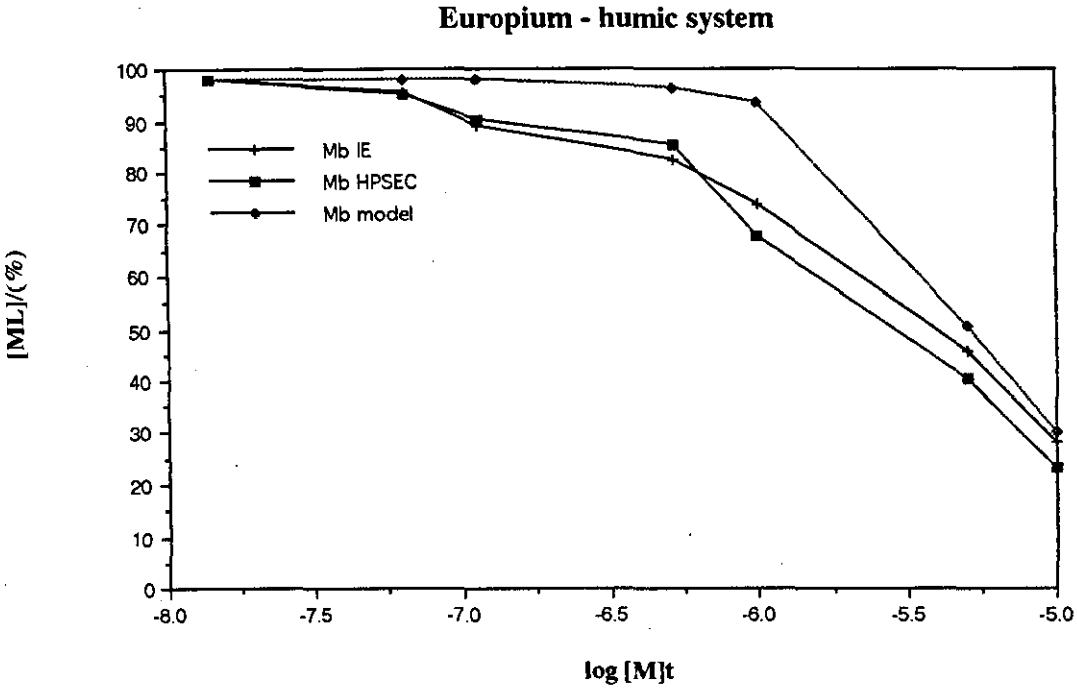


Figure 3.11: Comparision of HPSEC/Ion exchange/MINTEQA2 results on europium - humic acid system

Discussion

Over the metal concentration range used in the study there is good agreement between the ion exchange and HPSEC techniques. Such agreement was taken as validation of both techniques. However, both experimental techniques give consistently less metal bound than the MINTEQA2 model predictions. As with previous comparisons with MINTEQA2, discrepancies may be due to the heterogeneuous nature of the humic acid which results in a non-Gaussian distribution of log k values rather than a single value. Although MINTEQA2 attempts to mimic the heterogeneuous nature of humic acid it does so by adopting a mean log k = 6.4 with a standard deviation of $\sigma = 1.7$ with a Gaussian spread of log k values. Analysis of Scatchard plots suggests that a Gaussian spread is inappropriate to mimic the complexing ability of sites on a humic molecule.

3.5.4 Ion exchange pH studies by column cation exchange

To investigate the effect of pH on the interaction of europium with humic acid and hence to determine the amount of metal bound, a series of samples were prepared with each sample containing 50ppm humic acid and an approprate amount of analar europium (III) chloride such that each series contained an europium concentration range from 10^{-8} to 10^{-4}mol l^{-1}. All solutions were prepared in a background electrolyte of 0.1mol l^{-1} $NaClO_4$ and 0.01mol l^{-1} MES, PES, CAPS buffer solutions as appropriate for the pH. The series covered a pH range of 3.5 - 7.5. The amount of metal bound as a function of the total metal concentration is shown in figure 3.12.

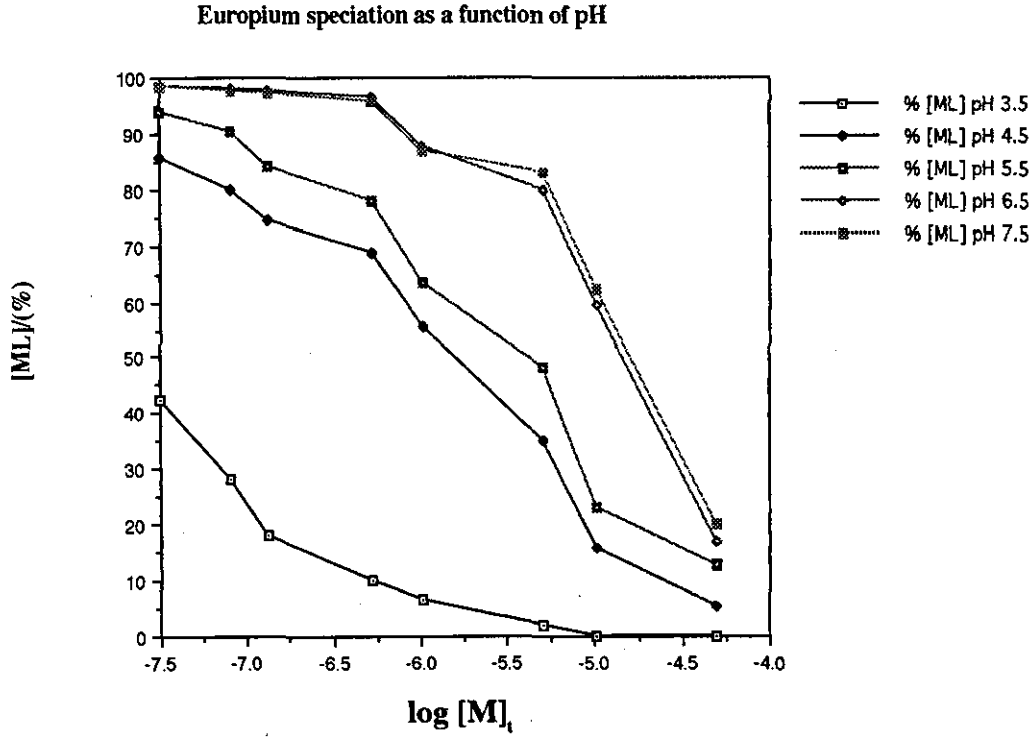


Figure 3.12: Ion exchange studies on the pH effect of europium with humic acid

From the results the following conclusions can be made:

1. There is a significant increase in the amount of europium bound from pH 3.5 to 4.5. This is inevitably due to the deprotonation of carboxylate groups on the humic molecule. This deprotonation will have two effects on the binding behaviour of the humic molecule with europium. Firstly, theincrease in pH from 3.5 to 4.5 lessens the competitive effect of protons for the carboxylate groups and allows the europium to bind. Secondly the deprotonation of the groups on the humic molecule will cause the molecule to uncoil, thereby allowing easier access to binding groups on the molecule.

2. At the low metal concentrations between the pH range of 5.5 - 7.5 over 95% of the total metal concentration is bound. Within this pH range the competition effect due to protons, hydroxide and carbonate ligands for the europium are negligible.

3. As the metal concentration is increased there is a gradual decrease in the amount of metal bound to the humic acid. This indicates that strong binding groups on the humic molecule complex with the europium first and as the europium concentration is increased weaker binding groups on the humic molecule become complexed.

4. Between the pH range 5.5 to 6.5, at high metal concentrations there is a significant increase in the amount of metal bound. This is due to the dissociation of all carboxylate groups on the humic molecule. Unfortunately, the technique could not be applied at higher pHs due to the presence of anionic negative complexes, hence the dissociation of phenolic groups and their effect on metal binding could not be investigated.

3.5.5 Further studies - a comparison between batch and column

Background /theory

A mechanism has been proposed to describe the binding between metal and humic substances [62,63]. The mechanism involves two types of binding, localised and non-localised. Localised involves binding between the metal and complexing groups on the humic molecule, where as non-localised binding is the electrostatic attraction only of the positive metal ion with the negative field that is associated with the polyelectrolytic humic molecule.

As a result of this proposed mechanism, cation column exchange experiments were carried out to determine the equilibria between the non-localised metal bound and the localised metal bound. As a metal - humic acid solution was passed down a column of cation exchanger the resin 'stripped' off the non-localised bound metal. The collected eluent was left to re-equilibrate and then passed down through another column of resin. Again the same amount of metal was stripped off from the humic, indicating the equilibria between non-localised and localised binding.

To further investigate the proposed mechanism, batch ion exchange experiments were performed for both nickel and europium and the results compared with corresponding column data. The aim was to validate the data from both techniques and to gather insight into the mechanism of metal - humic acid behaviour.

In addition, batch experiments may also provide further information on the binding of metals to humic substances since the batch technique is an equilibrium process as opposed to the dynamic process of column techniques.

3.5.6 Batch experimental - general procedure

All solutions used in the batch experiments were prepared using the following general procedure.

Stock solutions of 0.01mol l^{-1} MES buffer in 0.1mol l^{-1} $NaClO_4$ at pH 6.5 were prepared. 10^{-2}mol l^{-1} stock solutions of Ni^{2+} and Eu^{3+} were made up using the above buffer solution, in 250cm^3 volumetric flasks. From these metal stock solutions, accurate dilutions of metal solutions were prepared in the 0.01mol l^{-1} MES buffer/0.1mol l^{-1} $NaClO_4$ solution. A 250cm^3 solution of 100ppm Aldrich humic acid was also prepared in the buffer solution.

Radioactive Eu-152 and Ni-63 were prepared in 20ml buffer stocks:

Ni-63	- 27µl from 37MBq in 1000µl
Eu-152	- 1100µl from 4.63MBq in 5000µl

Approximately 0.1g of Dowex 50x4 100-200 mesh size cation resin was weighed into a pre-weighed plastic vial. To the plastic vial 5.0ml of 100ppm Aldrich humic acid (where appropriate) was added or 5ml of buffer solution. The required amount of standard metal solution was added and the solution volume was adjusted to 9.5ml. The vial was weighed. To the solution 0.5ml of Eu-152 or Ni-63 from the above working radioactive stock solutions were added to make a total volume of 10ml and the vial was re-weighed. The samples were left to equilibrate for five days before 0.5ml aliquots were taken to determine the activity present.

3.5.7 Europium cation batch results

The following samples were prepared to determine the distribution coefficient of the metal when in the presence of the resin. A range of europium concentrations, as shown in table 3.13, were prepared as described above in section 3.4.6. For each metal concentration the distribution coefficient, D_o, was determined from the initial activity of the solution and after equilibration with the resin. A plot of D_o against log $[M]_{res}$ is shown in figure 3.13.

$[M]_t$	$[M]_{solution}$	$[M]_{res}$	D_o
3.678×10^{-8}	1.318×10^{-9}	3.553×10^{-7}	269.0
8.678×10^{-8}	3.553×10^{-9}	8.323×10^{-7}	234.3
1.368×10^{-7}	5.176×10^{-9}	1.316×10^{-6}	254.3
5.368×10^{-7}	2.257×10^{-8}	5.142×10^{-6}	227.8
1.037×10^{-6}	4.292×10^{-8}	9.941×10^{-6}	231.6
5.037×10^{-6}	3.567×10^{-7}	4.680×10^{-5}	131.2
1.004×10^{-5}	1.071×10^{-6}	8.969×10^{-5}	83.7
5.004×10^{-5}	1.225×10^{-5}	3.779×10^{-5}	30.8

Table 3.13: Batch ion exchange - D_o calibration results

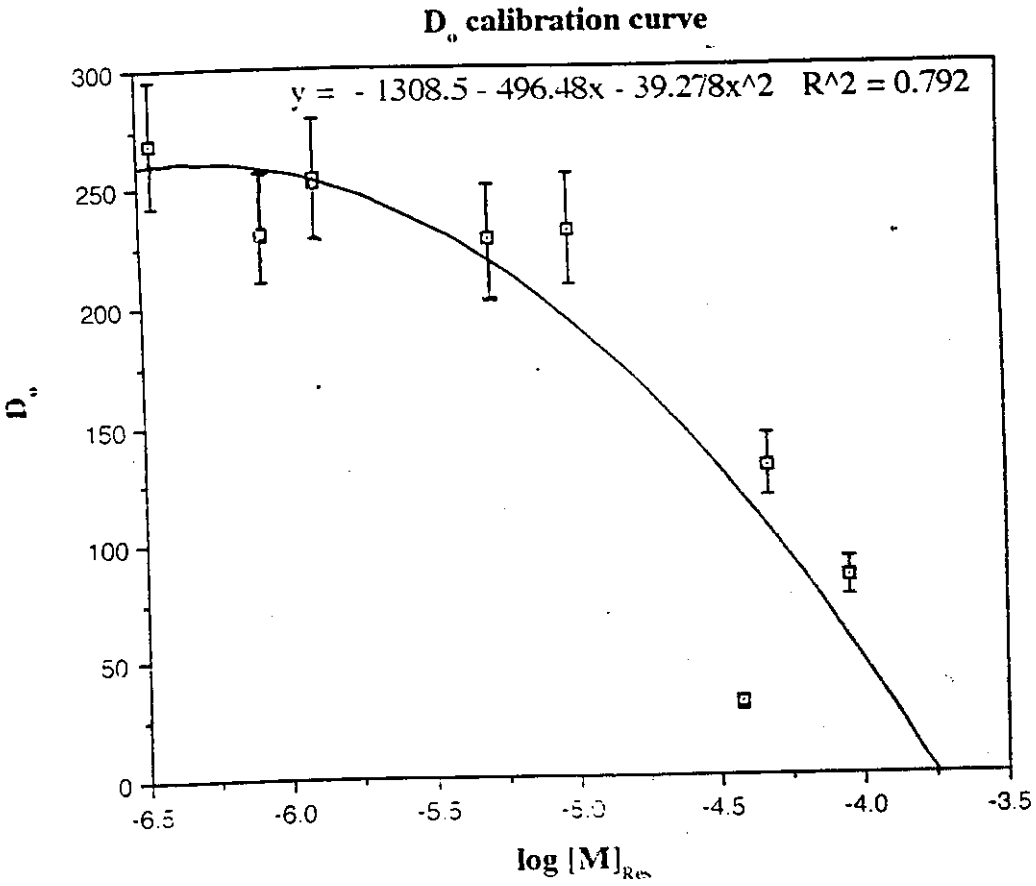


Figure 3.13: D_o curve for europium

As can be seen from the above graph, the D_o for europium is relatively constant, within 10% error until high concentrations of metal on the resin are reached. Hence when a complexing ligand is introduced, due to its ability to remove europium from the resin, the distibution coefficient will again remain constant for almost the entire europium concentration range.

For experiments where humic acid is present, a range of europium concentrations were used as shown in table 3.14, each with radiolabelled Eu-152. After 5 days equilibration aliquots of the solution were taken and the distribution coefficient, D_{Mres} was calculated.

$[M]_t$	$[M]_{solution}$	$[M]_{res}$	D_{MRes}	$[M^{x+}]$	[ML]
3.678×10^{-8}	3.670×10^{-8}	8.000×10^{-10}	271	2.952×10^{-12}	3.670×10^{-8}
8.678×10^{-8}	8.676×10^{-8}	2.000×10^{-10}	271	7.380×10^{-13}	8.676×10^{-8}
1.368×10^{-7}	1.359×10^{-7}	9.000×10^{-9}	271	3.321×10^{-11}	1.359×10^{-7}
5.368×10^{-7}	5.355×10^{-7}	1.300×10^{-8}	271	4.797×10^{-11}	5.355×10^{-7}
1.037×10^{-6}	1.021×10^{-6}	1.600×10^{-7}	271	5.904×10^{-10}	1.020×10^{-6}
5.037×10^{-6}	5.010×10^{-6}	2.700×10^{-7}	271	9.963×10^{-10}	5.009×10^{-6}
1.004×10^{-5}	9.281×10^{-6}	7.590×10^{-6}	213	3.568×10^{-8}	9.245×10^{-6}
2.504×10^{-5}	1.932×10^{-5}	5.680×10^{-5}	131	4.329×10^{-7}	1.889×10^{-5}
5.005×10^{-5}	3.535×10^{-5}	1.469×10^{-4}	79	1.864×10^{-6}	3.344×10^{-5}
7.504×10^{-5}	5.328×10^{-5}	2.172×10^{-4}	40	6.725×10^{-6}	4.655×10^{-5}

Table 3.14: Batch ion exchange results for europium humate

Aliquots of the prepared samples were also injected onto a column of ion exchange resin using the technique as described in section 3.4.1. The results of these experiments are shown below in table 3.15.

$[M]_t$	$[M]_{bound}$	$[M]_{free}$
3.678×10^{-8}	3.608×10^{-8}	7.000×10^{-10}
8.678×10^{-8}	8.469×10^{-8}	1.820×10^{-9}
1.368×10^{-7}	1.320×10^{-7}	4.780×10^{-9}
5.368×10^{-7}	5.153×10^{-7}	2.148×10^{-8}
1.037×10^{-6}	8.885×10^{-7}	1.483×10^{-7}
5.037×10^{-6}	3.989×10^{-6}	1.047×10^{-6}
1.004×10^{-5}	5.420×10^{-6}	4.617×10^{-6}

Table 3.15: Column ion exchange results for europium humate

The aim of the experiment was to compare the batch and column ion exchange techniques, specifically in terms of the amount of europium bound to the humic acid. This comparison of the two techniques is shown in figure 3.14.

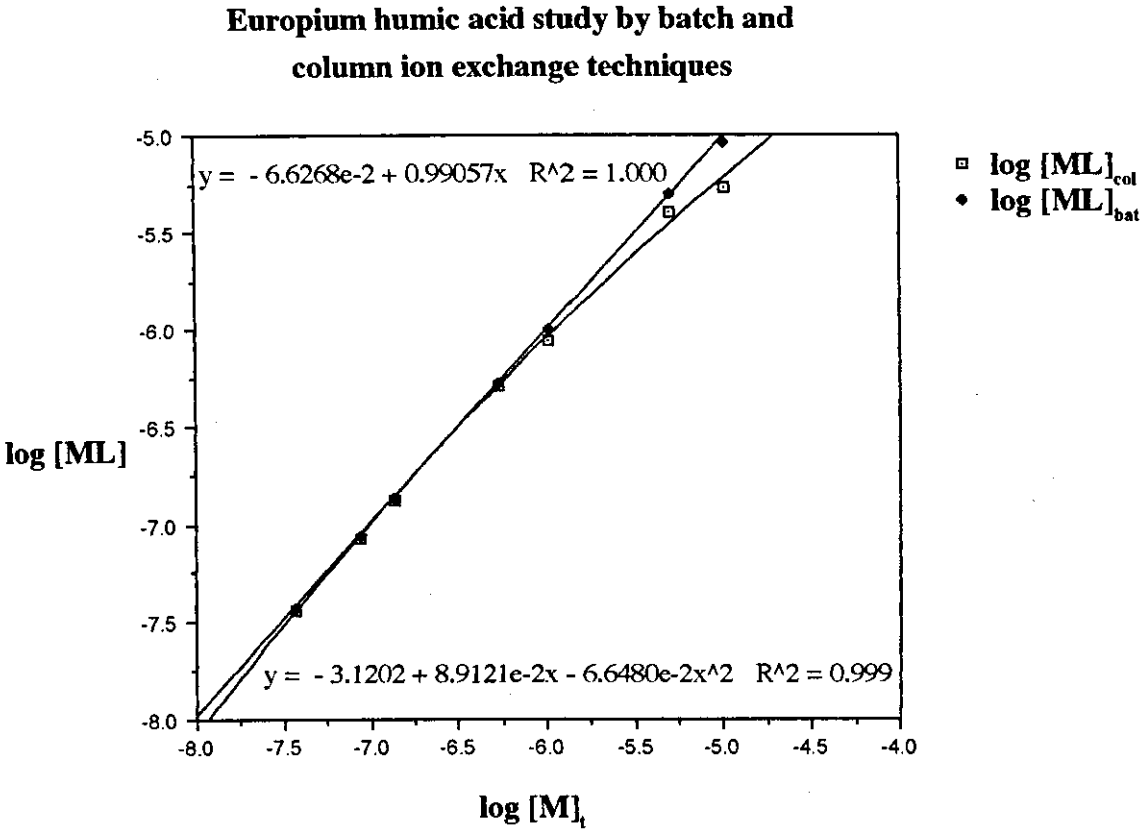


Figure 3.14: Europium humic acid binding - comparison between batch and column techniques

From figure 3.14 it can be seen that both batch and column ion exchange techniques correspond over most of the europium concentration range. However, at high metal concentrations there is a divergence between the metal bound by both techniques. This indicates that in the column technique at high metal loadings on the humic acid, the europium is stripped from the humic by the column of resin.

3.5.8 Nickel cation experiments

To further investigate the comparison between column and batch techniques a series of experiments were carried out to investigtae the binding of nickel with humic acid.

A set of nickel - humic acid samples were prepared in a background of 0.1 mol l^{-1} $NaClO_4$ + 0.01 mol l^{-1} MES buffer at pH 6.5. The humic acid concentration was 50ppm, and the nickel concentrations were as shown in table 3.16. In each sample Ni-63 (from Amersham International, 37Mbq in 0.093 mol l^{-1} HCl) was added as described in the batch general procedure. Results are shown in 3,16.

$[M]_t$	$[M]_{bound}$	$[M]_{free}$	% M bound
5.440×10^{-8}	5.152×10^{-8}	2.883×10^{-9}	94.7
1.540×10^{-7}	1.404×10^{-7}	1.355×10^{-8}	91.2
5.544×10^{-7}	4.840×10^{-7}	7.041×10^{-8}	87.3
1.054×10^{-6}	8.443×10^{-7}	2.097×10^{-7}	80.1
2.554×10^{-6}	1.739×10^{-6}	8.147×10^{-7}	68.1
5.054×10^{-6}	2.396×10^{-6}	2.658×10^{-6}	47.4
1.005×10^{-5}	2.643×10^{-6}	7.407×10^{-6}	26.3
2.505×10^{-5}	4.559×10^{-6}	2.049×10^{-5}	18.2
5.005×10^{-5}	6.857×10^{-6}	4.319×10^{-5}	13.7

3.5.9 Nickel cation batch results

The following samples were prepared to determine the distribution coefficient of the metal when in the presence of the resin. A range of nickel concentrations, as shown in table 3.17, were prepared as described in the general procedure. For each metal concentration the distribution coefficient, D_o, was determined from the initial activity of the solution and after equilibration with the resin. A plot of D_o against log $[M]_{res}$ is shown in figure 3.15.

