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ISOLATION AND CHARACTERIZATION OF HUMICS FROM NATURAL WATERS

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ABSTRACT

A method has been developed for quantitative recovery of humic substances from aqueous systems based on ion exchange on DEAE-cellulose. A scheme is suggested for the characterization of dissolved humic substances (UV-, IR- and ^1H NMR-spectroscopy, elemental analysis, molecular weight determination, ^{14}C-age, functionality, carbohydrate content and acid-base properties) as a routine in the chemical analysis of natural waters

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1 INTRODUCTION

The mobility and distribution of trace metals in the aqueous environment are highly related to the hydrochemical conditions in the system, determining the chemical speciation, besides hydrological factors determining the water flow rate. Species in solution will interact with solid media, e.g. stationary geologic phases or suspended matter of a colloidal nature, Fig. 1-1. The mechanisms of these interactions (physical adsorption, chemisorption, electrostatic interaction, substitution, precipitation etc.) reflect the chemical state of the trace elements in solution as well as the properties of the solid surfaces. Most important hydrochemical parameters are

- hydrogen ion concentration (pH), determining surface charge properties as well as the degree of hydrolysis (for multivalent metals)
- redox potential (for redox sensitive elements)
- the presence of inorganic complexing agents (primarily carbonate; occasionally sulphate, fluoride and phosphate)
- the presence of complexing natural organics - humic and fulvic acids

Complexation with humics is probably significant, or even dominant, for many divalent transition elements at low or intermediate pH in typical surface waters (more than 1 mg/l of humics). Mercury in particular is likely to be completely dominated by humate complexation. For trivalent elements (like lanthanides and actinides) formation of organic complexes will dominate over hydrolysis and carbonate complexation even in groundwaters with low contents of humics (0.01-0.1 mg/l - level).

Thus, the presence of humics will effect the mobility of trace elements by

- changing the sorption properties due to the formation of metal humates (usually enhancing the sorption at pH below PZC and reducing the sorption above this pH)

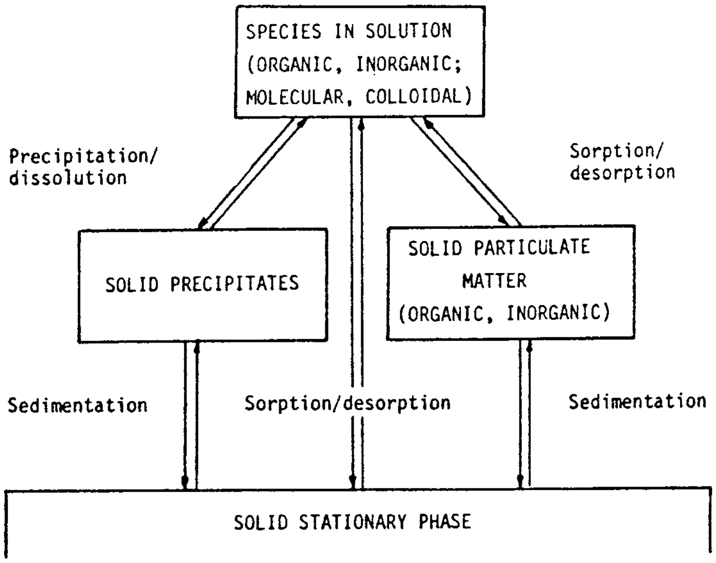

Fig. 1-1 Distribution of trace elements between solid phases and solution phase in natural water systems

- creating a "new" surface with other sorption properties due to the formation of an organic film on inorganic surfaces
- formation of macro molecular aggregates of colloidal nature

The present study has the following purpose:

- Development of a technique for recovery and isolation on a routine basis of humics from natural waters at representative concentration levels
- Design of a scheme for chemical analysis of natural humic substances, suitable for routine analysis of water containing humics in the 1-100 mg/l-level
- Isolation of large quantities of humic materials of various origin (surface waters, shallow groundwaters, deep groundwaters) for subsequent use in model studies of metal complexation and mobility

The concentration of a very heterogeneous material from large amounts of water raises chemical as well as technical problems.

2.1 USE OF XAD (HYDROPHOBIC INTERACTIONS)

Several methods for the concentration of aqueous humic substances have been reported. The method most widely used today /Malcolm, 1986/ is outlined in Fig. 2-2. By this method the water is first filtered through 0.45 um filters to remove suspended organic material. The filtered water is acidified to pH 2 with concentrated hydrochloric acid making the humic and fulvic acids less charged and hence more hydrophobic. In a first concentration step the humic substances are immobilized on a column packed with XAD-8, a crosslinked polymeric methacrylate ester, by means of hydrophobic interactions. Before a substantial break-through is obtained, the humic substances are eluted from the column by a 0.1 M NaOH-solution as the mobile phase. The eluent, applied to the column in the reversed direction, transfers the humic and