$[M]_t$	$[M]_{super}$	$[M]_{res}$	D_o
5.440×10^{-8}	5.382×10^{-9}	4.902×10^{-7}	91.07
1.540×10^{-7}	1.580×10^{-8}	1.386×10^{-6}	87.72
5.544×10^{-7}	5.691×10^{-8}	5.487×10^{-6}	96.42
1.054×10^{-6}	1.107×10^{-7}	9.433×10^{-6}	85.21
2.554×10^{-6}	2.638×10^{-7}	2.291×10^{-5}	86.82
5.054×10^{-6}	5.394×10^{-7}	4.515×10^{-5}	83.70
1.005×10^{-5}	1.251×10^{-6}	8.799×10^{-5}	70.34
2.505×10^{-5}	4.229×10^{-5}	2.082×10^{-4}	49.23
5.005×10^{-5}	1.415×10^{-5}	3.590×10^{-4}	25.37
1.000×10^{-4}	7.421×10^{-5}	2.579×10^{-4}	3.475

Table 3.17: Batch ion exchange results for nickel humate

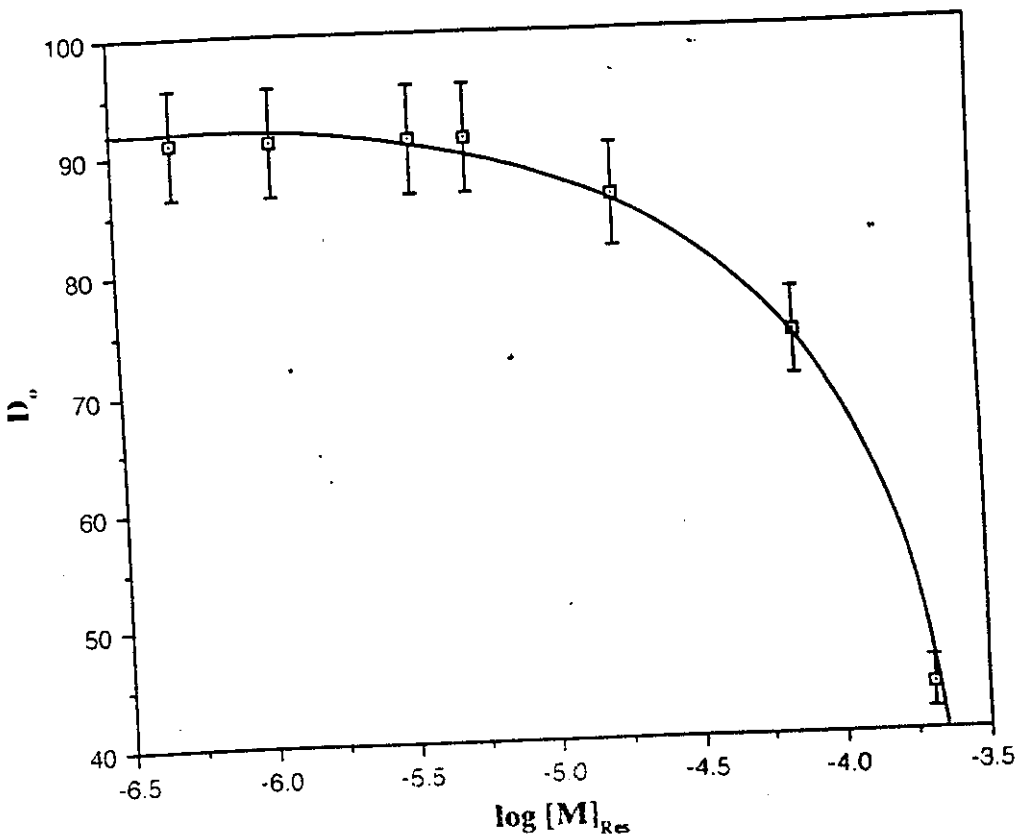


Figure 3.15: D_o calibration curve for nickel

As can be seen from the calibration curve, the D_o for nickel remains fairly constant until high concentrations of metal are on the resin. As with the europium case, when humic acid is introduced into such a system, due to its ability to remove the metal from the resin, the distribution constant will again remain constant for almost the entire concentration range.

For experiments involving humic acid present, a range of nickel concentrations were used as shown in table 3.19, each with radiolabelled Ni-63. After 5 days equilibration aliquots of the solution were taken and the distribution coefficient, D_{Mres} was calculated.

$[M]_t$	$[M]_{solution}$	$[M]_{res}$	D_{MRes}	$[M^{x+}]$	[ML]
5.440×10^{-8}	5.210×10^{-8}	2.300×10^{-8}	91	2.527×10^{-10}	5.185×10^{-8}
1.540×10^{-8}	1.486×10^{-7}	5.400×10^{-8}	91	5.934×10^{-10}	1.480×10^{-7}
5.544×10^{-7}	5.030×10^{-7}	5.140×10^{-7}	91	5.648×10^{-9}	4.974×10^{-7}
1.054×10^{-7}	9.401×10^{-7}	1.139×10^{-6}	91	1.252×10^{-8}	9.276×10^{-7}
2.554×10^{-6}	2.205×10^{-6}	3.490×10^{-6}	91	3.835×10^{-8}	2.167×10^{-6}
5.054×10^{-6}	4.502×10^{-6}	5.520×10^{-6}	91	6.066×10^{-8}	4.441×10^{-6}
1.005×10^{-5}	8.279×10^{-6}	1.771×10^{-5}	86	2.059×10^{-7}	8.073×10^{-6}
2.505×10^{-5}	1.773×10^{-5}	7.320×10^{-5}	74	9.892×10^{-7}	1.674×10^{-5}
5.005×10^{-5}	2.990×10^{-5}	2.015×10^{-4}	44	4.580×10^{-6}	2.532×10^{-5}

Table 3.18: Batch ion exchange results for nickel humate

The aim of the experiment was to compare the batch and column ion exchange techniques, specifically in terms of the amount of nickel bound to the humic acid. This comparison is shown in figure 3.16.

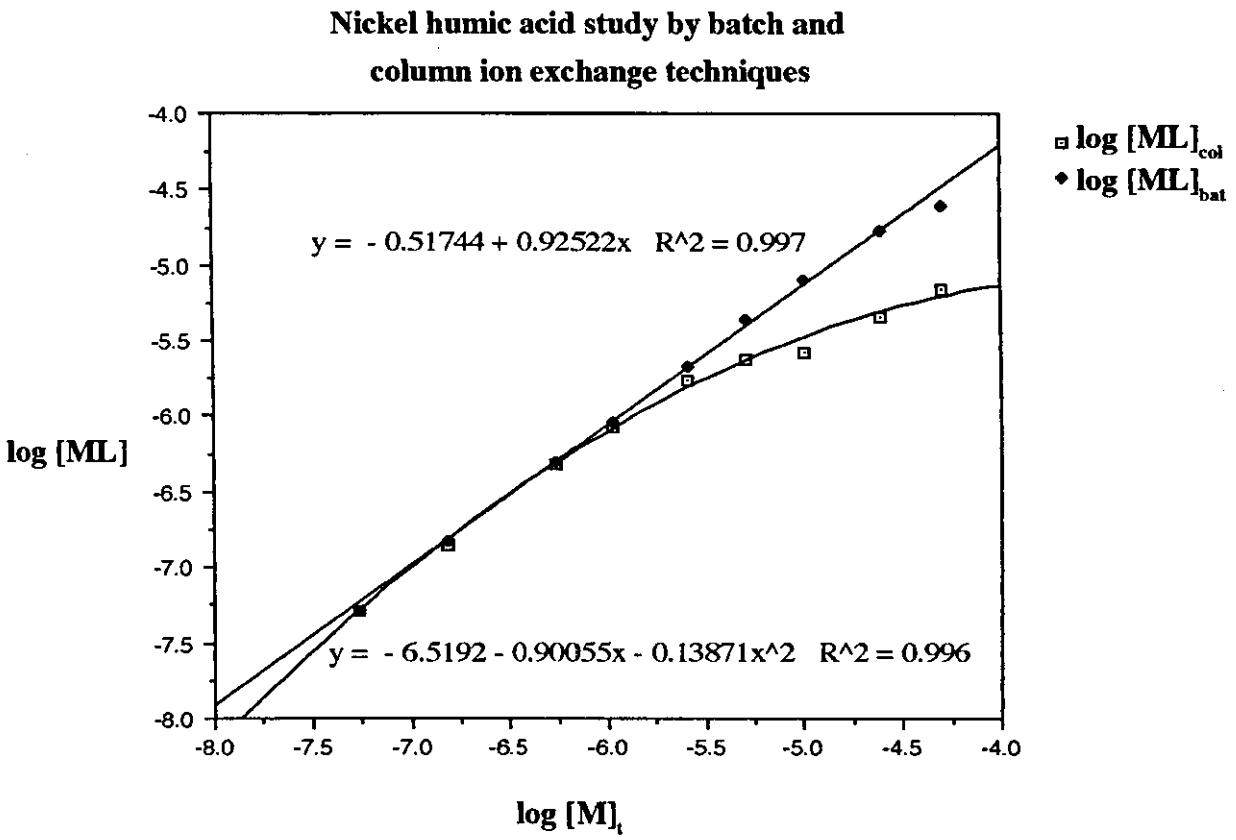


Figure 3.16: Nickel humic acid binding - comparison between batch and column techniques

From the above figure it can be seen that, as in the europium case there is a divergence between the column and batch results as the metal concentration is increased. The divergence between the two techniques occurs at lower metal concentrations than in the europium case. Again the results indicate the removal of weakly bound nickel from the humic acid as the solution passes through the column of ion exchange resin. Since nickel forms weaker complexes than europium with the humic acid, the stripping by the resin will occur at lower nickel concentrations than for europium.

3.6 Conclusions on column and batch ion exchange results

The aim of the experiments was to investigate the batch and column techniques. At low concentrations of metal, the two techniques give similar results of metal bound. However, as the total metal concentration is increased there is a divergence between the two techniques with the cation column exchange technique giving lower metal bound results. To explain these results, it is suggested that at high metal concentrations more of the metal bound becomes attached to weak sites on the humic acid. As a sample proceeds down the column of resin, these weak sites undergo dissociation and thus europium is removed by the resin. For nickel this phenonema is observed at lower total metal concentration due to the weaker binding of nickel with humic acid.

It should also be noted that the affinity for europium by the cation exchange resin will be greater than for nickel due to the higher charge of the europium ion. This should have the effect of removing the europium from the humic acid at lower total metal concentrations. However the experimental results suggest that this does not occur and this is due the high stability of the europium humate complex over a large range of metal concentrations.

A comparison of both techniques for both europium and nickel is shown in figure 3.17.

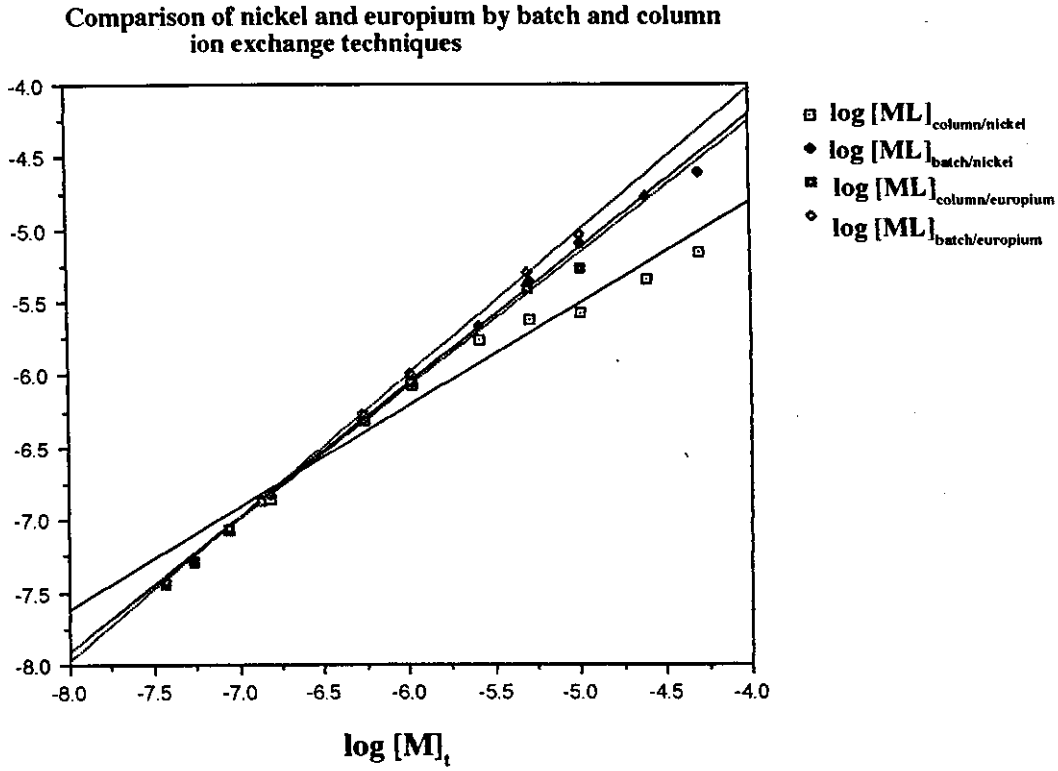


Figure 3.17: Europium and nickel humic acid binding studies by both batch and column ion exchange

CHAPTER FOUR

The role of low molecular weight organic acids on metal - humic interactions

4.1 Introduction

The aim of the work was to investigate equilibria involving the interactions of a metal when in the presence of both high molecular weight organics and low molecular weight organic acids. Specifically, the investigations were designed to ascertain the possible formation of mixed ligand complexes. If such complexes exist, it is essential that their interaction with metals are quantitatively understood and are incorporated into geochemical speciation codes. Since in aquifer systems there a large number of ligands to complex with toxic metal ions the probability of mixed complexes being formed is high. In the literature there is a lack of sufficient data for such species, particularly for natural organic acids. The only thorough investigation has been in the study of the mixed complexes of the amino acids [64].

For simple ligands, it is possible to calculate the stability constant of a mixed ligand system from the individual stability constants:

$$MA_2 + MB_2 \rightleftharpoons 2MAB \qquad (4.1)$$

Therefore,

$$K = \frac{[MAB]^2}{[MA_2]\,[MB_2]} = \frac{(\beta^{AB})^2}{\beta_2{}^A\beta_2{}^B}$$

where β^{AB}, $\beta_2{}^A$, and $\beta_2{}^B$, are the overall formation constants for the complexes MAB, MA_2, and MB_2 respectively.

The formation of mixed complexes can further be promoted by a number of effects:

a) Steric factors

If a metal is coordinated to a bulky ligand, then it is more likely that a smaller ligand will occupy the second coordination position.

b) **π-bond formation**

Log K will be greater if the ligands A and B possess π-acceptor systems.

c) **Intramolecular bonds**

If the two ligands are sufficiently large, bonds will develop between them which will be independent of the metal ion. These bonds can be divided into:

i. **Hydrogen bonds.**
ii. **Covalent bonds** formed specifically between A and B in the coordination sphere of the cation, which serves as a catalyst for the formation of this bond.
iii **Ionic interactions** between A and B when each possesses a charged lateral chain.
iv. **Aromatic ring packing.**
v. **Hydrophobic interaction** between aliphatic and aromatic lateral chain.

Also within such systems it is possible to form bonds without the presence of metal. For this to occur the bond energies of (i) - (v) must be sufficiently high. Such bonds can be termed as intermolecular and the two ligands, A and B, behave as a single entity, AB, and offer two complexing sites. If A and B are large enough then they can act as two independent sites.

Thus it can be seen that many of the above factors may be applicable when dealing with metal complexation equilibria involving humic substances. Consequently, there is a clear need to determine whether mixed complex formation does occur between humic acid and other organic complexing agents, or that they behave as discrete entities. Conversely, even if mixed complexes do not exist can systems involving mixtures of organic acids be successfully predicted with current individual equilibrium constants as used by geochemical speciation codes?

4.2 Experimental

The possibility of mixed ligand complex formation, resulting from the interaction of europium with mixtures of high and low molecular weight naturally occuring organic acids was investigated. Three systems were studied containing europium with humic acid in the presence of (i) acetic acid (ii) oxalic acid and (iii) citric acid. The various mixtures were analysed using high performance size exclusion chromatography (HPSEC). To facilitate the measurements C-14 labelled low molecular weight organic acids and Eu-152 were used.

Seven series of samples were prepared, all in 0.01mol l^{-1} NaCl at pH 6.0. Within each series the Eu^{3+} concentrations ranged from ~ 1 $x10^{-8}$ to 1 x 10^{-4} mol l^{-1}, but the acid concentrations were held constant as shown in table 4.1. At higher europium concentrations precipitation of europium-humate was observed. The C-14 labelled acids were obtained from Amersham International (citric acid), NEN Research products (oxalic acid) and ICN Radiochemicals, Inc. (acetic acid) with specific activities of 4.07GBq/mmol, 0.19GBq/mmol and 0.592GBq/mmol respectively. All concentrations were calculated taking into account dilutions and where appropriate the presence of radioactive tracer material. The composition of the samples is shown in table 4.1.

Results from the europium - humic acid system only have been given in section 3.4.

Series*	humic /(gl^{-1})	acetic /($mol\ l^{-1}$)	oxalic /($mol\ l^{-1}$)	citric /($mol\ l^{-1}$)
1	0.010	------	------	------
2	------	------	------	1.05×10^{-6}
3	0.010	------	------	1.05×10^{-6}
4	------	------	1.04×10^{-5}	------
5	0.010	------	1.04×10^{-5}	------
6	------	1.04×10^{-2}	------	------
7	0.010	1.04×10^{-2}	------	------

Table 4.1: Composition of samples

* Within each series Eu^{3+} concentrations ranged from 10^{-8} to $10^{-4}mol\ l^{-1}$. The europium contained trace amounts of Eu-152 and the three low molecular weight acids, acetic, oxalic and citric contained C-14. The humic acid was not labelled.

4.3 Experimental procedures

The HPSEC experiments were conducted using the technique described in section 3.4. The eluate flowed through a Canberra Packard Flo-one/Beta Radiometric A140 detector, containing a 500µl flow through cell. Liquid scintillation cocktail (Ecoscint A: National Diagnostics) was mixed with the column eluate to permit the eluted β activity to be monitored. Upstream of the mixing chamber a splitter, within the detector, was used to divert part of the eluate to a LKB Redifrac fraction collector. The collected fractions were monitored for γ activity using a Philips PW 4800 γ counter. Elution profiles of both the β activity associated with the low molecular weight organic acid species and the γ activity of the Eu^{3+} containing species were obtained. The 100µl injected samples and the bulk mobile phases were 0.45µm filtered with Acro discs and filters. Using the previous europium stepped elution scheme the organically complexed europium species were eluted in HPLC grade water and the free europium in $0.1mol\ l^{-1}$ EDTA, at a constant flow rate of $0.75ml\ min^{-1}$. The chromatographic peak areas were used to determine the percentage of bound europium in each sample.

4.4 Europium - citric acid system

The HPSEC method was used to investigate the equilibria between europium and citric acid. A range of europium chloride solutions was prepared in a background electrolyte of 0.01mol l^{-1} sodium chloride. To each of these solutions 1ml of 1 x 10^{-5}mol l^{-1} citric acid was added to make a total citric acid concentration of 1 x 10^{-6}mol l^{-1}. The pH of each solution was adjusted to 6.0 by the dropwise addition of sodium hydroxide and/or hydrochloric acid. Conditions of the column and DAD detector are summarised below:

Flow rate	- .75ml/min
Pressure	~ 38 bars
Mobile phase	- HPLC water/0.1mol l^{-1} EDTA
UV absorption wavelength	- 210nm

The previously used stepped and injection scheme as shown in figure 3.2 was employed to achieve separation of the organically bound and 'free' europium. Initial control experiments, in the absence of citric acid, showed that all the europium was desorbed by the EDTA mobile phase. In addition, control experiments were performed to determine the retention time of the citric acid. A DAD chromatogram of citric acid is shown in figure 4.4 and table 4.3 shows the results of the europium - citric acid system.

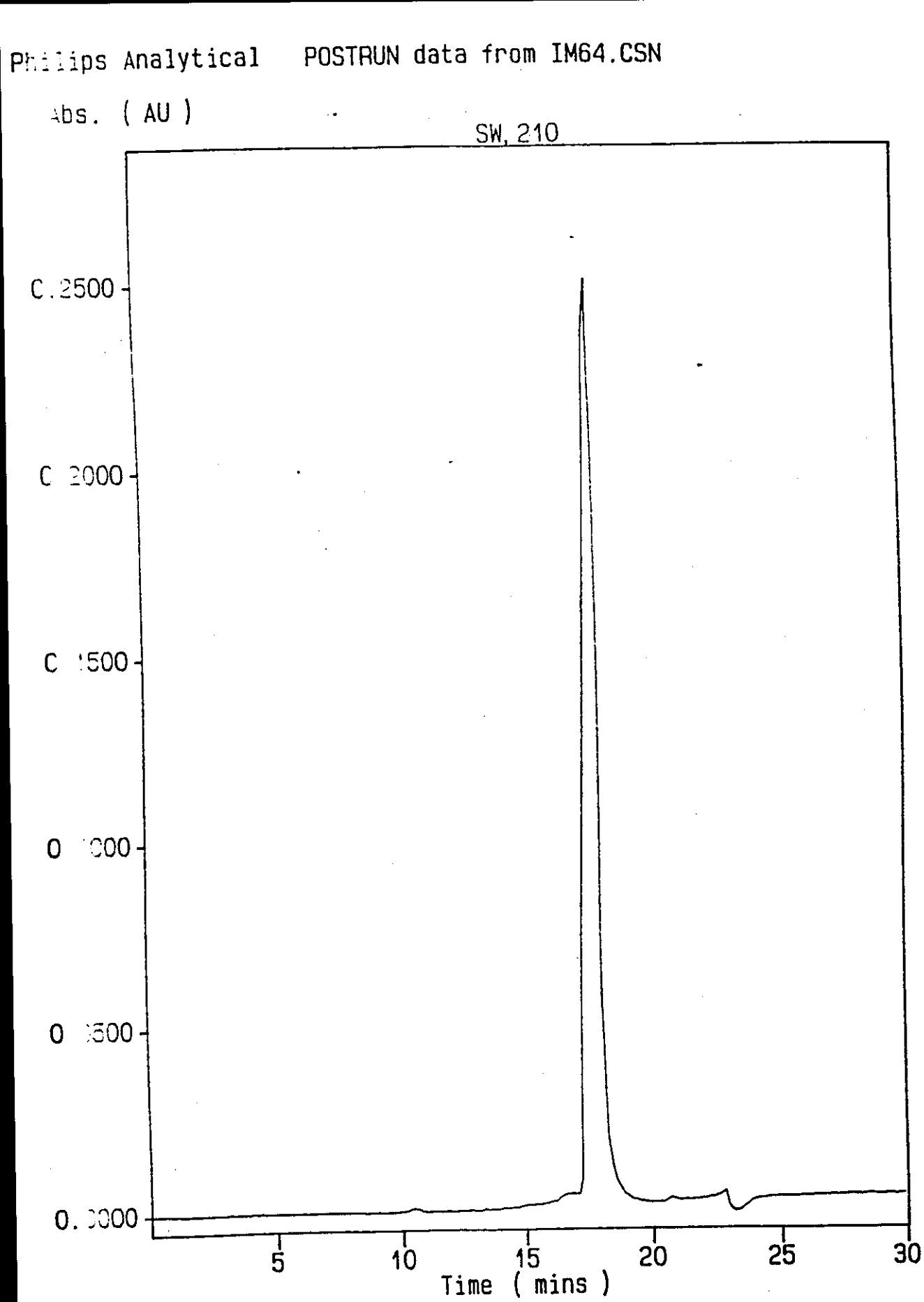


Figure 4.4: An UV-Vis chromatogram of citric acid

Results

$[M]_t$ Total Eu concentration (mol l^{-1})	[ML] Eu-Citrate complex (mol l^{-1})	[M] Eu-free (mol l^{-1})	$\frac{[ML]}{[M]}$	log [ML]	log [M]
1.41×10^{-8}	1.40×10^{-8}	0.10×10^{-9}	141.0	-7.854	-10.00
6.41×10^{-8}	6.17×10^{-8}	2.40×10^{-9}	25.71	-7.210	-8.620
1.14×10^{-7}	1.08×10^{-7}	6.00×10^{-9}	18.00	-6.967	-8.222
5.14×10^{-7}	4.73×10^{-7}	4.10×10^{-8}	11.54	-6.325	-7.387
1.00×10^{-6}	8.86×10^{-7}	1.14×10^{-7}	7.772	-6.053	-6.943
5.00×10^{-6}	9.15×10^{-7}	4.09×10^{-6}	0.224	-6.039	-5.388
1.00×10^{-5}	8.30×10^{-7}	9.17×10^{-6}	0.091	-6.081	-5.038
5.00×10^{-5}	5.00×10^{-7}	4.95×10^{-5}	0.010	-6.301	-4.305
1.00×10^{-4}	n/a				

Table 4.3: Results of europium - citric acid system

A typical radiochromatogram of these experiments is shown in figure 4.4.

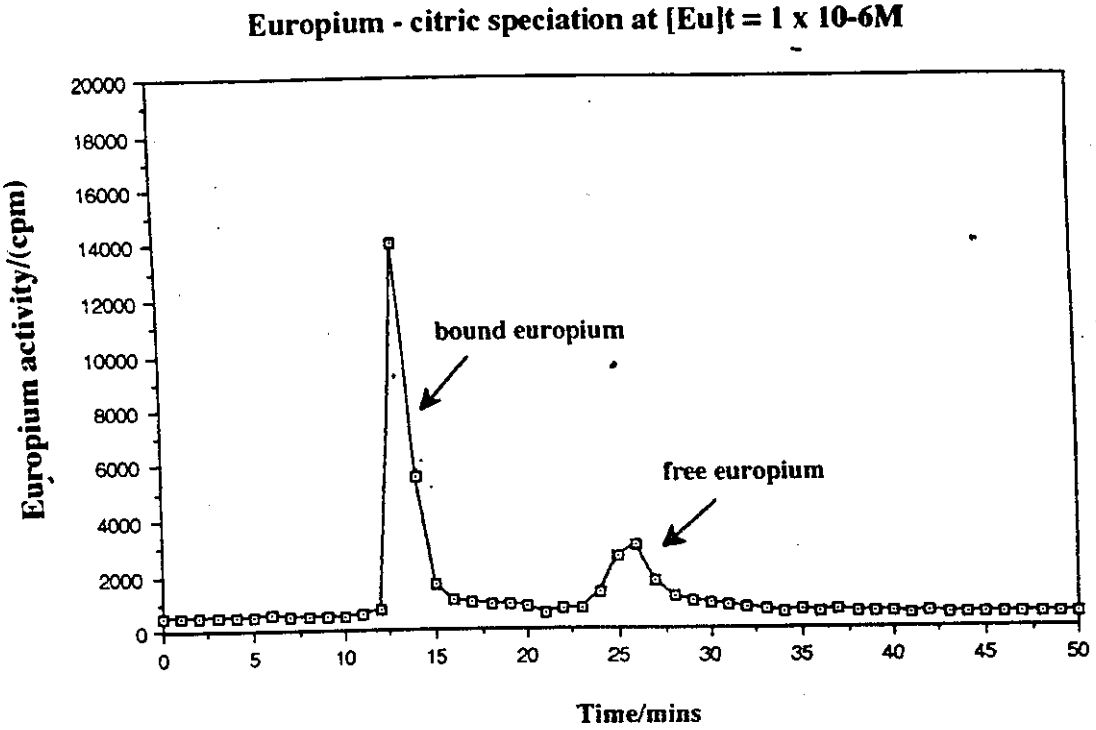


Figure 4.4: Radiochromatogram of europium - citric acid system

An important observation can be made from the above chromatogram and the citric acid chromatogram shown in figure 4.3. The retention time of the citric acid is much shorter than one would expect if the column was behaving purely as a size exclusion column. This has also been observed for citric acid with TSK columns in the literature [65]. The effect has been attributed to electrostatic repulsion between the citric acid and the silica gel column packing. In the context of this study the repulsion exhibited by the citric acid is beneficial since it provides clear resolution between the europium citrate complex and the free europium.

The experimental data shown in table 4.3 was modelled using MINTEQA2. The comparision between experimental results and model results is shown in figure 4.5:

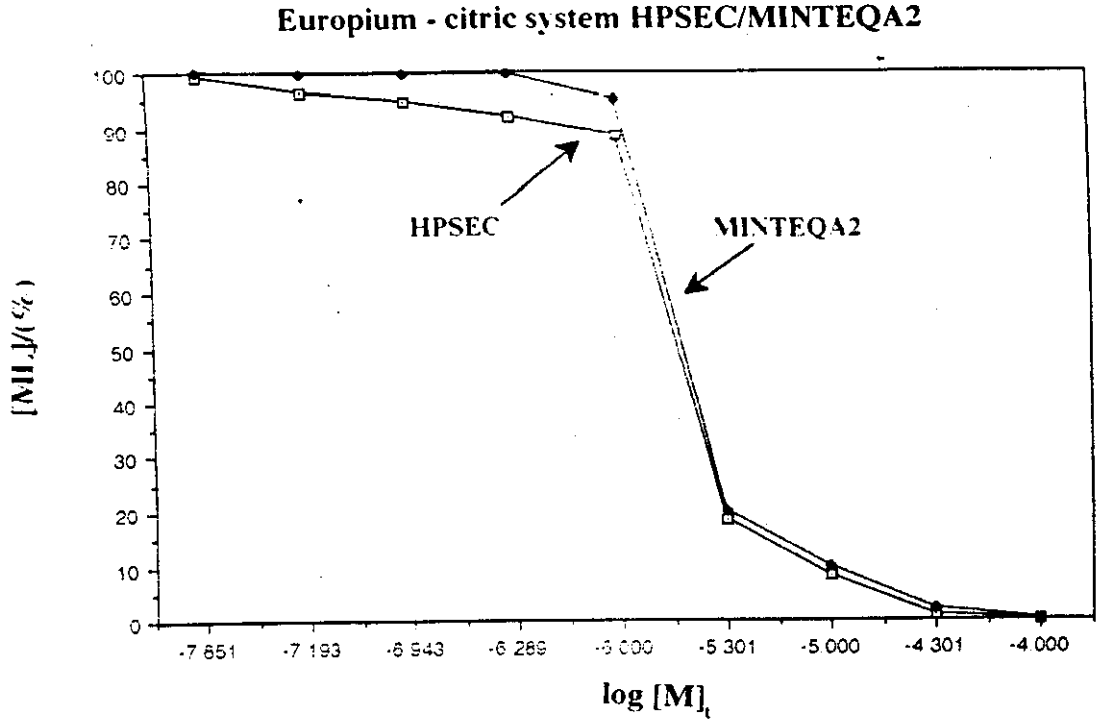


Figure 4.5: Europium - citric acid speciation

The agreement between the model results and the HPSEC experimental results is very good over the metal concentration range. However, as in the previous europium-humate study, the experimentally determined percentage of bound metal is consistently below that determined by the model. Nevertheless the close agreement is taken as evidence of the validity of the HPSEC technique and its usefulness in the speciation of metal - ligand interactions, providing the complexes formed are sufficiently stable.

4.5 Europium - humic acid - citric acid system

The experimental set up, as described in sections 4.1 was again used in this investigation. In addition, the citric acid was spiked with radioactive C-14 labelled citric acid. This radiolabelled citric acid was monitored using a Flo-one Beta radio detector. The results of the europium/humic/citric system are shown in table 4.4.

$[M]_t$ Total Eu concentration (mol l^{-1})	[ML] Eu-bound complex (mol l^{-1})	[M] Eu-free (mol l^{-1})	$\frac{[ML]}{[M]}$	log [ML]	log [M]
1.41×10^{-8}	1.39×10^{-8}	2.00×10^{-10}	69.500	-7.857	-9.699
6.41×10^{-8}	6.33×10^{-8}	8.00×10^{-10}	79.125	-7.199	-9.097
1.14×10^{-7}	1.12×10^{-7}	2.00×10^{-9}	56.000	-6.951	-8.699
5.14×10^{-7}	5.00×10^{-7}	1.40×10^{-8}	35.714	-6.301	-7.854
1.00×10^{-6}	8.54×10^{-7}	1.46×10^{-7}	5.849	-6.069	-6.836
5.00×10^{-6}	2.54×10^{-6}	2.46×10^{-6}	1.033	-5.595	-5.609
1.00×10^{-5}	2.99×10^{-6}	7.01×10^{-6}	0.426	-5.524	-5.154
5.00×10^{-5}	ppt				
1.00×10^{-4}	ppt				

Table 4.4: Results of europium - humic acid - citric acid system

A radiochromatogram of these experiments is shown in figure 4.6.

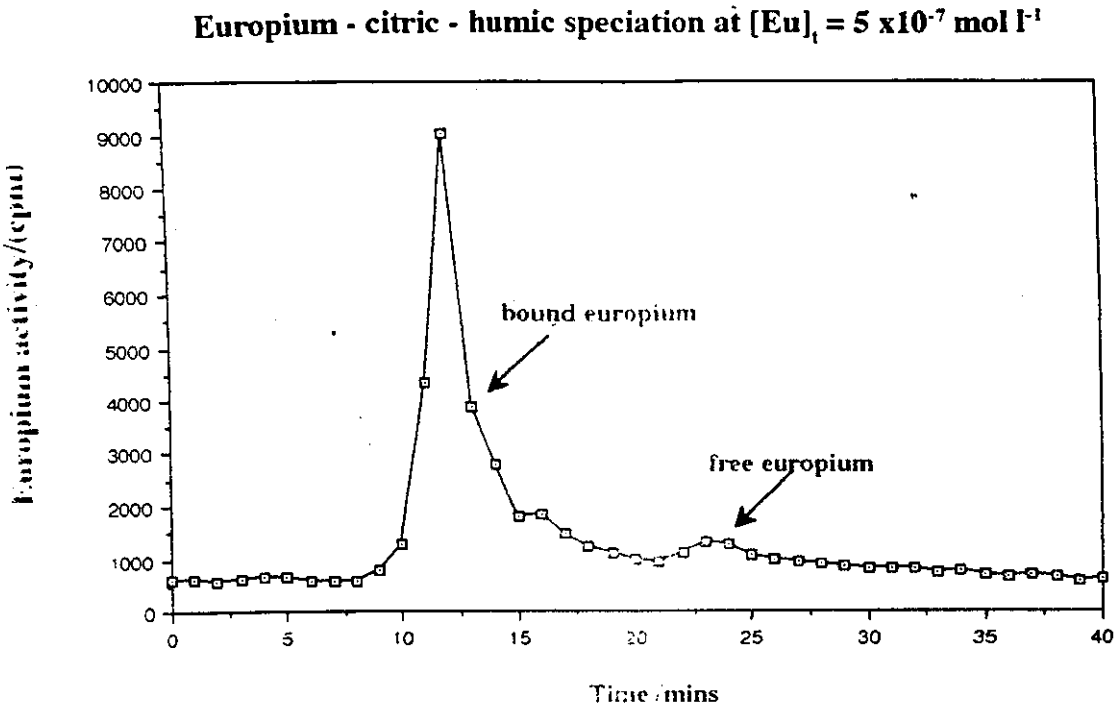


Figure 4.5: Radiochromatogram of europium/humic/citric acid system

The experimental data was modelled using MINTEQA2. The comparision between experimental data and model predictions is shown in figure 4.7.

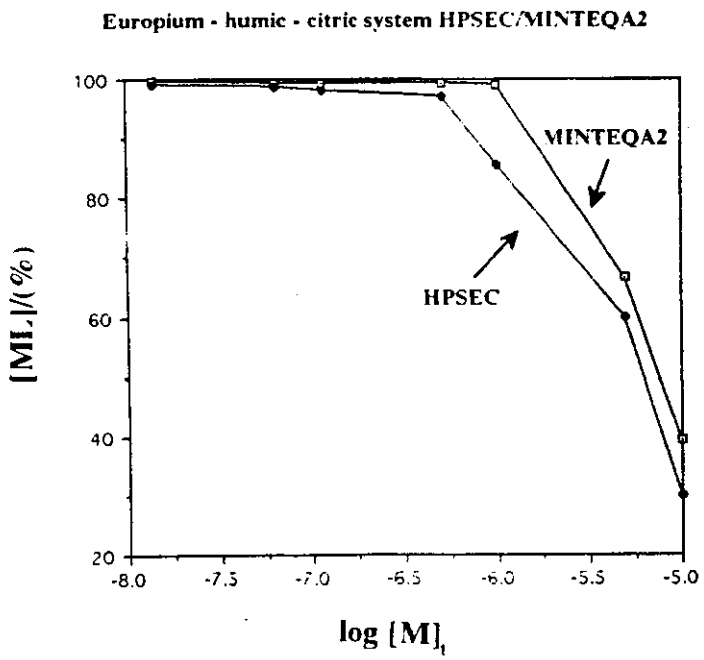


Figure 4.7: Speciation plot of europium - humic acid - citric acid

The objective of these experiments was to determine whether europium in the presence of humic and citric acids will form mixed complexes. The HPSEC experimental results suggested that mixed complexes did not exist within the range of europium, citric and humic concentrations used in the investigation. Evidence for the absence of mixed ligand complex formation was obtained by comparing the HPSEC experimental data with speciation modelling results obtained by using the MINTEQA2 code. This code does not contain equilibrium data for mixed ligand complexes, and successfully modelled the experimental results using individual stability constants for europium - humate and europium - citrate.

Unfortunately, poor resolution between citric and humic acids was observed. However, there was no change in the retention time of the radiolabelled citric acid both in the absence and presence of humic acid which suggested that there was no interaction between the two acids. Further evidence for the lack of interaction was also shown by the Gaussian symmetry retained by the citric acid chromatogram when in the presence of humic acid.

4.6 Europium - oxalic acid system

The experimental conditions were as previously described in section 4.1, with 1ml of 1 $x10^{-4}$mol l^{-1} oxalic acid added to make a total oxalic acid concentration of 1 $x10^{-5}$mol l^{-1}. The results of the europium - oxalic acid system are shown in table 4.5:

Results

$[M]_t$ Total Eu concentration (mol l^{-1})	[ML] Eu-oxalate complex (mol l^{-1})	[M] Eu-free (mol l^{-1})	$\frac{[ML]}{[M]}$	log [ML]	log [M]
1.41×10^{-8}	1.26×10^{-8}	1.50×10^{-9}	8.400	-7.900	-8.824
6.41×10^{-8}	5.26×10^{-8}	1.15×10^{-8}	4.574	-7.279	-7.940
1.14×10^{-7}	9.29×10^{-8}	2.11×10^{-8}	4.403	-7.032	-7.676
5.14×10^{-7}	4.17×10^{-7}	9.70×10^{-8}	4.299	-7.013	-6.456
1.00×10^{-6}	7.96×10^{-7}	2.04×10^{-7}	3.902	-6.099	-6.690
5.00×10^{-6}	3.41×10^{-6}	1.59×10^{-6}	2.145	-5.467	-5.799
1.00×10^{-5}	5.91×10^{-6}	4.09×10^{-6}	1.445	-5.228	-5.388
5.00×10^{-5}	7.10×10^{-6}	4.29×10^{-5}	0.166	-5.149	-4.368
1.00×10^{-4}	6.90×10^{-6}	9.31×10^{-5}	0.074	-5.161	-4.031

Table 4.5: Results of europium - oxalic acid system

An example of a radiochromatogram recorded as part of these experiments is shown in figure 4.8.

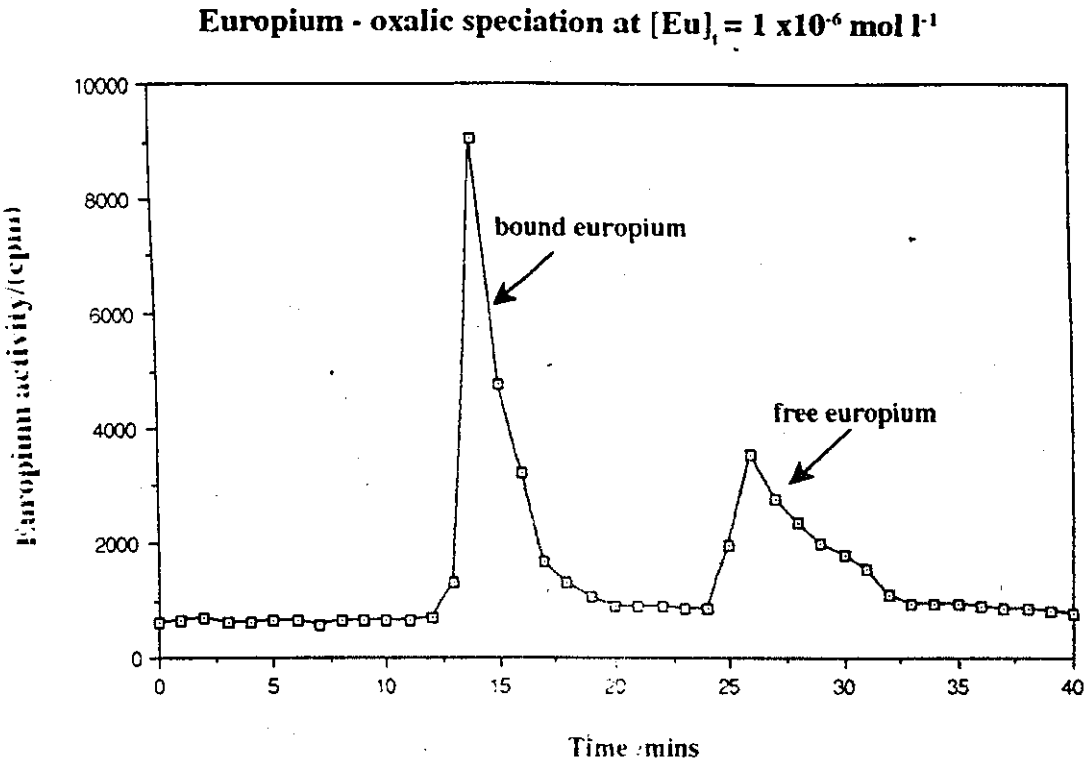


Figure 4.8: Radiochromatogram of europium oxalic acid system

The experimental data was modelled using MINTEQA2. The comparision between experimental and modelling is shown in figure 4.9.

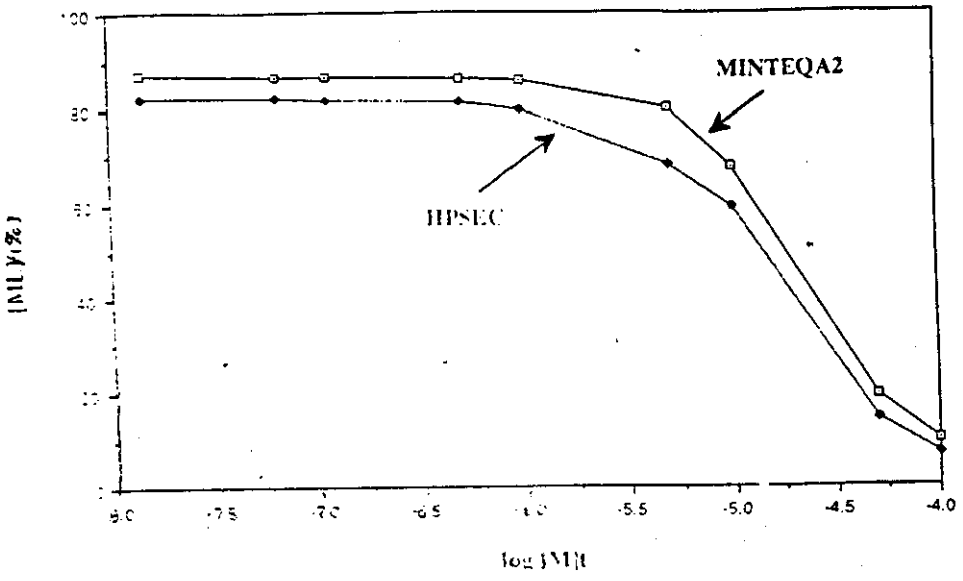


Figure 4.9: Europium - oxalic acid speciation

Agreement between the HPSEC experimental data and MINTEQA2 model predictions is good over the metal concentration range, although as in the previous europium/humic/citric investigation the experimentally determined metal bound is consistently less than model predictions.

4.7 Europium - humic acid - oxalic acid system

The same experimental procedure that was used as in the europium - citric - humic system was used in this investigation. The radiolabelled oxalic acid was monitored using the Flo-one Beta radio detector. Results from the europium/humic/oxalic acid system are shown in table 4.6.

Results

$[M]_t$ Total Eu concentration (mol l^{-1})	[ML] Eu-bound complex (mol l^{-1})	[M] Eu-free (mol l^{-1})	$\frac{[ML]}{[M]}$	log [ML]	log [M]
1.41×10^{-8}	1.37×10^{-8}	4.00×10^{-10}	34.298	-7.863	-9.398
6.41×10^{-8}	6.18×10^{-8}	2.30×10^{-9}	26.870	-7.209	-8.638
1.14×10^{-7}	1.07×10^{-7}	7.00×10^{-9}	15.286	-6.971	-8.155
5.14×10^{-7}	4.70×10^{-7}	4.40×10^{-8}	10.682	-6.328	-7.357
1.00×10^{-6}	7.92×10^{-7}	2.08×10^{-7}	3.808	-6.101	-6.682
5.00×10^{-6}	3.38×10^{-6}	1.62×10^{-6}	2.086	-5.472	-5.790
1.00×10^{-5}	5.63×10^{-6}	4.37×10^{-6}	1.288	-5.249	-5.360
5.00×10^{-5}	ppt				
1.00×10^{-4}	ppt				

Table 4.6: Results of europium - humic acid - oxalic acid system

An example radiochromatogram, showing the speciation of the europium, of these experiments is shown in figure 4.10.

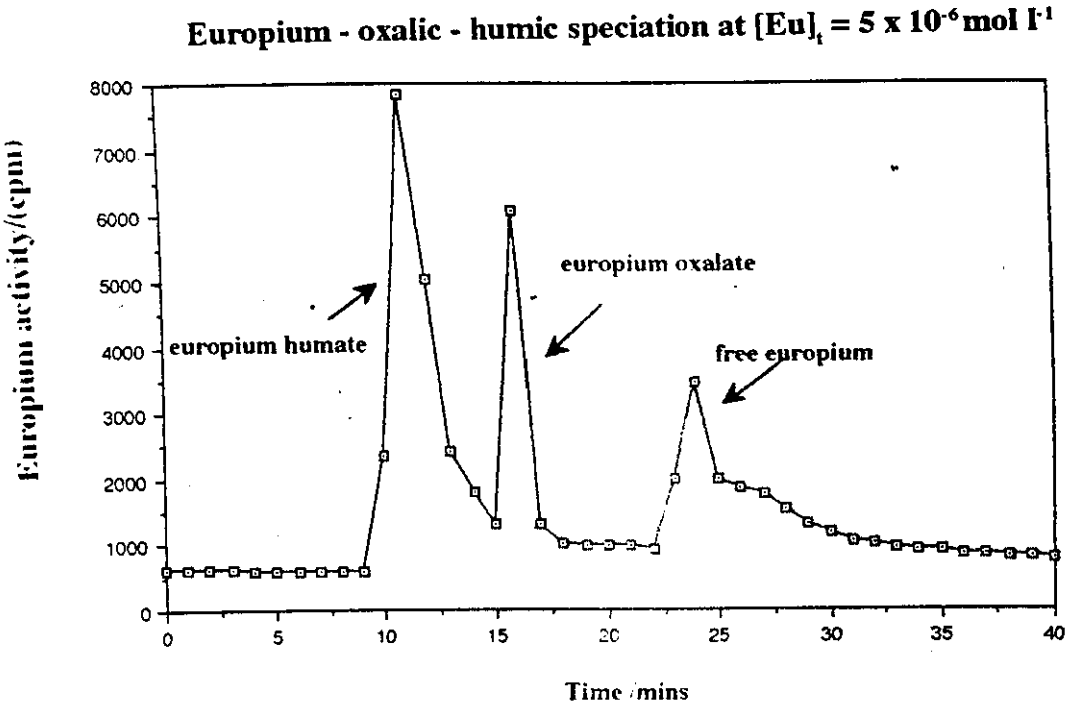


Figure 4.10: Radiochromatogram of europium/humic/oxalic acid system

The results of the HPSEC experiments for the europium - humic acid - oxalic acid systems are shown with the MINTEQA2 predictions in figure 4.11:

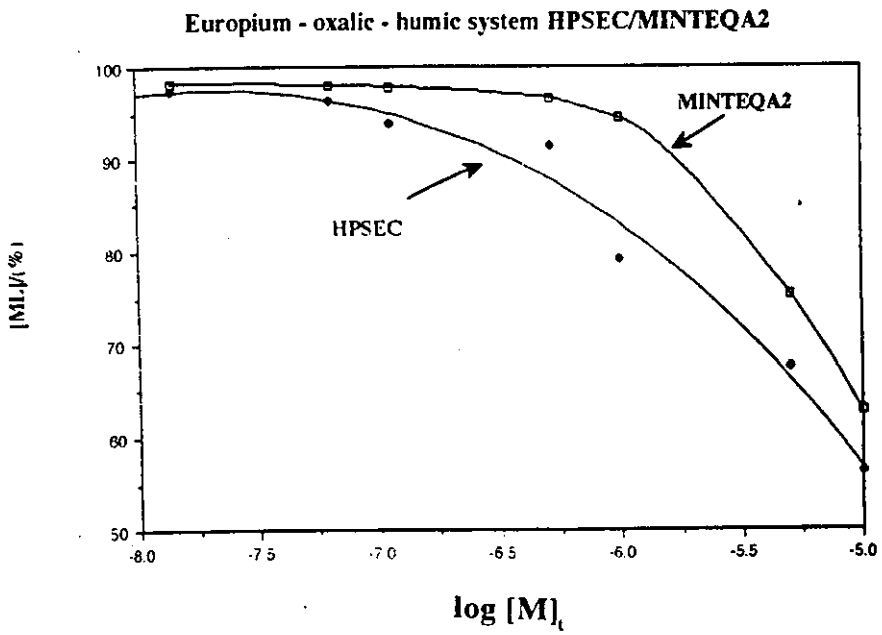


Figure 4.11 : Speciation for europium - humic acid - oxalic acid system

Agreement between experimental data and model predictions over the metal concentration range is reasonable. Monitoring of the radiolabelled oxalic acid showed no shift in retention time and therefore no interaction with the europium humate complex suggesting again, that mixed ligand formation was not evident. The system was also modelled using SPE, a general purpose speciation program. These results are shown in table 4.7:

HPSEC			SPE		
log $[Eu]_t$	**%EuOx**	**%EuHu**	**log $[Eu]_t$**	**%EuOx**	**%EuHu**
-7.851	0.0	97.3	-7.851	0.0	99.0
-7.193	0.0	96.4	-7.193	0.0	99.0
-6.943	0.0	93.8	-6.943	0.0	99.0
-6.289	2.3	89.1	-6.289	3.0	91.5
-6.000	5.1	84.1	-6.000	5.3	84.7
-5.301	22.0	45.5	-5.301	24.0	47.0
-5.000	27.9	28.4	-5.000	32.5	29.0

Table 4.7: Comparison of HPSEC results and SPE model predictions for the europium/humic/oxalic acid system

The parameters inputted into SPE were obtained from the individual europium - humic acid and europium oxalic systems. Again the agreement between experimental results and SPE predictions indicates that mixed ligand formation in the europium - humic - oxalic system does not occur.

4.8 Europium - acetic acid system

The same experimental conditions that were used previously in section 4.3. were also used in this investigation. A 1ml solution of 1 x 10^{-1}mol l^{-1} acetic acid was added to give a total acetic acid concentration of 1 x 10^{-2}mol l^{-1}. Experimental results showed that a significant amount of the europium complex was dissociating during separation of the bound and free europium.

4.9 Conclusions

Mixed ligand complex formation was not observed under the conditions employed and speciation modelling of the mixed systems was successfully achieved using appropriate combinations of single ligand constants. The absence of mixed complex formation could be attributable to a number of parameters. The process being observed in the systems studied indicates direct ligand exchange of low molecular weight organic acids for the humic acid with no stable intermediate mixed complex formation. Further investigations should be directed into the study of interactions between humic acid and amino acids since amino acids have been shown to form mixed complexes between themselves.

Further analysis of the data indicates that under groundwater conditions, humic acids would dominate the speciation of toxic metals.

4.10 Previous work

The work presented in this chapter does not support the findings of Maes et al [67,68] who, employing a modified Schubert method, found evidence for mixed complex formation. To investigate any possible discrepancies in the previous work, the Maes et al theory and data was reviewed.

The work involved a large number of ion exchange experiments. The Schubert method was based on the following relationship:

$$\frac{K^{\circ}_{D}}{K_{D}} - 1 = \frac{\beta_{1}[HA] + ... + \beta_{m}[HA]^{m}}{A} = \frac{M_{b}}{M_{f}*A} \qquad (4.2)$$

Where K°_{D} and K_{D} are the distribution coefficients of the metal ion in the absence and in the presence of humic acid (HA). β_{1} and β_{m} represent the overall stability constants, where m is the maximum number of ligands that can be bound by the metal. The 'A' term is the side reaction coefficient which accounts for the contribution of ligands, other than the humic acid, which have the capacity to complex with the metal. For example, in the presence of a ligand, L, the side reaction coefficient A equals:

$$A = 1 + \Sigma \beta^{L}_{n} [L]^{n} \qquad (4.3)$$

Experimentally the concentration ratio of the metal bound by the humic acid to the free metal (M_{b}/M_{f}) is calculated from the distribution measurements and taking into account the side reaction coefficient. The slope of the plot $\log (K^{\circ}_{D}/K_{D} - 1) * A$ versus log [HA] is close to unity indicating a stoichometry of 1:1 for the metal humate complex. Consequently, equation 4.1 can be reduced to:

$$\log\left(\frac{K^{\circ}_{D}}{K_{D}} - 1\right) = \log \beta_{1} + \log [HA] - \log A \qquad (4.4)$$

From the above the stability constant can be calculated from the distribution

data, the HA concentration and the calculated side reaction coefficient.

Typically the experimental procedure involves approximately 0.1g resin equilibrated with, for a set period of time, a known volume and concentration Eu spiked with Eu-152 solution and complexing ligand with a background electrolyte to adjust the ionic strength. A K_d measurement is made and then varying humic acid concentrations are added.

If we consider the equilibria in solution when only europium (concentration ~ 10^{-6} mol l^{-1}) and the competing ligand (in this case oxalic acid) are present, the speciation of europium as a function of the oxalic concentration will be as shown in figure 4.12:

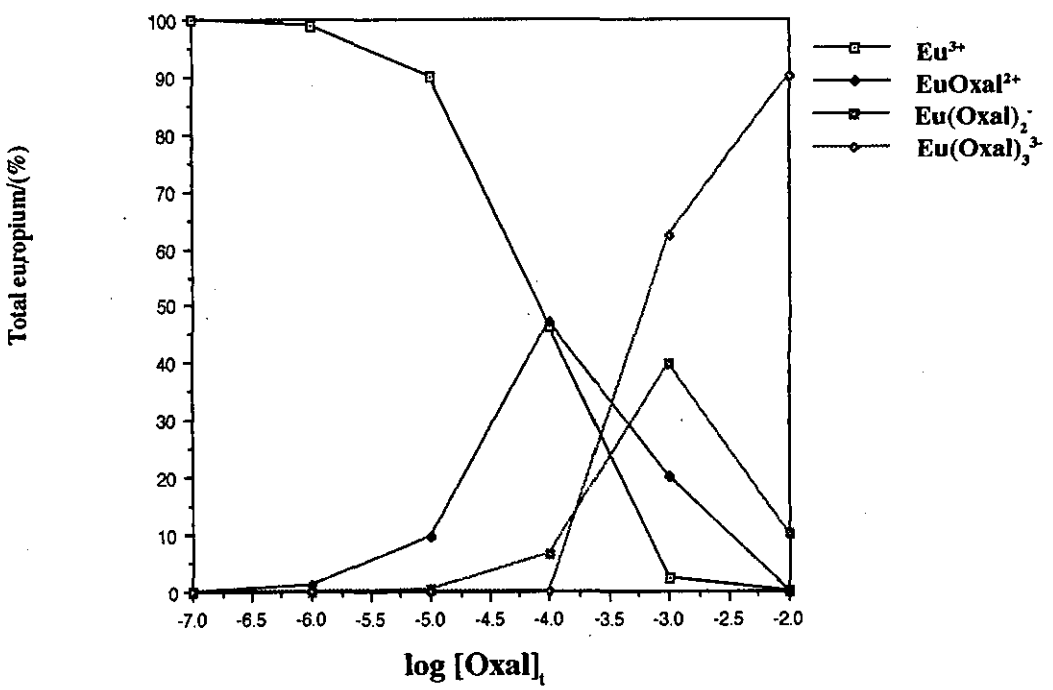


Figure 4.12: Europium speciation as a function of oxalic acid concentration

As can be seen from the above speciation plot the overall charge of the europium species changes as the oxalic acid concentration is increased. This will have a profound effect on the K°_d measurement. The ion exchange utilises

a cation exchanger in batch equilibrium with the solution under study. Clearly at low oxalic acid concentrations when the europium is present as cationic species the K°_{D} value will be large. However with increasing oxalic acid concentration less europium will be bound to the resin and subsequently the measured K°_{D} will decrease. At such large oxalic acid concentrations the amount of europium bound to the resin will be minimal. Upon the addition of humic acid to the system the distribution coefficient will change little since the majority of the europium is present in solution in the form of anionic oxalate complexes. Consequently the ratio K°_{D}/K_{D} will tend to unity. Refering to equation 4.3:

$$\log\left(\frac{K^{\circ}_{D}}{K_{D}} - 1\right) = \log \beta_{1} + \log [HA] - \log A \qquad (4.4)$$

As a function of increasing oxalic acid concentration the expression $\log K^{\circ}_{D}/K_{D}$ -1 will tend to unity and thus remain constant. Also the amount of humic acid added is constant (typically 50ppm). Hence the stability constant, β_{1}, becomes a direct function of A, the side reaction coefficient.

The authors state that mixed complex formation only becomes significant at critical concentrations of competing ligands. For a europium concentration of 10^{-6} mol l^{-1} and 50ppm humic acid the critical concentration for oxalic acid before mixed complex formation is observed is ~10^{-3} mol l^{-1}. If the A term is calculated for 1 x 10^{-5} mol l^{-1} oxalate concentration we obtain:

$$A = 1 + \Sigma \beta^{L}_{n} [L]^{n}$$

$$A = 1 + \{354813 * 1 \times 10^{-5}\} + \{7413102413 * [1 \times 10^{-5}]^{2}\}$$

$$A = 2.096$$

$$\log A = 0.32$$

If the A term is recalculated using an oxalate concentration of 1 x 10^{-2} mol l^{-1}.

$$A = 1 + \Sigma \beta^{L}_{n} [L]^{n}$$

$$A = 1 + \{354813 * 1 \times 10^{-2}\} + \{7413102413 * [1 \times 10^{-2}]^{2}\} + \{3.09 \times 10^{13} * [1 \times 10^{-2}]^{3}\}$$

$$A = 31644858$$

$$\log A = 7.50$$

Obviously, the stability constant β_1 will change greatly over the oxalic acid concentration range of 10^{-5} to 10^{-2} mol l^{-1}. However, this does not appear to be due the formation of a mixed complex but a consequence of the limits of experimental design i.e: the distribution coefficient has a limiting factor, so as more oxalic acid is added to the system the only term in equation 4.4 is the 'A' the side reaction coefficient. This rise in the 'A' term accounts for the observed increase in β.

CHAPTER FIVE

The role of humic acid at alkaline pH

5.1 Introduction and review

Virtually all equilibrium studies performed on humic substances have been at acidic or neutral pH. This is to eliminate the presence of any other equilibrium reactions involving other complexing agents. Unfortunately, in geochemical systems and waste repository sites it is necessary to consider the presence of other agents and in particular, the possible formation of hydroxides and carbonates at pHs above neutral. The competitive effect of these inorganic ions on metal-humate complexes will determine the speciation, and ultimately the migration of metal ions in high pH environments.

At present, it is unclear whether humic substances can effectively compete for the speciation of a toxic metal at alkaline pH. If they are able to do so, it is likely they are able to because of two mechanisms: humic substances have phenolic structures which show very strong complexation with metal ions. It is envisaged [69] has the pH is increased these phenolic groups become deprotonated and participate in binding with metals. Alternatively, humic substances, via either phenolic or carboxylate groups form mixed complexes with inorganic ligands such as carbonate or hydroxide [68].

The aim of this study is to investigate the role of humic substances at alkaline pH and to determine if humic substances play any significant part in the speciation of a toxic metal at such high pHs, since this will ultimately effect the metals migration through a groundwater system. If humic substances are important, can current geochemical models accurately predict their role.

5.2 Humic acid studies

Due to their heterogenity and polymeric nature, humic substances undergo changes in geochemical configuration if conditions of a particular system change.

The aim of this study was to determine whether humic substances change their shape as a function of pH. This change may have important consequences, since it may open up more complexing sites on a humic molecule and hence increase the complexing ability of the humic acid. Although studies have been performed on humic substances to look at their size [70,71], none have been performed as a function of pH.

To investigate this phenomena, two techniques were employed; atomic force microscopy and ultrafiltration.

5.2.1 Atomic Force Microscope

The atomic force microscope (AFM) was invented by Binning, Quate and Gerber in 1986[72]. Essentially it consists of a sharp, stylus tip connected to a cantilever. This cantilever is deflected as the tip interacts with the surface. The deflection is measured by means of a laser beam that is deflected off the cantilever to a photodiode. The ouput from the photodiode maintains a steady displacement by varying voltage to a piezoelectric control. Thus the varying voltage replicates the surface topography. As well as giving atomic resolution of surfaces the AFM has the added advantage in that the sample being scanned does not need to be a conductor.

Microscopical studies on humic substances have been limited and their is further scope for work in this field. Early morphological studies on humic and fulvic acids utilised transmission and scanning electron microscope (TEM, SEM) [73,74]. The problems with these electron microscope techniques was in sample preparation. For example the need for uniform distribution of the sample over a support surface as well as the problems of surface tension force.

which tend to agglomerate the samples. To overcome these problems involved in sample preparation, Stevenson and Schnitzer [75]developed new preparatory techniques and obtained high resolution TEM images. From the results five major types of structure were observed with both humic and fulvic acids.

Initial investigations on the structure of humic acid were performed by a scanning tunneling microscope. However, due to the non-conductive nature of the humic acid sample, the AFM technique was preferred. To investigate the use of AFM a concentrated (>200ppm) Aldrich sodium humate samples were prepared in HPLC grade water. The samples were then pipetted onto cleaned glass microscope slide. The samples were allowed to dry and then scanned using the AFM at the Company Research Laboratories (CRL) at the BNFL Springfields site. An AFM image of the samples is shown in figure 5.1.

From the figure small spheroid structures can be seen, varying in diameter from between 50nm to 200nm diameter. Due to the concentration of the sample the glass slide was coated with humic acid and therefore discrete structures could not be identified. Nevertheless, the AFM had identified and measured discrete structures of the humic acid sample.

Further studies were carried out on two Aldrich sodium humate samples deposited on glass substrates and under pHs of 2.6 and 11.2. AFM images of these samples are shown in figures 5.2 and 5.3.

Although it appears their is a change in shape of the humic acid as a function of the pH it is in fact due to dragging of the humic acid by the AFM tip, thereby causing elongation of the sample as it is dragged by the tip. Nevertheless, with future refinement of both the equipment and technique AFM has the potential to clearly define humic acid geometry.

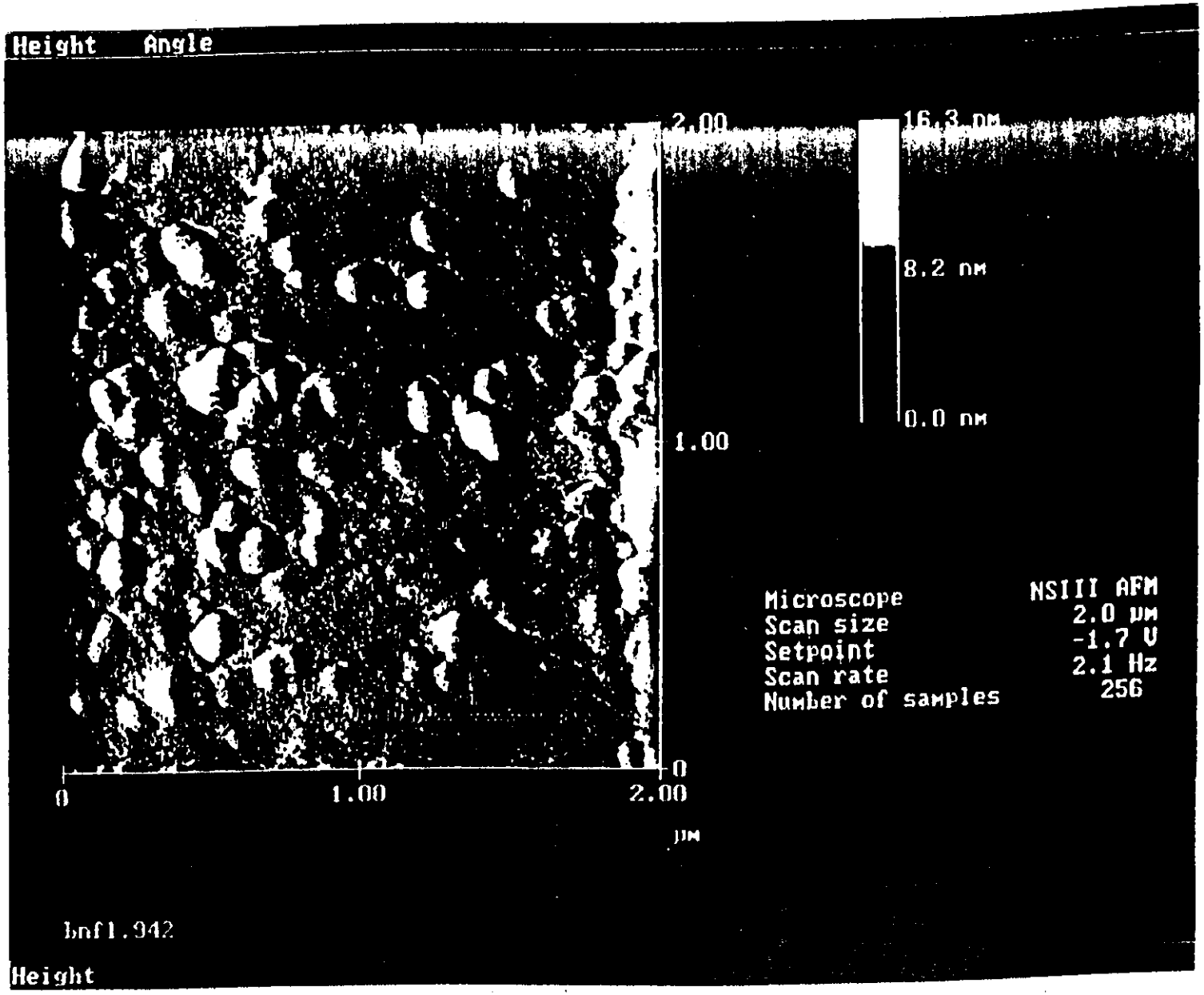


Figure 5.1: Concentrated aldrich sodium humate on glass substrate

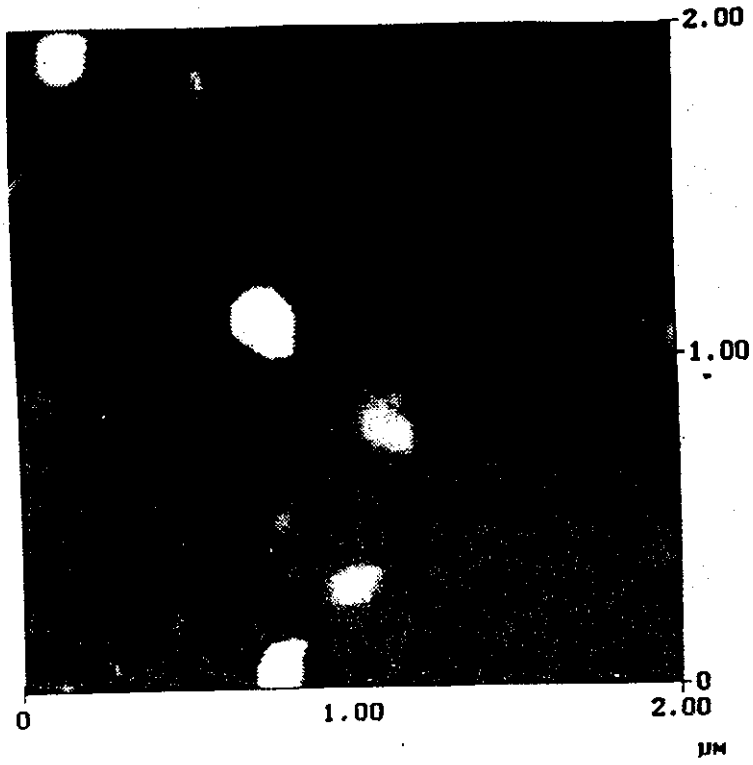


Figure 5.2: Aldrich sodium humate sample at pH 2.6

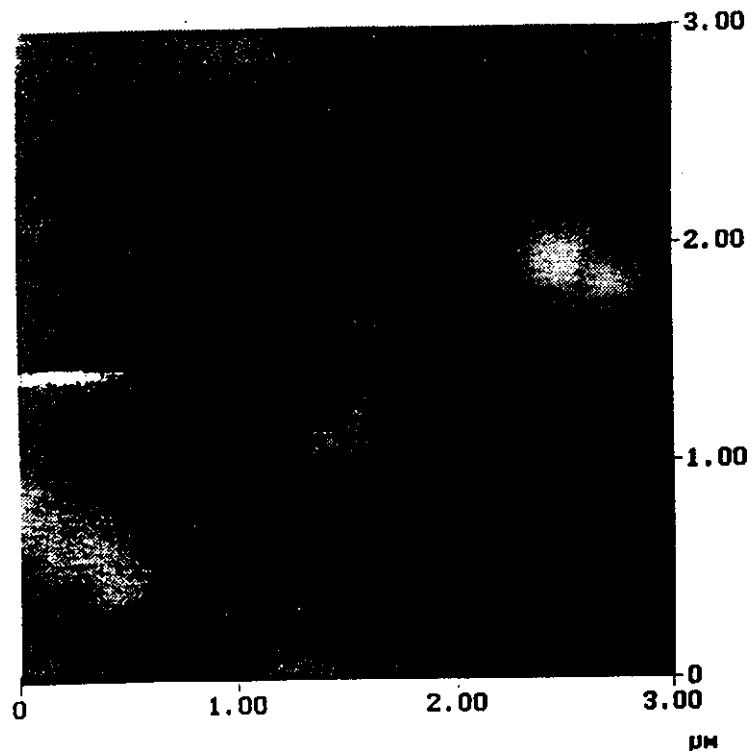


Figure 5.3: Aldrich sodium humate sample at pH 11.2

5.3 Ultrafiltration studies

A series of ultrafiltration experiments were performed to observe any change in size of humic acid as a function of pH. Three 250ml samples of 50ppm Aldrich humic acid were prepared at pHs 4.2, 6.5 and 11.6. All samples were filtered using the following general procedure.

100ml of the sample solution was placed into an Amicon 8050 stirred ultrafiltration unit, above a filter membrane (45mm in diameter cut from a 150mm membrane disc). All filter membranes were soaked in sodium chloride (5% w/v) solution then in copious amounts of distilled water. The unit was assembled and a nitrogen atmosphere applied.

The first 10ml collected from the cell was discarded, the next 10ml was kept for analysis and the remainder of the solution was filtered. The membrane filter was changed after each run for the next pore size membrane and the experiment repeated.

The membrane pores sizes were:

Membrane Code	Pore Size
YC05	<1nm
YM1	~1nm
YM5	<1.3nm
YM10	<1.7nm
YM30	<2.1nm
YM100	<5nm

The ultra-violet absorbance at 254nm of each of the initial three samples was measured and of all the subsequent filtrates. From the results the percentage of humic acid at the three pHs passing through each filter could be calculated.

Table 5.1 shows the results obtained from the ultrafiltration experiments.

pore size	pH 4.2	pH 6.5	pH 11.6
5nm	95.56%	95.19%	83.29%
2.1nm	80.49%	84.03%	38.12%
1.7nm	59.74%	56.11%	26.36%
1.3nm	32.47%	27.93%	15.66%
~1nm	26.01%	9.01%	6.00%
<1nm	7.11%	1.49%	1.86%

Table 5.1: Percentage of total humic acid concentration passing through filters

From the above table it can clearly be seen that as the pH is increased the amount of humic passing through the 1.7nm filter pores size is markedly decreased. This suggests that at alkaline pH the humic acid has uncoiled in shape and therefore is unable to filter through the smaller filter pore sizes as easily as at acidic neutral pHs. Such an observation leads to the theory of phenolic groups dissociating at high pH, thus causing electrostatic repulsion and allowing the humic acid molecule to uncoil. A column graph of the above results is shown in figure 5.4. Such a result will have important implications for metal - humic acid binding since at high pH more complexing sites on the humic acid molecule may become available for binding.

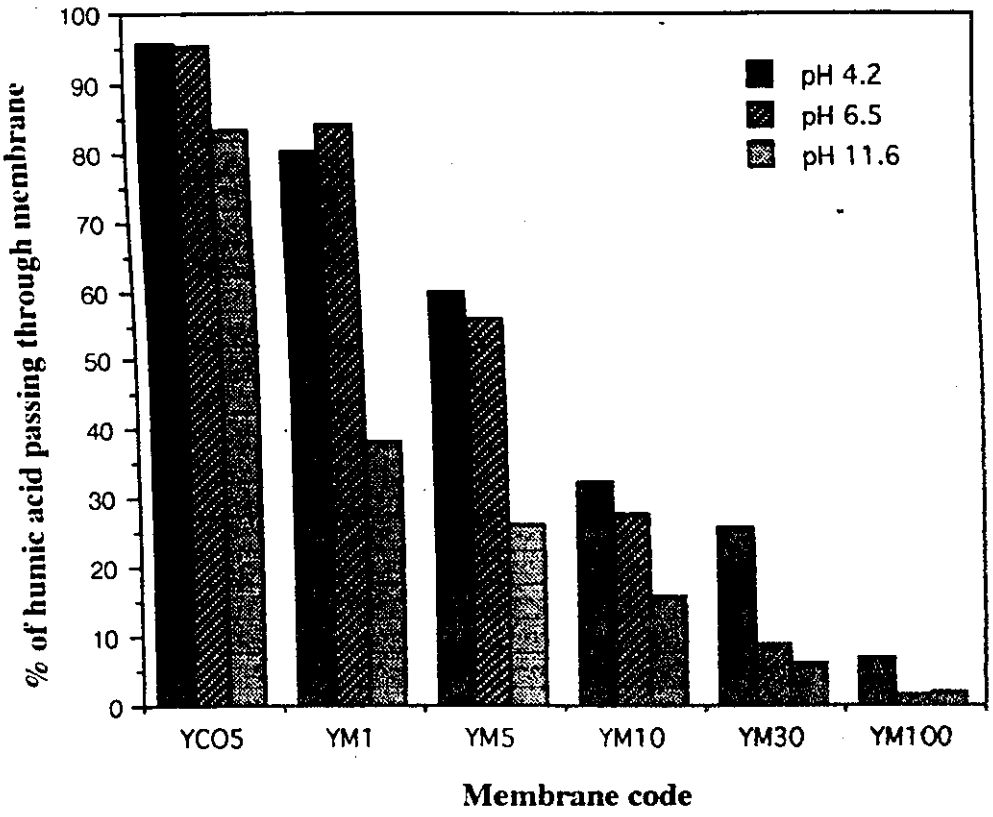


Figure 5.4: A column graph showing humic acid passing through filters as a function of pH

5.4 Preliminary investigations in the study of metal - humic acid complexation at alkaline pH

Initial studies into the study of the complexing ability of humic acid at alkaline pH involved the use of a number of techniques. All the techniques used proved ineffective in the determination of equilibria at high pH. Below is a summary of the techniques and a brief description of the problems encountered in the application of these techniques.

5.4.1 Ion exchange - cation

The ion exchange technique as described in section 3.4.1 was attempted at pHs of 10, 11, 12 in 0.01mol l^{-1} CAPS buffer in a background electrolyte of

0.1mol l^{-1} $NaClO_4$. The test solution contained 1 x 10^{-5}mol l^{-1} Eu^{3+} + radiolabelled Eu-152 in the presence of 50ppm Aldrich humic acid. and 0.01mol l^{-1} analar sodium carbonate. In all experiments over 98% of the injected europium activity was eluted with 5ml of the 0.01mol l^{-1} CAPS/ 0.1mol l^{-1} $NaClO_4$ background buffer solution. Clearly at high pH the europium is forming anionic complexes but due to all the species in the test solution being anionic, the cation column ion exchange experiments were unable to differentiate between species.

5.4.2 Ion exchange - anion

Scoping experiments were performed by column exchange using a weak anion exchanger, diethylaminoethylcellulose (DEAE) resin (from Whatman). The resin was pretreated using the following procedure. 50g of dry DEAE cellulose was mixed in 1000ml of 0.5mol l^{-1} HCl for 1 hour, by flushing the acid solution through a glass chromatographic column. The resin was then rinsed with copious amounts of distilled water before being resuspended in 0.5mol l^{-1} NaOH for 1 hour. The resin was then again rinsed with distilled water until the pH was neutral. Fines were removed by suspending small amounts of the resin (~5-10g dry weight) in a 1000ml graduated cylinder of distilled water and settling for 1 hour before removing the supernatent. The pretreated resin was stored in darkness.

Control experiments were performed using three test solutions prepared in a buffer solution of 0.01mol l^{-1} CAPS/0.1mol l^{-1} $NaClO_4$ at pH 10.1. The three tests solutions contained C-14 sodium carbonate (supplied by ICN Radiochemicals 2.1GBq/mmol), a 50ppm solution of Aldrich humic acid and a solution of 1 x 10^{-5}mol l^{-1} Eu^{3+} + radiolabelled Eu-152, respectively. In both the sodium carbonate and humic acid solutions the resin retained both the radiolabelled C-14 sodium carbonate and the 50ppm Aldrich humic acid (determined by UV-Vis measurement). Unfortunately, the resin also retained a significant proportion (~30%) of the solution containing the Eu^{3+} solution only. The failure of the europium to be totally excluded from the column mean't that quantitative experiments could not be carried out.

5.4.3 Dialysis

Although dialysis experiments have been reported in the literature for the measurement of europium - humic acid equilibria, problems were encountered in the use of dialysis membranes due to sorption of the europium on the dialysis membrane and on the clips.

Similar experiments using ultrafiltration and potentiometry also proved ineffectual due to sorption and hydrolysis problems, respectively.

5.5 HPSEC studies on the role of humic acid on the complexation of europium at high pH

Due to the ineffectiveness of the above techniques it was decided to undertake a major study using the HPSEC technique. Unfortunately due to the integrity of the silica gel columns the pH of the mobile phase could not be greater than 8.5.

The aim of the experiments was to compare the complexing ability of humic acid at two pHs, 6.5 and 8.5. At pH 6.5 competition for the metal ion from other complexing agents other than humic acid is minimal. Specifically at pH 8.5 increasing amounts of sodium bicarbonate would be added to the system as a competing ligand. By knowing the stability constants of europium carbonate complexes it would indicate the stability of the europium humate complex.

5.5.1 HPSEC study of europium - humic acid at pH 6.5

Using the HPSEC method described in section 3.2.1 a range of concentrations of analar grade europium (III) chloride solutions, spiked with radioactive Eu-152 (from Amersham 251MBq ml^{-1} with 0.538mg ml^{-1} inactive Eu carrier), were prepared in a background electrolyte of 0.01mol l^{-1}MES/0.1mol l^{-1} $NaClO_4$ at pH 6.5. To each of these solutions 50ppm (w.v) Aldrich humic acid was added. Conditions of the column and DAD detector are summarised below:

Flow rate	-.50ml/min
Pressure	~ 27 bars
Mobile phase	- HPLC water/0.1 mol l^{-1} EDTA
UV absorption wavelength	- 254nm

The stepped and injection scheme as shown in figure 3.2 was used to achieve separation of the organically bound europium and 'free' europium. Results of the experiments are shown below in table 5.1.

$[M]_t$ Total Eu concentration (mol l^{-1})	[ML] Eu-AHA complex (mol l^{-1})	[M] Eu-free (mol l^{-1})	$\frac{[ML]}{[M]}$	log [ML]	log [M]
3.678×10^{-8}	3.601×10^{-8}	7.724×10^{-10}	46.619	-7.444	-9.112
8.678×10^{-8}	8.461×10^{-8}	2.170×10^{-9}	39.000	-7.073	-8.664
1.368×10^{-7}	1.326×10^{-7}	4.241×10^{-9}	31.258	-6.878	-8.373
5.368×10^{-7}	5.121×10^{-7}	2.469×10^{-8}	20.739	-6.291	-7.607
1.037×10^{-6}	8.400×10^{-7}	1.970×10^{-7}	4.263	-6.076	-6.705
5.037×10^{-6}	3.682×10^{-6}	1.355×10^{-6}	2.717	-5.434	-5.869
1.004×10^{-5}	5.100×10^{-6}	4.940×10^{-6}	1.033	-5.292	-5.306
5.004×10^{-5}	7.106×10^{-6}	4.293×10^{-5}	0.166	-5.148	-4.367

Table 5.1: Results of europium - humic acid system at pH 6.5

From the above data, a Scatchard plot was obtained as shown in figure 5.5. From the plot, stability constants were obtained corresponding to the europium interaction with strong and weak sites of the humic acid.

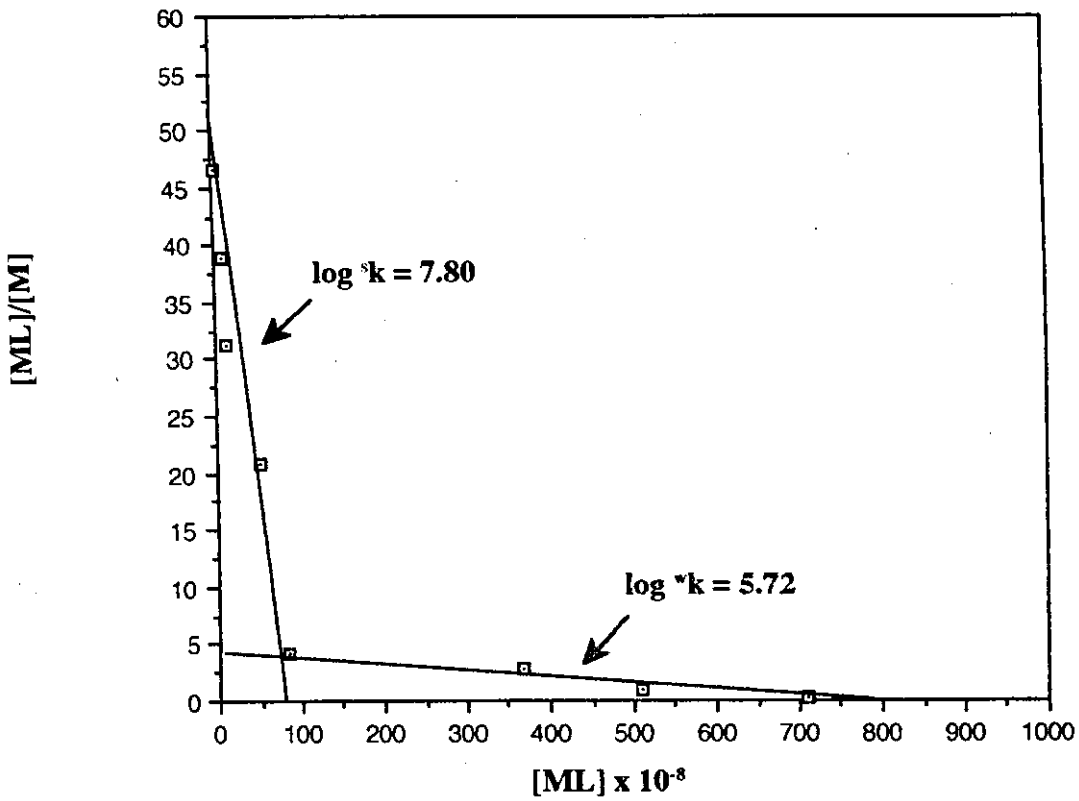


Figure 5.5: Scatchard plot of europium humic acid

5.5.2 HPSEC study of europium - humic acid at pH 8.5

To investigate the role of humic acid at pH 8.5 in the presence of sodium bicarbonate the HPSEC was modified to achieve separation of the bicarbonate/carbonate from the humic acid. To prevent the use of EDTA in the mobile phase a low europium concentration was used which would allow for negligible free europium in the system.

A single mobile phase was used in this HPSEC investigation. The mobile phase consisted of 0.01mol l^{-1} CAPS/0.1mol l^{-1} $NaClO_4$ at pH 8.5. Initial control experiments were perfomed to determine the retention times of humic acid and sodium bicarbonate through the chromatographic columns and detectors.

The retention time of a 50ppm humic acid solution is shown below in figure 5.6. The conditions of the column are shown below:

Flow rate	- .50ml/min
Mobile phase	- 0.01mol l^{-1} CAPS/0.1mol l^{-1} $NaClO_4$
Pressure	~ 24 bars

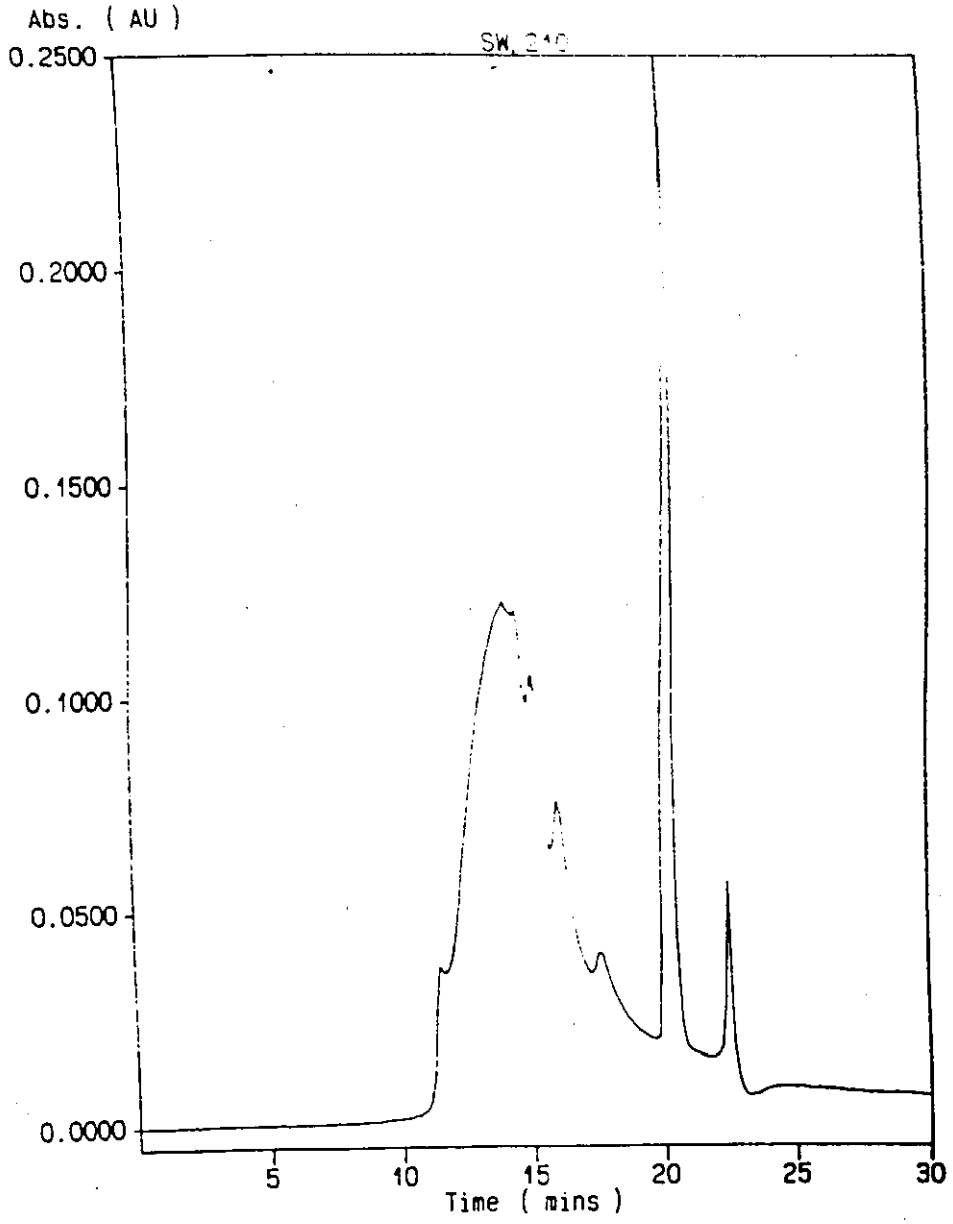


Figure 5.6: A DAD chromatogram of 50ppm humic acid

Once the humic acid retention time was established, a 10ml volume test solution was prepared containing 1 x 10^{-5} mol l^{-1} sodium bicarbonate which contained 25µl aliquot of radiolabelled C-14 sodium bicarbonate (from ICN Radiochemicals, 2.1GBq/mmol) and was injected into the HPSEC under the same conditions as the previous humic acid sample. A radiochromatogram of the sodium bicarbonate is shown in figure 5.7.

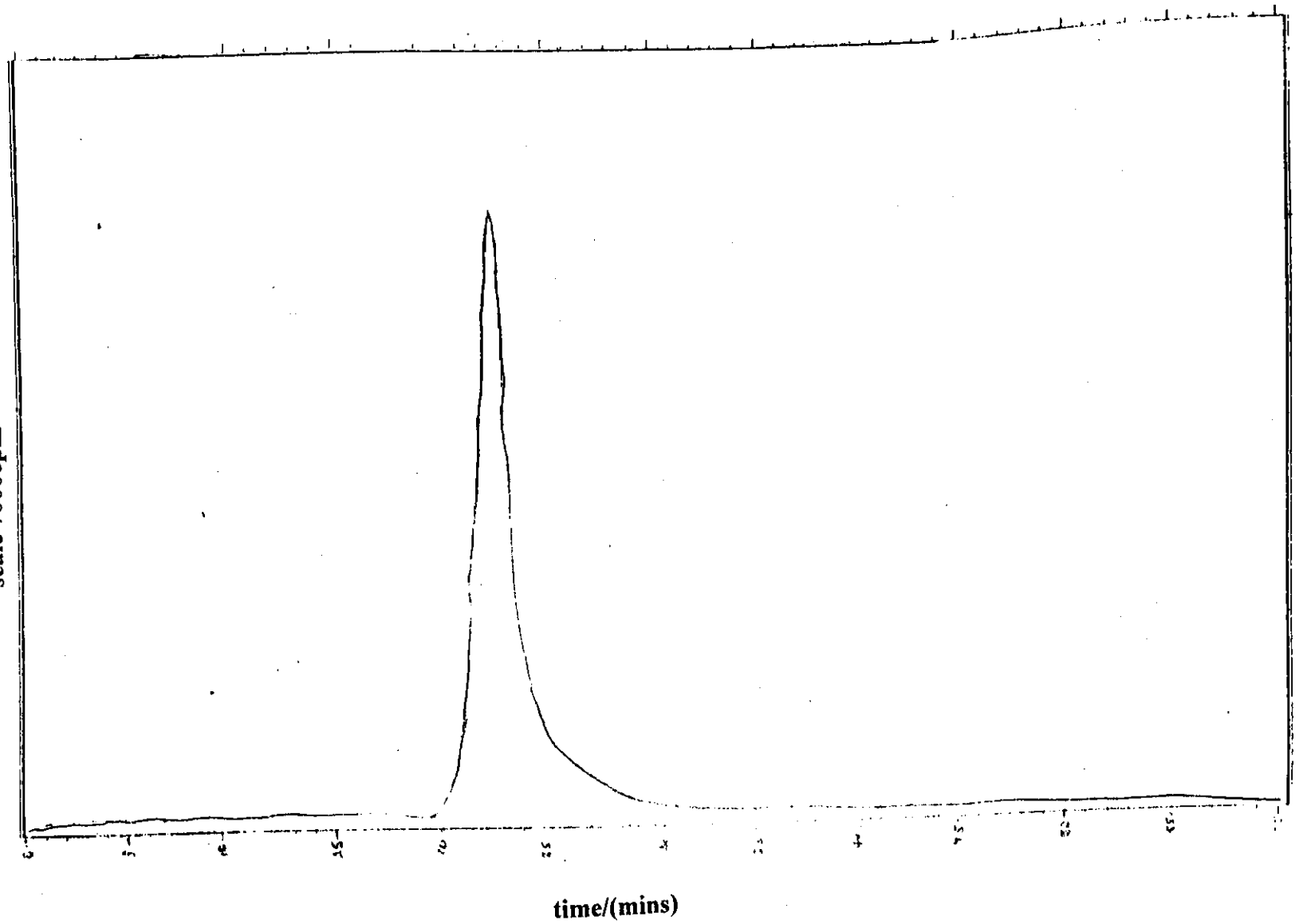


Figure 5.7: Radiochromatogram of C-14 sodium bicarbonate

As can be seen from figures 5.6 and 5.7 there is good resolution between the humic acid and the sodium bicarbonate with maximum peak retention times of 13min 50secs and 22min 23secs respectively.

To investigate the equilibria of the above ligands with europium a range of solutions were prepared containing 1x 10^{-7}mol l^{-1} analar europium (III) chloride + radiolabelled Eu-152, 50ppm (w.v) Aldrich humic acid and a series of sodium bicarbonate concentrations ranging from 10^{-7} to 10^{-2} mol l^{-1} with each series also containing radiolabelled C-14 sodium bicarbonate. (from ICN Radiochemicals 2.1GBq/mmol). All samples were prepared in the 0.0mol l^{-1} CAPS/0.1 mol l^{-1} $NaClO_4$ buffer solution at pH 8.5.

As described in section 4.3, in the Canberra Packard Flo-one/Beta Radiometric A140 detector liquid scintillation cocktail (Ecoscint A: National Diagnostics) was mixed with the column eluate to permit the eluted β activity to be monitored. Upstream of the mixing chamber a splitter, within the detector, was used to divert part of the eluate to an LKB Redifrac fraction collector. The collected fractions were monitored for γ activity using a Philips PW 4800 γ counter.

The conditions of the HPSEC and the DAD are summarised below:

Flow rate	- .50 ml/min
Pressure	~ 24 bars
Mobile phase	- 0.01mol l^{-1} CAPS/0.1mol l^{-1} $NaClO_4$
UV absorption wavelength	- 210nm

A radiochromatogram of the europium activity at a sodium bicarbonate concentration of 1 x 10^{-5} mol l^{-1} is shown in figure 5.8.

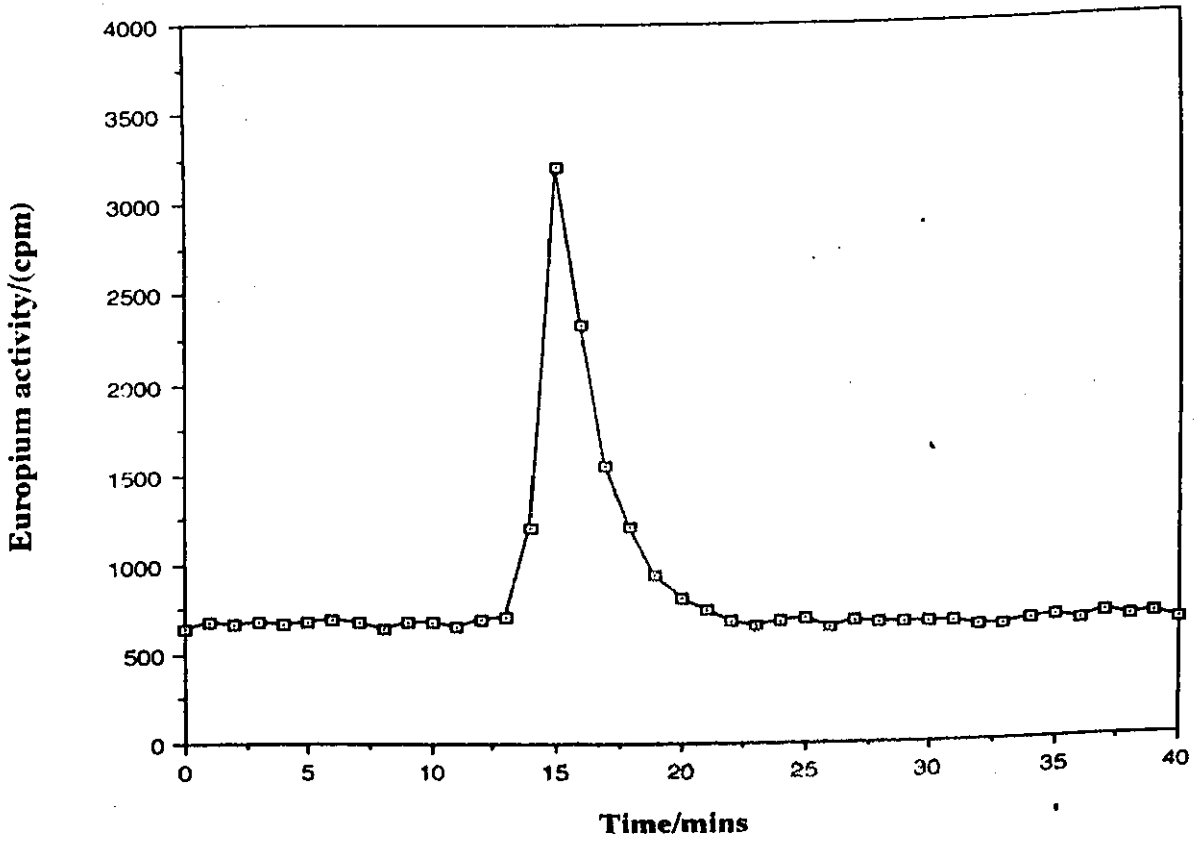


Figure 5.8: Radiochromatograom of europium - humic acid - sodium bicarbonate (1 x 10^{-5} mol l^{-1})

Results for the series are shown below in table 5.2.

$[NaHCO_3]_t$	% europium humate	%europium carbonate
$1.00 \times 10^{-}$	100.0	0.0
$1.00 \times 10^{-}$	100.0	0.0
1.00×10^{-5}	100.0	0.0
1.00×10^{-4}	100.0	0.0
$1.00 \times 10^{-}$	62.5	37.5
$1.00 \times 10^{-}$	27.0	82.0

Table 5.2: Speciation of europium as a function of sodium bicarbonate concentration

To illustrate the change in speciation of the europium as a function of the increasing concentration of sodium bicarbonate a radiochromatogram of the radiolabelled europium is shown in figure 5.9.

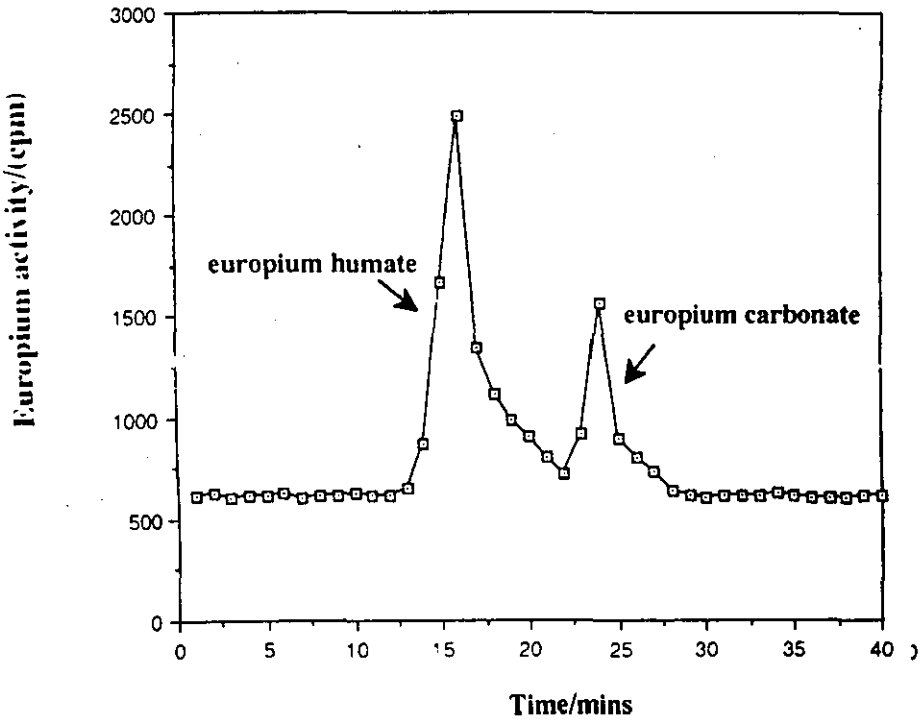


Figure 5.9: Radiochromatogram of europium - humic acid - sodium bicarbonate (1×10^{-3} mol l^{-1})

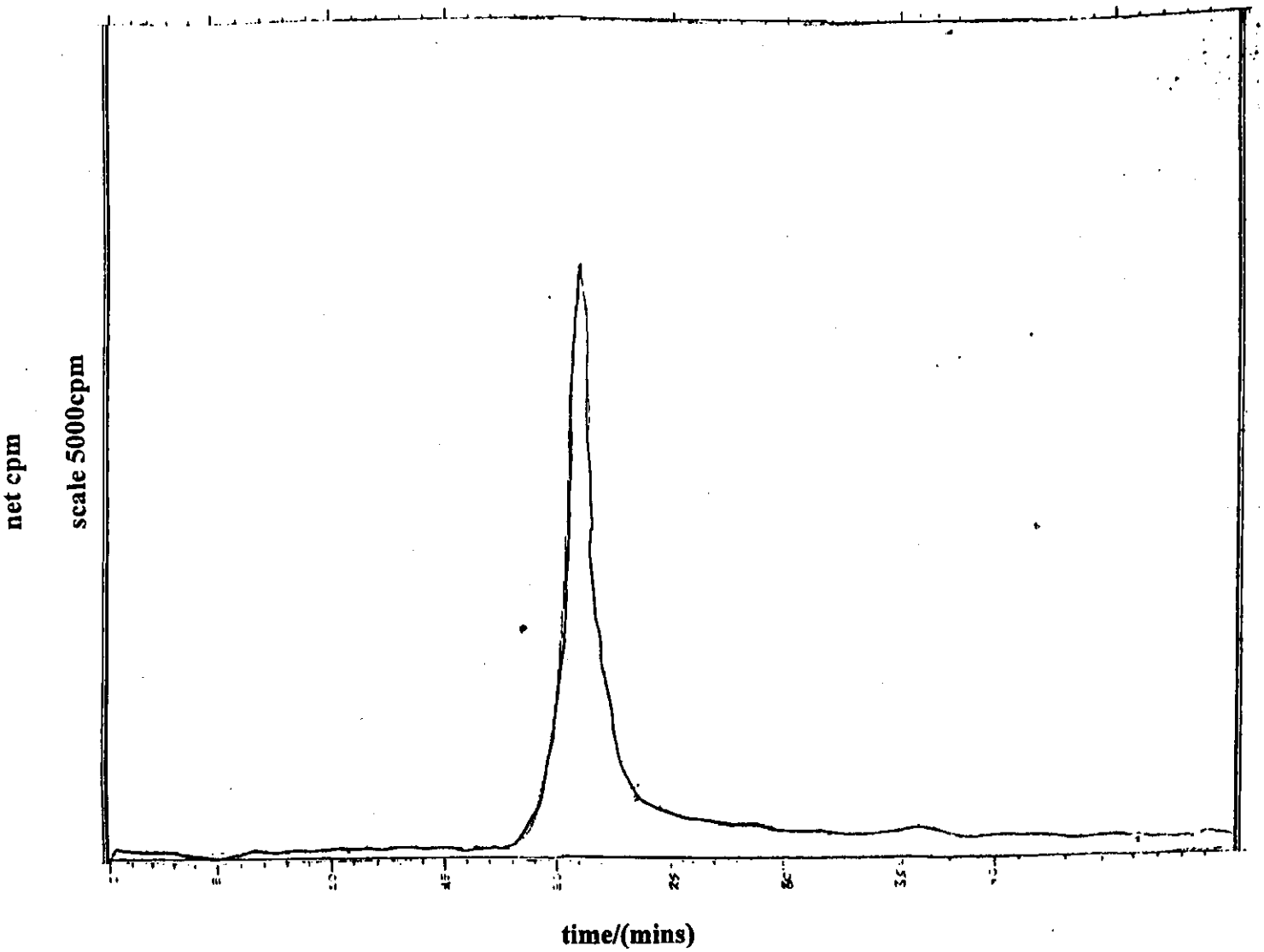


Figure 5.10: Radiochromatogram of europium - humic acid - sodium bicarbonate (1×10^{-3} mol l^{-1})

In addition to the europium chromatogram, figure 5.10 shows a radiochromatogram of the C-14 labelled sodium bicarbonate.

From figure 5.10 it can be seen that there is no change in the retention time of the radiolabelled sodium bicarbonate. Since no actvity has eluted with the humic acid, this is strong evidence for the absence of mixed complexes, since any significant mixed complex formation would have resulted in some of the C-14 activity eluting from the chromatographic columns with the same retention times as the humic acid. Therefore, in the system studied the humic acid and the sodium bicarbonate behave as single ligands. This conclusion again does not support the findings of Maes et al [69] who observed mixed complex formation at high concentrations of carbonate.

A criticism against the HPSEC technique employed in these investigations is that the ratio of inactive to active sodium bicarbonate is extremely high and no C-14 activity is associated with the humic acid is may not be due to the lack of formation of mixed complex formation but may be a consequence of the high inactive to active ratio.

To test this hypothesis, a highly active sample of sodium bicarbonate was prepared. at a total sodium bicarbonate concentration of 1×10^{-3} mol l^{-1}. The activity was such that if any mixed complex was formed then there was sufficent radiolabelled sodium bicarbonate to confirm this. Figure 5.11 shows the C-14 radiochromatogram for this experiment. Clearly, activity is not associated with the humic acid retention time. This again confirms thelack of mixed complex formation in the system.

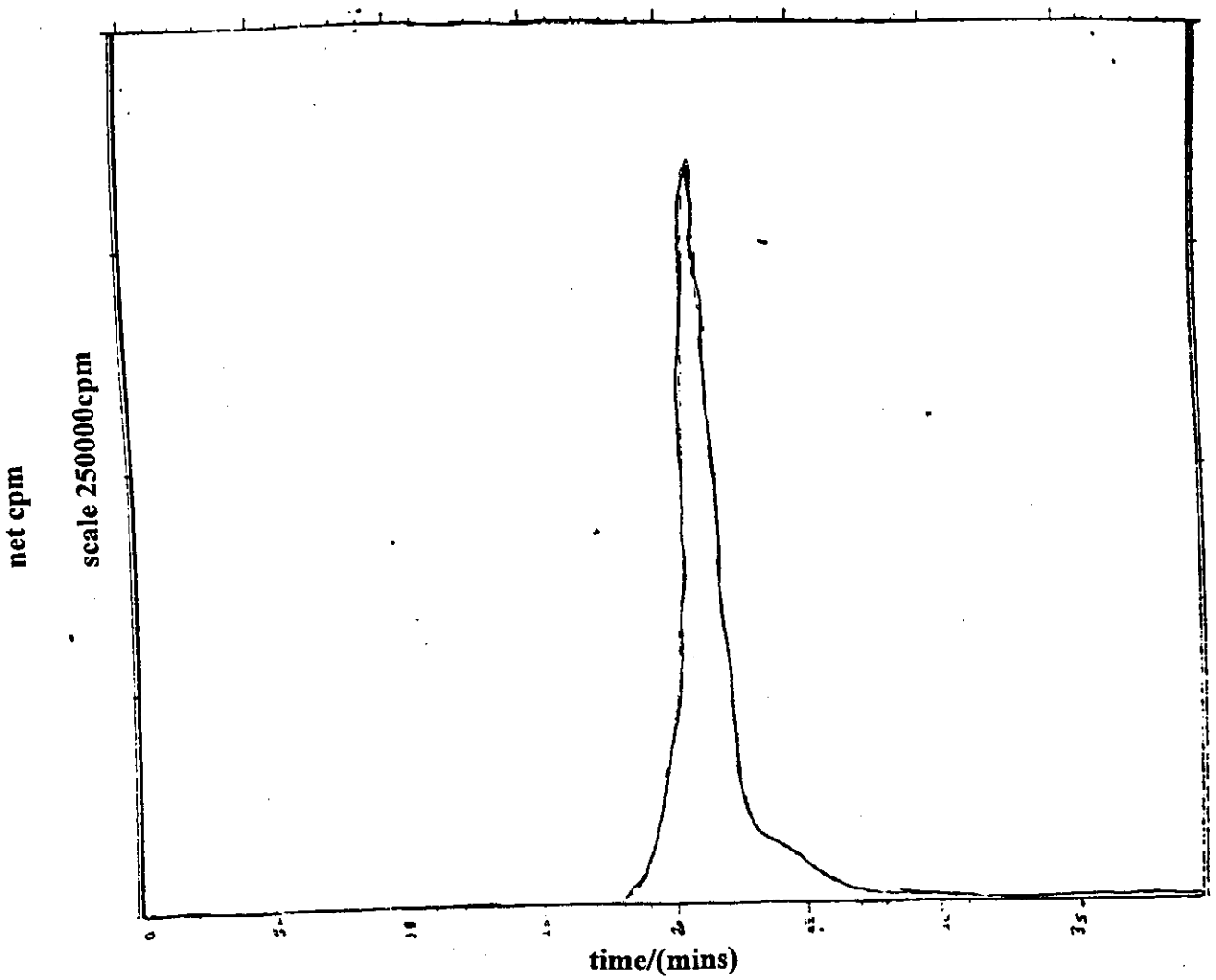


Figure 5.11: Radiochromatogram of europium - humic acid - sodium bicarbonate (1x 10^{-3} mol l^{-1})

Conclusions

From figure 5.11 and 5.12 it appears that there is no evidence for the formation of mixed complexes. To further investigate the following parameters were inputted into MINTEQA2 so that the speciation of the system could be modelled.

europium - humic acid:

strong site concentration	= 8.1 x 10^{-7} mol l^{-1}
strong site interaction, log ^{s}K	= 7.80
weak site concentration	= 7.8 x 10^{-6} mol l^{-1}
weak site interaction, log ^{w}K	= 5.72

europium carbonate [53]:

$\log k_1 = 6.96$

$\log k_2 = 7.22$

The above parameters were inputted into MINTEQA2 and the system was modelled as a function of increasing sodium bicarbonate concentration. The comparison between the model predictions and the experimental data is shown in figure 5.12.

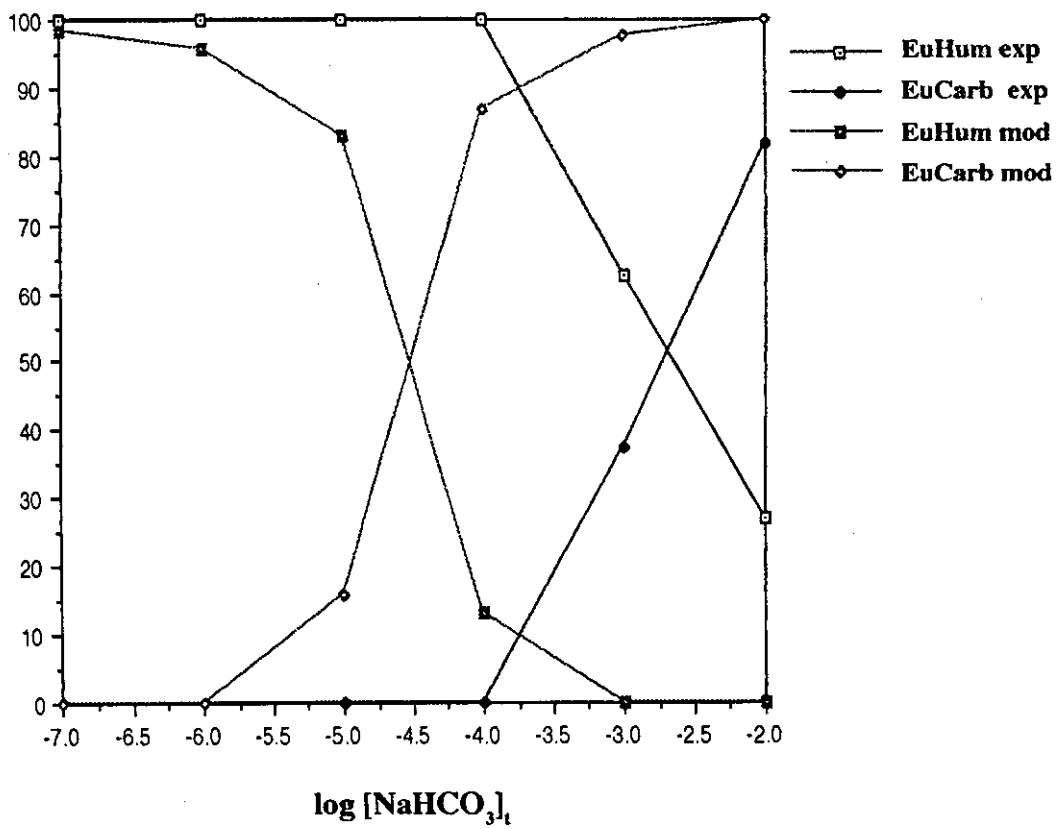


Figure 5.12: Speciation of europium - humic acid - sodium bicarbonate systems. Comparison of experimental data with model predictions

It can be seen that there is poor correlation between experimental data and model predictions. At a sodium bicarbonate concentration of 1 $x10^{-4}$ mol l^{-1}, the model predicts ~13% of the total europium bound to the humic acid, where as experimentally it is found to be >99%.

Clearly the inputted parameters used to model europium - humic acid binding,at pH 6.5 are not appropriate at pH 8.5, since they provide a far lower estimate of the metal bound than was experimentally found. This is in contrast to previous systems in the research project which have been modelled and where model predictions have consistently predicted more metal bound than was experimentally observed.

The conclusion is that at pH 8.5 humic acid shows a marked increase in its binding for europium and therefore humics can compete with high levels of carbonate concentrations.

CHAPTER SIX

Summary

6.1 Summary and Conclusions

This research project has identified a number of important reactions that are likely to occur if a toxic metal breaches a waste disposal site and enters soils and groundwaters. It has generated quantitative data for these reactions unde a variety of conditions.

Natural organic acids have been recognised as important agents in the speciation, and hence migration, of toxic metals. A literture search identified organic acids such as acetic and citric acids has being both sufficiently abundant within the Geosphere and having the ability to form complexes with metals. Additionally, high molecular weight organics, such as fulvic and humic acids are in sufficient concentration within the environment to have a major effect on the speciation of toxic metals.

Initial experimental investigations were concerned with potentiometric titrations of selected low molecular weight organic acids with the metal ions, nickel and europium. Results have shown the benefits of potentiometry over other analytical techniques. It is fast, requires simple equipment, and involves the measurement of a variable (i.e: H^+) over several orders of magnitude. The application of state of the art computational methods for analysis of the data allows for accurate determination of both protonation constants for acids and stability constants for metals. Additionally, it provides an efficient method for other metal-ligand equilibria and may be used to derive values for organic pollutants in the environment and their interactions with metals.

The values produced for nickel and europium are in good agreement with IUPAC critically assesed literature data. Such values can be incorporated into risk assessments and groundwater speciation models of the Drigg low level waste site and assist in a better understanding of the solution chemistry. It should be noted that such values must always be used in conjunction with the concentrations of ligands. For example,

the values for acetic acid with both nickel and europium illustrate the weak complexing beahaviour of the acetate ion. However, if the acetic acid is in sufficent excess over both the toxic metal and other competing ligands (as may well be the case for Drigg leachates), then the acetic acid will have major influenec on the speciation of the metal ion and a significant percentage of the metal may well be present as acetate complexes.

Salicylic and phthalic acids were studied as simple analogues to functional groups that are thought to occur on humic acids. Both for nickel and europium the stability constants highlighted the strong complexation of the salicylate anion, and the correspondingly weak complexation of the phthalate anion.

Extension of the potentiometric technique to competing ligand systems showed a number of significant results. Firstly, in the systems studied there was no evidence of any synergistic effects and the values obtained for individual metal-ligand equilibria were in a agreement with earlier experimental results. Additionally, overall equilibrium constants were derived and could be applied to such competing systems. Speciation plots of such systems allow for accurate determination of the concentrations for the various metal complexes that exist.

A comprehensive evaluation of High Performance Size Exclusion Chromatography (HPSEC) and its suitability for the study of equilibria between metals and humic acids highlighted the problems of dissociation with nickel - humate complexes. However for europium the technique proved suitable and provided a range of data for various europium concentrations. By application of the Scatchard Plot, stability constants were obtained for europium with humic acid. Such values agree with earlier studies[76,77].

Further comparison of the HPSEC results was carried out by using a column ion exchange technique. There was good agreement with both techniques and with the MINTEQA2 speciation model.

Stability constants generated from the HPSEC experimental data indicated the strong complexing ability of humic acid. It should be borne in mind that the concentrations of metal used in these experiments are likely to be several orders of magnitude higher than concentrations of toxic metals found in soils and groundwaters. As a result the stability constants generated from the experimental data are likely to be an under-estimation of the even stronger binding that will occur under environmental conditions. At very low metal concentrations the metal will bind to the strongest sites on the humic acid. As the metal concentration is increased weaker sites on the humic acid will be become complexed, hence producing a lower average stability constant.

Further work on metal acid equilibria was performed by ion exchange using two techniques, batch and column. Since metal - humate complexes are negative, ion exchange provides a fast and easy way to determine the amounts of 'free' and bound metal. Column cation exchange was used to study the complexation between europium and humic acid as a function of pH. Analysis of the data illustrated the evidence for carboxylate groups with pKa's of between 4-5 on the humic acid molecule.

A comparison was carried out between batch and column ion exchange techniques for both nickel and europium. With both metals as the metal concentration was increased, column ion exchange gave lower metal bound for a given total metal concentration than batch. The conclusion from this observation was that weak complexed sites on the humic acid molecule dissociated as the 'free' and bound metal were separated down the column of resin. The result again indicates the theory that progressievly weaker sites on the humic acid are taken by increasing metal loading. However, it cannot be concluded that these weak sites are metal bound to the electric field associated with the humic acid.

The HPSEC was developed to monitor experiments involving competing high and low molecular weight organic acids and their interactions with europium. The use of radiolabelled europium allowed for a powerful technique and quantitative speciation of the europium. The overall conclusion from these experiments was no evidence for mixed complexes. A possible exaplanation for this could be that in a system containing europium - citric acid - humic acid the europium will first bind to the citrate anion to form a neutral or negatively charged complex. Due to the negative charge of the humic acid, by electrostatic repulsion, the europium citrate is repelled and cannot approach the binding sites on the humic acid molecule. However the usefulness of the HPSEC technique for investigating such systems should be extended to look at other systems involving organic acid, such as amino acids and organic pollutants with humic acids.

Application of MINTEQA2 to the mixed systems produced reasonable estimates of the amounts of metal bound as a function of the total metal concentration.

Investigation of the role of humic acid at alkaline highlighted the problems of applying a number of analytical techniques at high pH. Ultrafiltration indicated the increase in size of humic acid molecules as the pH is increased. This is attributed to the dissociation of the functional groups on the humic acid, thereby causing electrostatic repulsion.

HPSEC, as with the previous mixed systems, was used to investigate the equilibria of europium when in the presence of humic acid and increasing concentrations of carbonate. Again there was no evidence of mixed complexes between the metal and the humic acid and carbonate. However, the humic acid did show enhance binding with the europium at pH 8.5 relative to the stability constants that were determined at pH 6.5. As a consequence of this stronger complexation the majority of the total concentration of europium was bound to the humic acid until high

concentrations of carbonate. It is envisaged that as the pH is increased phenolic groups on the humic acid become dissociated and are therefore available to bind with the europium. Additionally, as the humic acid uncoils with the change in pH more functional groups on the molecule become accessible for binding with the europium.

Under these alkaline conditions, MINTEQA2 failed to predict the speciation of the europium. This highlights the need to correctly interpret stability constants involving humic substances and to define rigorously the parameters under which the constants are operable. The results have shown that MINTEQA2 is able to model experimental data at pH 6.5 but at higher pH is unable to do so.

In conclusion, the study of natural organic acids in this research has indicated their importance as complexants for metals in groundwaters. Their effects have been determined under varying conditions of metal concentration, pH and competing ligands. From these studies quantitative data has been generated concerning the equilibria with metal ions.

CHAPTER SEVEN

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APPENDIX ONE

Below is given a number of stability constants for the reaction of metal ions with humic acid under the conditions used.

Ion	Acid	$\log \beta_1$	$\log \beta_2$	α	pH	μ/M
UO_2^{2+}	HA	5.1	8.9	0.47	4.04	0.1
		7.6	11.5		8	
		6.7	11.5		6	0.01
		7.8			3.5-7	0.1
	FA	7.4	13.0		6	0.01
U^{4+}	HA	7.0	11.5		6	0.01
	FA	6.6	11.6		6	0.01
Th^{4+}	HA	13.2	18.4	0.54	5.03	0.1
		10.7	15.8	0.37	3.99	0.1
		17		1.0		0.1
	FA	10.8	15.1	0.84	5.00	0.1
Pu^{4+}	HA	18.8	20.0	1.0	8	
		12.4	17.2		~4	
Am^{3+}	HA	14.4	15.7	1.0	8	
		6.8	10.6		4.5	0.1
		6.4	10.6		6.5	
Eu^{3+}	HA	13.3	14.6	1.0	8	0.1
		7.4	10.3		4.5	0.1
		6.2			4.47	0.05
		5.9			4.48	0.1
Ca^{2+}	HA	2.25		0.44	3.88	0.1
		3.32		0.65	5.01	0.1

APPENDIX TWO

THE ROLE OF LOW MOLECULAR WEIGHT NATURAL ORGANICS IN METAL HUMATE INTERACTIONS

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Europium / Humic acid / Complexation / Acetic acid / Oxalic acid / Citric acid

Abstract

The possibility of mixed ligand complex formation, resulting from the interaction of europium with mixtures of high and low molecular weight naturally occurring organic acids has been investigated. Three systems were studied containing europium with humic acid in the presence of (i) acetic acid (ii) oxalic acid and (iii) citric acid. The various mixtures were analysed using both high performance size exclusion chromatography (HPSEC) and ion exchange (I. Ex). To facilitate the measurements C-14 labelled low molecular weight acids and Eu-152 were used. The observed amounts of complexation in the mixed systems were compared with predicted amounts. Predictions were based both on the complexation parameters derived from analyses of the individual ligand systems and on modelling experiments using MINTEQA2. The agreement of the observed and predicted results was taken as evidence that mixed ligand complexes were absent under the conditions employed.

Introduction

The mobility of radionuclides in the environment is partly determined by their speciation. Consequently their complexation by naturally occurring high molecular weight humic and fulvic acids (HA and FA) has been the focus of much research. However, the role of low molecular weight acids has received less attention. Low molecular weight acids, eg acetic

(HOAc), oxalic (H_2Ox) and citric (H_3Cit) arise from processes such as plant degradation, rhizosphere activity and microbial metabolism (1). These acids also complex metal ions and column studies, for example, have shown that in soil matrices the complexes formed possess enhanced mobilities compared to the original non-complexed metal species (2). Evidence of mixed ligand complexes containing both high and low molecular weight acids also exists (3). Recently certain geochemical speciation codes, eg PHREEQE (4) and MINTEQA2 (5) have been modified to include provision for metal HA and FA interactions. The possible existence of mixed ligand complexes if not taken into account may introduce errors in speciation modelling.

For these reasons, an investigation has been conducted into the complexation behaviour of europium (Eu-III), a typical trivalent metal often used as an analogue of trivalent actinides, with the aforementioned ligands. The evidence for mixed ligand complex formation has been assessed primarily by comparing the observed amounts of complexation in the mixed systems with amounts predicted using, experimentally derived, conditional stability constants (K values) for the single systems. For the physico-chemical significance of the derived K values the reader is referred to the Appendix. Close agreement between observed and predicted complexation behaviour, within the analytical range investigated, has been taken to mean that either mixed ligand complexes are absent or that their impact on speciation modelling is insignificant.

Comparisons between observed and predicted levels of complexation were also made by modelling the complexation behaviour of the various systems with MINTEQA2.

Preparation of samples

Seven series of samples were prepared, all in 0.01mol l^{-1} NaCl at pH = 6.0. Within each series the Eu-III concentrations ranged from ~ 1 x 10^{-8} to 1 x 10^{-4}mol l^{-1}, but the acid concentrations were held constant as shown in Table 1. At higher europium concentrations precipitation of Eu-humate was observed. Apart from the technical grade Aldrich sodium humate used all the chemicals were of A.R. quality or better. The C-14 labelled acids were obtained from Amersham International (citric acid), NEN Research products (oxalic acid) and ICN Radiochemicals, Inc. (acetic acid) with specific activities of 4.07GBq/mmol, 0.19GBq/mmol and 0.592GBq/mmol respectively. All concentrations were calculated taking

into account dilutions and where appropriate the presence of radioactive tracer material.

Experimental procedures

A variety of methods are available for determining metal humic complexation parameters. Unfortunately different methods can give different results. To validate the current study two techniques were used , High Performance Size Exclusion Chromatography (HPSEC) and Ion Exchange (I. Ex).

HPSEC technique

The HPSEC experiments were conducted using a previously described technique (6) but important details are included here for convenience. A Philips PU 4000 series liquid chromatograph was used. The eluate flowed through a Canberra Packard Flo-one/Beta Radiometric A140 detector, containing a 500μl flow through cell. Liquid scintillation cocktail (EcoScint A: National Diagnostics) was mixed with the column eluate to permit the eluted β activity to be monitored. Upstream of the mixing chamber a splitter, within the detector, was used to divert part of the eluate to an LKB Redifrac fraction collector. The collected fractions were monitored for γ activity using a Philips PW 4800 γ counter. Elution profiles of both the β activity associated with the low molecular weight acid species and the γ activity of the Eu-III containing species were obtained. The 100 μl injected samples and the bulk mobile phases were 0.45μm filtered with Acro discs and filters. Using a stepped elution scheme the organically complexed europium species were eluted in HPLC grade water and the free europium in 0.1mol l^{-1} EDTA, at a constant flow rate of 0.750ml min^{-1}. The chromatographic peak areas were used to determine the percentage of bound Eu-III in each sample.

I. Ex technique

The I.Ex procedure was based on a technique described by Choppin (7) designed to be a rapid technique allowing labile metal ligand complexes to be analysed. The Dowex 50x4, 100-200 mesh cation exchange resin was prepared by successively washing with concentrated HCl, distilled water, 0.1mol l^{-1} EDTA, distilled water again and finally 0.1mol l^{-1} NaOH solution. Each 500μl sample was rapidly separated on a short column of

freshly prepared resin (12mm i.d x 38mm length). Columns were pre-equilibrated by washing with sodium acetate buffer (pH = 6.0, 0.001mol l^{-1}) and the samples were eluted with 5ml of the same buffer. The eluates were monitored for γ and/or β activity as appropriate. From a knowledge of the initial activity of each sample and the eluted activities the percentage of bound Eu-III in each solution was calculated.

Results and Discussion

(a) Single ligand systems

The results of the Eu-citric acid (Eu-Cit), Eu-oxalic acid (Eu-Ox), Eu-acetic acid (Eu-OAc) and Eu-humic acid (Eu-Hu) experiments are depicted graphically in Figure 1. The graphs show the variation of the bound Eu-III as a function of the total Eu-III added, as determined using the HPSEC and I. Ex. techniques and predicted using MINTEQA2. With the exception of the Eu-OAc, HPSEC, results the close agreement of the two sets of experimental results in each case and the reasonable agreement with MINTEQA2 predictions was taken as evidence of the reliability of the procedures. The low results for the Eu-OAc system, obtained using HPSEC, were attributed to the lability of the Eu-OAc complex, so for this system only the I.Ex. results were subsequently used. The conditional stability constants (K values) required for modelling purposes were obtained from plots of [ML]/[M] v [M] which were constructed for each system. ML represents the bound europium, M the free europium and [ ] denotes concentration in mol l^{-1}. The slopes of the graphs gave the K values (see Appendix). A typical graph which illustrates the I. Ex. results for the Eu-Cit system is shown in figure 2.

Using the equations A6, A15, A16 and literature K_A values at pH 6.0 the following relationships can be demonstrated to hold:

For the HOAc system $K = 0.96\ K_{ML}$

for the H_2Ox system $K = 0.98\ K_{ML} + 0.018\ K_{MHL}$

and for the H_3Cit system $K = 0.275\ K_{ML} + 0.685\ K_{MHL} + 0.039\ K_{MH_2L}$

K_{MH_2L}, K_{MHL} and K_{ML} represent the stability constants of the possible 1:1 europium complexes produced in the different sytems due to the various protonated forms of the ligands. It can be seen that, with the exception of the H_3Cit system, the derived K values are very close to the K_{ML} values. Inspection of literature stability constants showed that the

derived conditional constants were consistent with the above equations. However, it must be emphasised that any agreement between the K and K_{ML} values is irrelevant with respect to the modelling approach adopted.

The major reason for adopting the graphical method for determining K values was to maintain consistency with the simplified Scatchard method used to interpret the Eu-Hu results. A modification of the Scatchard approach (9) was used because L_T values cannot be known initially or accurately for humic materials and metal humate stability constants vary with metal loading. For simplicity the humic material was assumed to possess only strongly (^{S}L) and weakly binding (^{W}L) sites. The slope of the early part of the $\frac{[ML]}{[M]}$ υ [ML] plot provided the strong site constant (^{S}K) whilst the latter part provided the weak constant (^{W}K). The derivation of the complexation parameters for the Eu-Hu system using HPSEC is shown in Figure 3. A reaction stoichiometry of one europium ion to one site was assumed. For a fuller explanation of the approach the reader is referred to reference (6). The derived ^{S}K and ^{W}K values do not have rigorous thermodynamic significance. Their use here was justified because they were used only to model the binding behaviour of the Eu-Hu system under the conditions employed.

The complete set of parameters derived for the individual ligand systems are collected in Table 2. The closeness of the experimental values was taken as vindication of the techniques. Except in the case of the Eu-QAC system which used the I.Ex. results only, the speciation of Eu in the mixed systems, was predicted using the averaged HPSEC and I.Ex. parameters. Predictions were made using SPE, a general purpose speciation program (10), with the acid parameter inputs modified to take account of the conditional nature of the derived stability constants.

Mixed ligand systems

The results of the mixed ligand experiments are presented in Figure 4. The closeness of the observed HPSEC and I. Ex results with the predicted complexation behaviour, using SPE with the experimentally derived parameters, and the MINTEQA2 model indicated that either mixed ligand complexes were absent or that their formation was insignificant in terms of successful speciation modelling.

The results of the individual analyses of the Eu-Hu-Ox samples using HPSEC are

presented in Table 3 along with the predicted speciation using the experimentally derived single ligand K values. The agreement between the observed and predicted speciation again pointed to the absence of mixed ligand species in the Eu-Hu-Ox system.

If mixed ligand complexes had been formed evidence would have emerged from the HPSEC β and γ elution profiles.

Figure 5 shows the β and γ chromatograms for a typical Eu-Hu-Ox sample. It can be seen that no observable β activity due to C-14 labelled oxalate species eluted with the humate peak. Similarly the β and γ chromatograms for the Eu-Hu-Cit and Eu-Hu-OAc systems showed no evidence of mixed activity species and therefore mixed ligand complexes.

Conclusions

Mixed ligand complex formation was not observed under the conditions employed and speciation modelling of the mixed systems was successfully achieved using appropriate combinations of single ligand constants. The results apparently do not support the findings of Dierckx et al (3). However these authors stated that mixed ligand complex formation may not occur until a certain critical concentration ratio of the competing ligands has been exceeded. Further studies with even higher levels of competing low molecular weight organic acids ligands are planned.

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Appendix

Physico-chemical siginficance of K values derived from [ML]/[M] v [ML] plots

The principles underpinning the graphical derivation of K values are explained below. Preliminary trials using MINTEQA2 to model the Eu-OAc, Eu-Ox and Eu-Cit systems indicated that only 1:1 complexes were important for the ligand concentrations used.

Monoprotic ligands eg HOAc

The formation of a 1:1 complex between a metal (M) and a simple monoprotic ligand (HL) requires two equilibria to be simultaneously satisfied, ie the acid dissociation of the ligand:-

$$HL \Leftrightarrow H^+ + L$$

which is governed by an acid dissociation constant (K_A) given by

$$K_A = \frac{[H^+][L]}{[HL]} \qquad (A1)$$

and the complexation reaction:-

$$M + L \Leftrightarrow ML$$

which is governed by a stability constant (K_{ML}) given by

$$K_{ML} = \frac{[ML]}{[M][L]} \qquad (A2)$$

(Apart from H^+ charges are omitted).

After equilibrium has been achieved in any such system the original total ligand concentration (C_L) is given by

$$C_L = [ML] + [L] + [HL] \qquad (A3)$$

$\therefore$ from equation A1

$$C_L = [ML] + [L] + \frac{[H^+][L]}{K_A}$$

$$= [ML] + \left(1 + \frac{[H^+]}{K_A}\right)[L]$$

$$\therefore [L] = \frac{C_L - [ML]}{1 + \frac{[H^+]}{K_A}} \qquad (A4)$$

Substitution of this expression for [L] into equation A2 and rearrangement gives

$$\frac{[ML]}{[M]} = \frac{K_{ML}}{1 + \frac{[H^+]}{K_A}} (C_L - [ML]) \qquad (A5)$$

It is apparent from equation A5 that for such a 1:1 system, at constant pH, a complexation graph of $\frac{[ML]}{[M]}$ υ [ML] will be linear with a slope (K) given by

$$K = \frac{-K_{ML}}{1 + \frac{[H^+]}{K_A}} \qquad (A6)$$

Equation A6 shows that K does not equal K_{ML}, but is related to it by the acid properties of the system, ie the pH and the pK_A ($pK_A = -\log_{10} K_A$) of the ligand.

Inspection of equation A5 shows that the x-intercept, which gives the maximum possible value of [ML], occurs when $[ML] = C_L$.

Diprotic ligands eg H_2Ox

The formation of only 1:1 complexes between a metal (M) and a simple diprotic ligand (H_2L) can be treated similarly. The various equilibria which co-exist and the corresponding equilibrium constants are:-

$$H_2L \Leftrightarrow H^+ + HL \quad \text{for which } K'_A = \frac{[H^+][HL]}{[H_2L]} \qquad (A7)$$

$$HL \Leftrightarrow H^+ + L \quad \text{for which } K''_A = \frac{[H^+][L]}{[HL]} \qquad (A8)$$

$$M + L \Leftrightarrow ML \quad \text{for which } K_{ML} = \frac{[ML]}{[M][L]} \qquad (A9)$$

and

$$M + HL \Leftrightarrow MHL \quad \text{for which } K_{MHL} = \frac{[MHL]}{[M][HL]} \qquad (A10)$$

The total concentration of ligand (C_L) after attainment of equilibrium in any such system is given by

$$C_L = [H_2L] + [HL] + [L] + [ML] + [MHL] \qquad \text{(A11)}$$

$$= \frac{[H^+][HL]}{K'_A} + [HL] + [L] + [ML] + [MHL] \qquad \text{from equation (A7)}$$

$$= \left(\frac{[H^+]}{K'_A} + 1\right)[HL] + [L] + [ML] + [MHL]$$

$$= \left(\frac{[H^+]}{K'_A} + 1\right)\left(\frac{[H^+][L]}{K''_A}\right) + [L] + [ML] + [MHL] \qquad \text{from equation (A8)}$$

$$= \left(\frac{[H^+]^2}{K'_A K''_A} + \frac{[H^+]}{K''_A} + 1\right)[L] + [ML] + [MHL]$$

$$\therefore [L] = \frac{C_L - \left([ML] + [MHL]\right)}{\left(\frac{[H^+]^2}{K'_A K''_A} + \frac{[H^+]}{K''_A} + 1\right)} \qquad \text{(A12)}$$

$$\text{Now } \frac{[ML] + [MHL]}{[M]} = K_{ML}[L] + K_{MHL}[HL] \qquad \text{from equations (A9) and (A10)}$$

$$= K_{ML}[L] + K_{MHL}\frac{[H^+][L]}{K''_A} \qquad \text{from equation (A8)}$$

$$= \left(K_{ML} + \frac{K_{MHL}[H^+]}{K''_A}\right)[L] \qquad \text{(A13)}$$

$\therefore$ substituting for [L] using equation 6.12

$$\frac{[ML]+[MHL]}{[M]} = \frac{\left(K_{ML} + \frac{K_{MHL}[H^+]}{K''_A}\right)}{\left(\frac{[H^+]^2}{K'_A K''_A} + \frac{[H^+]}{K''_A} + 1\right)} \left(C_L - ([ML] + [MHL])\right) \quad \text{(A14)}$$

Consequently a graph of $\frac{[ML]+[MHL]}{[M]}$ v $[ML]+[MHL]$ is linear at constant pH, with a slope (K) given by

$$K = \frac{K_{ML} + \frac{K_{MHL}}{K''_A}[H^+]}{\frac{[H^+]^2}{K'_A K''_A} + \frac{[H^+]}{K''_A} + 1} \quad \text{(A15)}$$

The x-intercept which is independent of pH and corresponds to the maximum possible value of [ML] + [MHL] = C_L once again.

Triprotic ligands eg H_3Cit

In an analogous fashion it can be shown that when only 1:1 complexes are important with a triprotic ligand the slope of the graph (K) is given by:

$$K = \frac{\left(K_{ML} + \frac{K_{MHL}[H^+]}{K_A'''} + \frac{K_{MH_2L}[H^+]^2}{K_A'' K_A'''}\right)}{\left(\frac{[H^+]^3}{K_A' K_A'' K_A'''} + \frac{[H^+]^2}{K_A'' K_A'''} + \frac{[H^+]}{K_A'''} + 1\right)} \quad \text{(A16)}$$

Captions for Tables and Figures

Table 1 Composition of samples

Table 2 Complexation parameters derived for the individual ligand systems

Table 3 HPSEC and SPE data for the europium - oxalic - humic system

Figure 1 Binding behaviour of the various Eu-III single acid ligand systems determined using HPSEC (■) and Ion Exchange (+) and predicted using MINTEQA2 (◆).

Figure 2 Derivation of the conditional stability constant using I.Ex. for the Eu-Cit system

Figure 3 Derivation of the complexation parameters for the Eu-Hu system using the HPSEC results

Figure 4 Binding behaviour of the mixed ligand systems determined using HPSEC (■) and I.Ex (+) and predicted using experimentally derived stability constants(Δ) and MINTEQA2 (◆).

Figure 5 Radiochromatograms of the Eu-Hu-Ox system at $[Eu]_{total} = 5 \times 10^{-6}$ mol l^{-1}

Table 1 Composition of samples

Series*	[HA] /(gl^{-1})	[HOAc] /(mol l^{-1})	[H_2Ox] /(mol l^{-1})	(H_3Cit] /(mol l^{-1})
1	0.010	–	–	–
2	–	–	–	1.05×10^{-6}
3	0.010	–	–	1.05×10^{-6}
4	–	–	1.04×10^{-5}	–
5	0.010	–	1.04×10^{-5}	–
6	–	1.04×10^{-2}	–	–
7	0.010	1.04×10^{-2}	–	–

* Within each series Eu-III concentrations ranged from 10^{-8} to 10^{-4}M.

The europium contained trace amounts of Eu-152 and the three low molecular weight acids, HOAc, H_2Ox and H_3Cit containd C-14. The HA was not labelled.

Table 2 Complexation parameters derived for the individual ligand systems

System	Complexation Parameters	
	HPSEC	I. Ex.
Eu-Cit	log K = 7.35 ± 0.08	log K = 7.37 ± 0.01
Eu-Ox	log K = 5.79 ± 0.10	log K = 5.94 ± 0.17
Eu-OAc	log K = 1.2*	log K = 2.20 ± 0.25
Eu-Hu	log ^{S}K = 8.54 ± 0.16	log ^{S}K = 8.34 ± 0.33
	log ^{W}K = 6.21 ± 0.05	log ^{W}K = 6.27 ± 0.04
	$^{S}L_T$ = 8.19 (± 1.18)x10^{-8} mol l^{-}	$^{S}L_T$ = 6.56 (± 1.62)x10^{-8} mol l^{-1}
	$^{W}L_T$ = 2.43 (± 0.08)x10^{-6} mol l^{-1}	$^{W}L_T$ = 1.58 (± 0.05)x10^{-6} mol l^{-1}

* only first data point calculated.

Table 3 HPSEC and SPE data for the europium - oxalic - humic system

HPSEC			SPE		
log [Eu]t	%EuOx	%EuHu	log [Eu]t	%EuOx	%EuHu
-7.851	0.0	97.3	-7.851	0.0	99.0
-7.193	0.0	96.4	-7.193	0.0	99.0
-6.943	0.0	93.8	-6.943	0.0	99.0
-6.289	2.3	89.1	-6.289	3.0	91.5
-6.000	5.1	84.1	-6.000	5.3	84.7
-5.301	22.0	45.5	-5.301	24.0	47.0
-5.000	27.9	28.4	-5.000	32.5	29.0

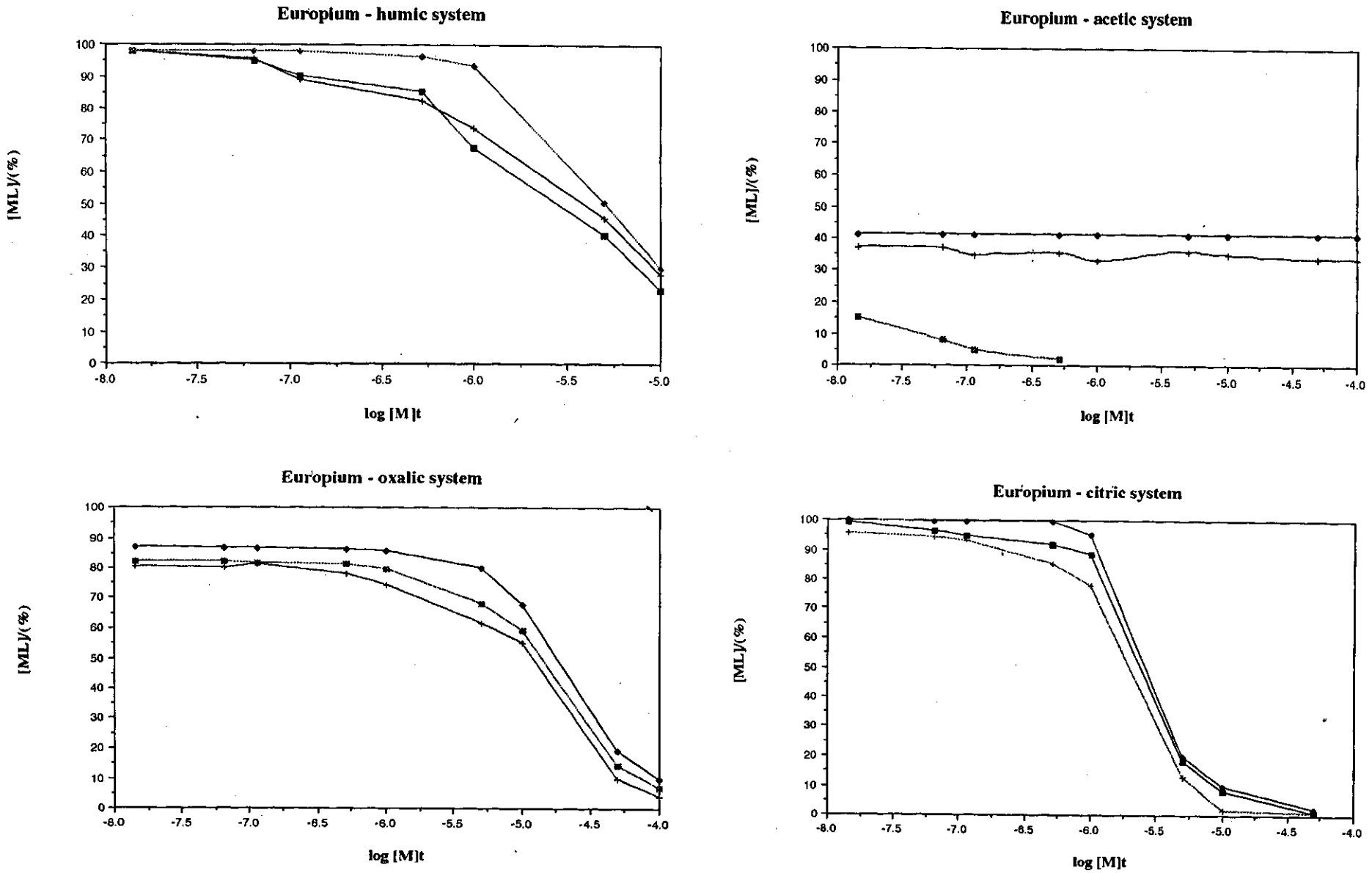


Figure 1: Binding behaviour of the various Eu-III single acid ligand systems, determined using HPSEC (■) and Ion Exchange (+) and predicted using MINTEQA2 (◆)

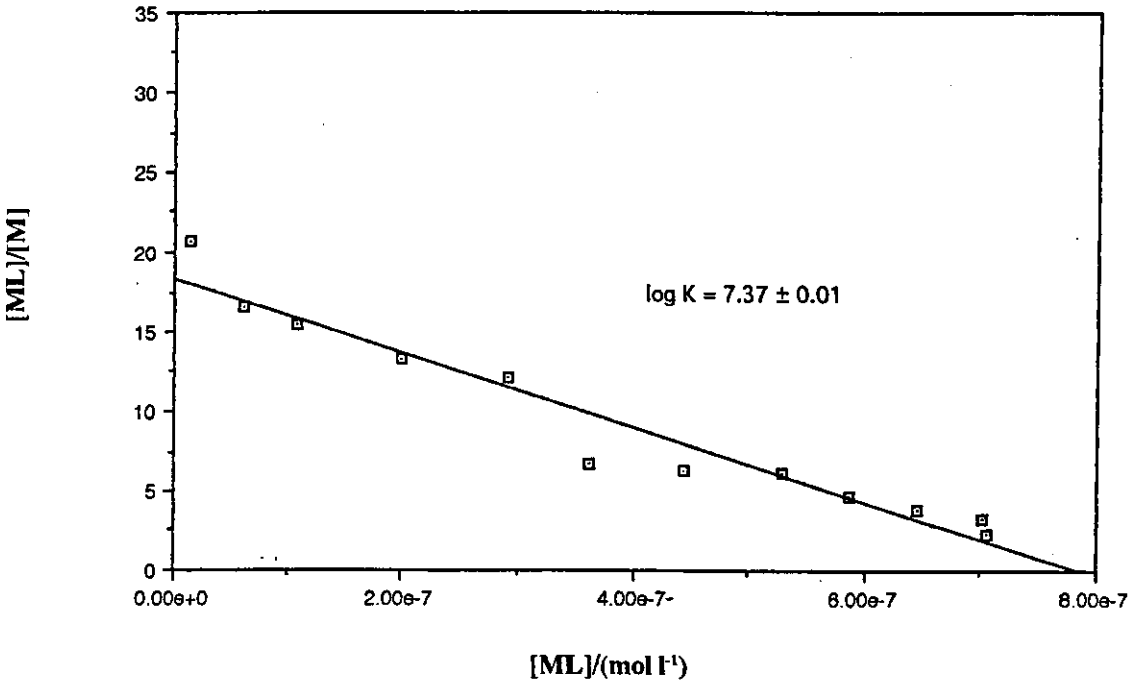
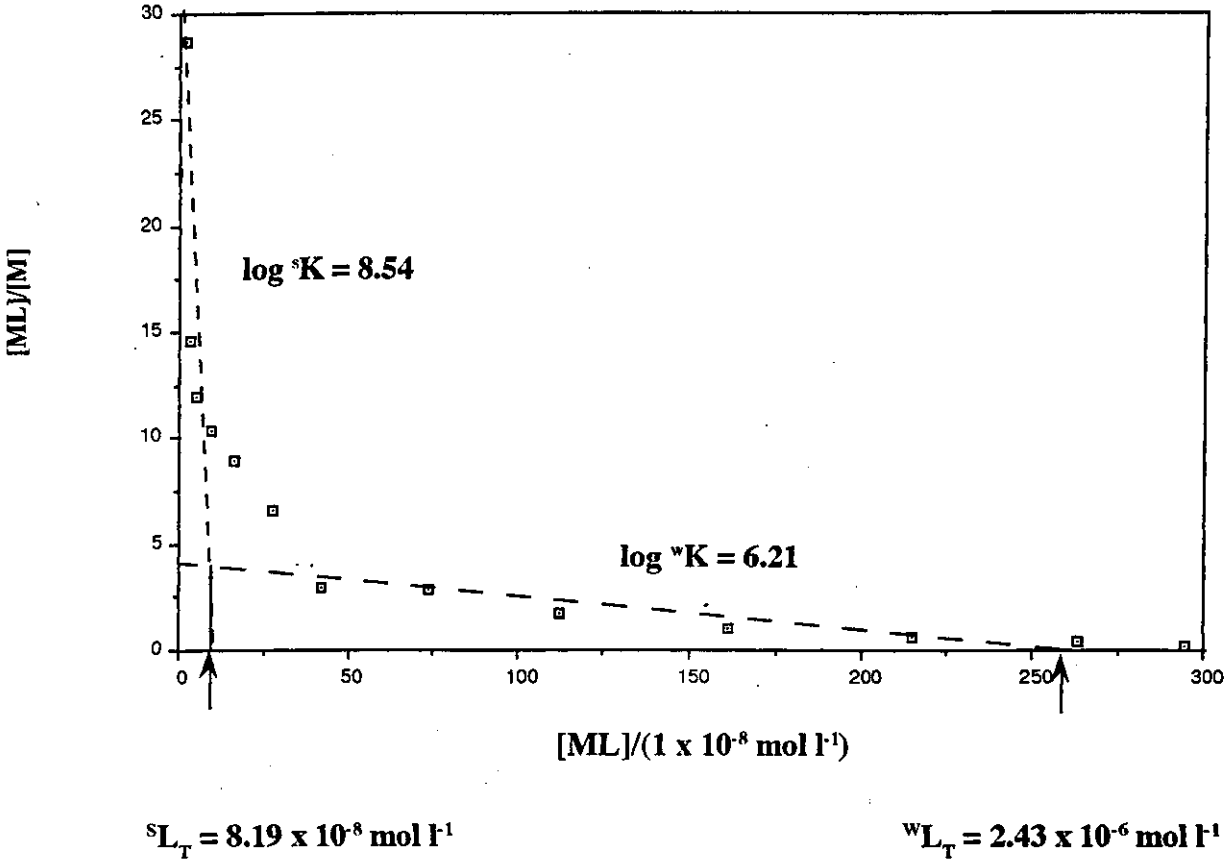


Figure 2: Derivation of the conditional stability constant using I.Ex. for the Eu-Cit system



$^{S}L_T = 8.19 \times 10^{-8}$ mol l^{-1} $^{W}L_T = 2.43 \times 10^{-6}$ mol l^{-1}

Figure 3 **Derivation of the complexation parameters for the Eu-Hu system using the HPSEC results**

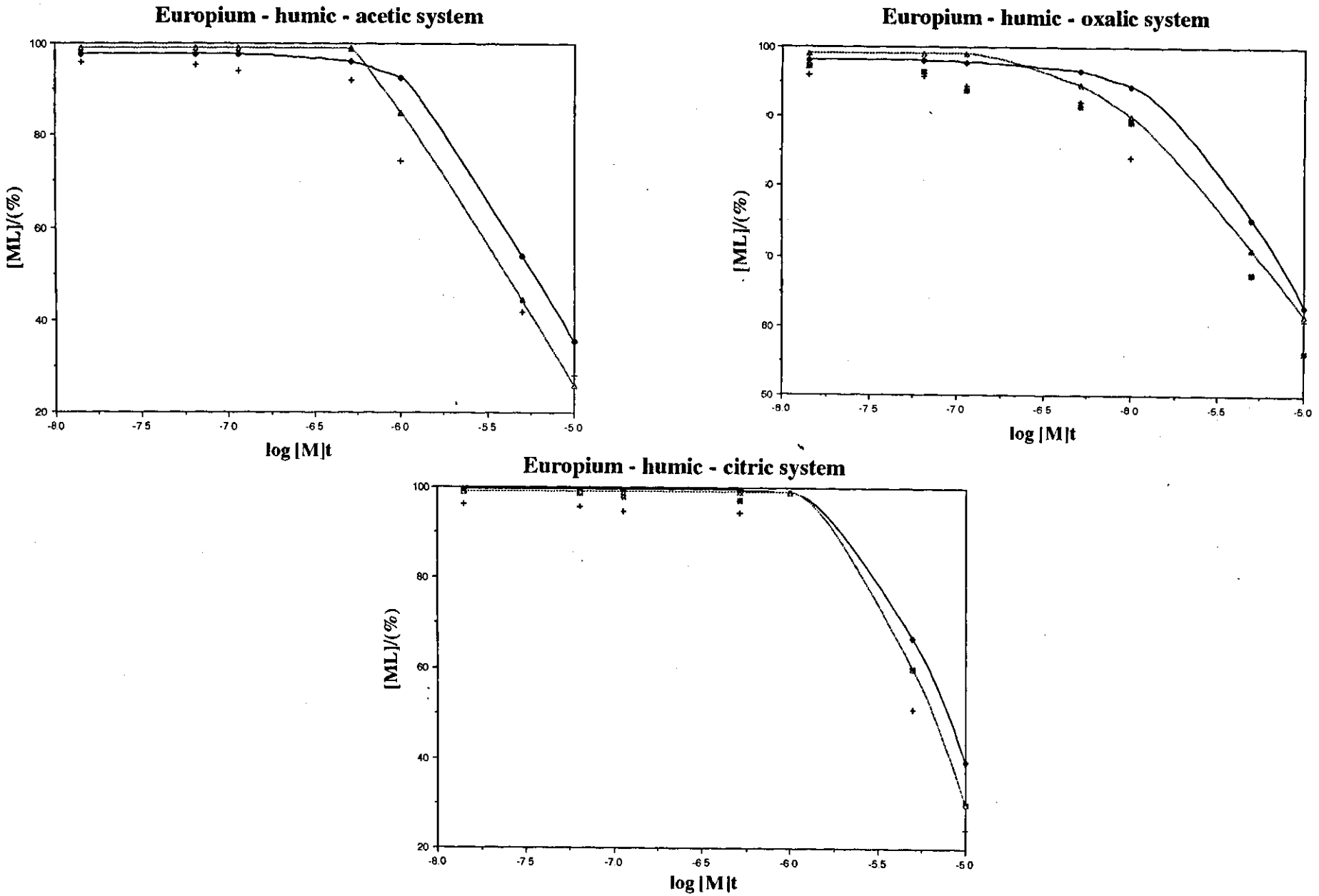


Figure 4: Binding behaviour of the mixed ligand systems determined using HPSEC (■) I. Ex (+) and predicted using experimentally derived stability constants (Δ) and MINTEQA2 (♦).

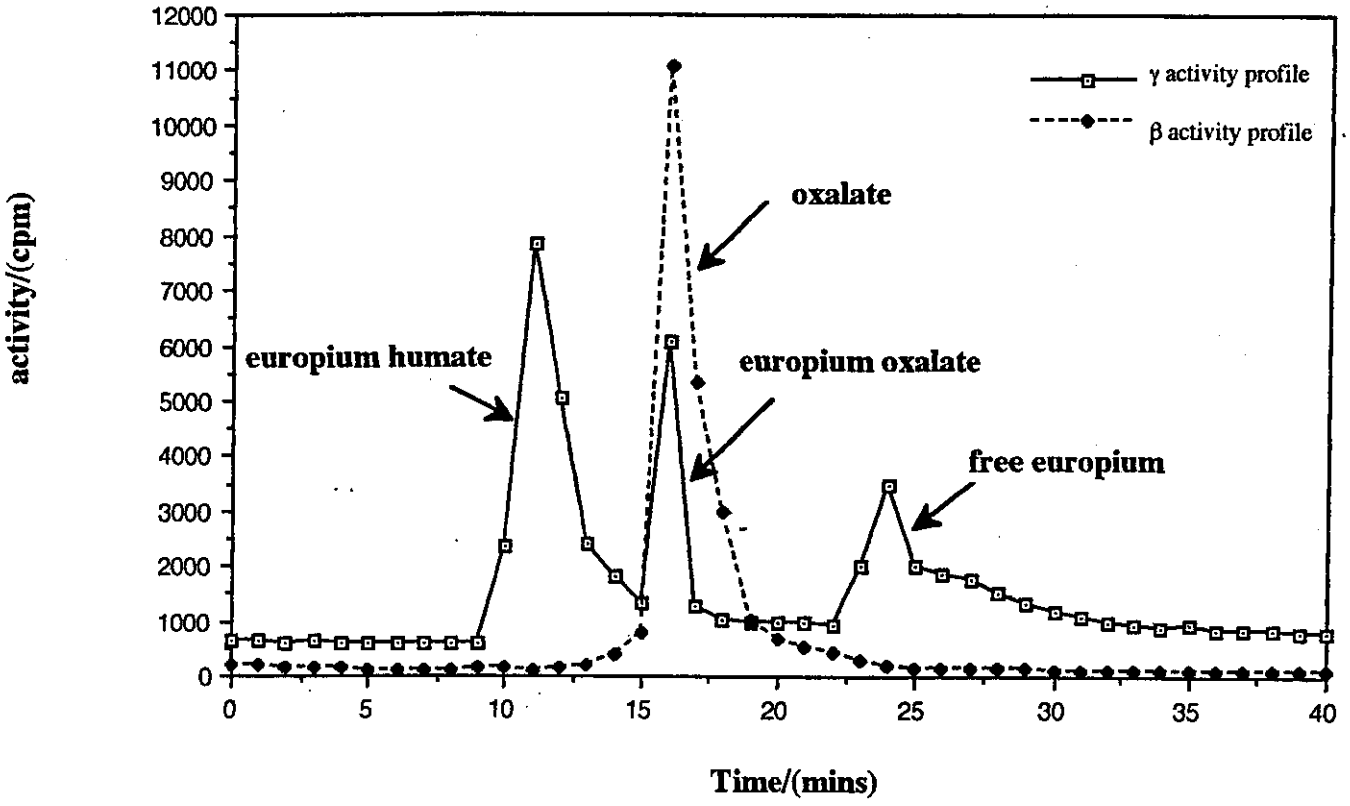


Figure 5 Radiochromatograms of the Eu-Hu-Ox system at $[Eu]_{total} = 5 \times 10^{-6}$ mol l^{-1}