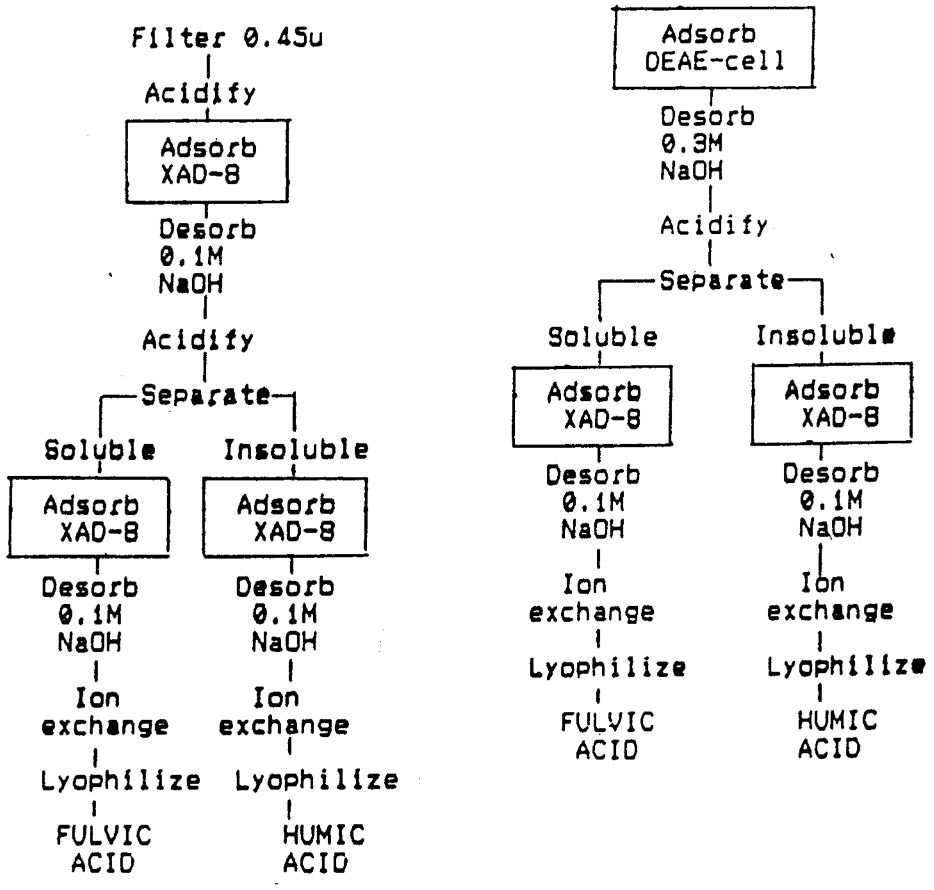

Fig. 2-2 Concentration of aqueous humic substances on XAD and DEAE

fulvic acids into their anionic forms hence making them less hydrophobic and consequently easily eluted from the column. The front of the new mobile phase is easily observed as the coloured humic substances are eluted. The eluate is immediately acidified as the humic substances are very easily oxidized in alkaline solution. The acidified solutions may be stored for weeks at low temperature. After elution of the humic and fulvic acids a faint yellow to brown colour may persist at the top of the column. This colour is due to nonionizable hydrophobic compounds still being bonded to the support by hydrophobic interactions. This fraction, mainly consisting of humin, may be eluted from the column by methanol.

After the first concentration step the humic acid is precipitated at pH 1 by the addition of concentrated hydrochloric acid. The humic acid is separated from the dissolved fulvic acid by centrifugation.

In the subsequent steps, the XAD-column is used again as a combined concentration and purification step. The acidic fulvic acid solution, a combined water phase from several first concentration steps, is again pumped onto the column. When the fulvic acid is immobilized on the column the mobile phase is changed for distilled water. The purpose of this cleaninig procedure is to desalt the sample. The conductivity of the eluate is measured and when it equals that of the distilled water the washing is ended. The fulvic acid is again desorbed by 0.1 M NaOH-solution applied to the column in the reversed direction. The XAD-column is now coupled in series with a strong cat-ion exchanger in the H^+-form. The concentrated fulvic acid solution thus obtained is lyophilized to obtain an amorphous substance which may be regarded as a "pure" fulvic acid.

The humic acid is reconcentrated and purified according to the same procedure as the fulvic acid. One problem that may be faced is precipitation of the humic acid in acidic solution when pumping it onto the column. This may be overcome by diluting the solution and by careful acidification of the solution just enough for immobilizing the material on the column without precipitation.

The general idea of the above strategy is based on the fact that acidic groups are predominant in the humic material. By changing the pH of the mobile phase the humic substances may be immobilized on the column or eluted. The humics are desalted by distilled water, desorbed from the XAD-column and protonated by an ion exchange column.

The technique described above is applicable for waters containing huge concentrations of humic materials. For waters of low concentrations, however, the method is time-consuming as compared to the amount of humics obtained. Large volumes of water have to be acidified, which can lead to practical problems. Also, this acidification could alter the original cation distribution in the system, exchanging bonded cations with hydrogen ions.

2.2 USE OF DEAE (ANION EXCHANGE)

An alternative method, designed for groundwater humic substances, may be used for natural waters without pretreatment /Paxeus, 1985/. According to this technique the near-neutral water sample is pumped through a DEAE-cellulose adsorbent, a weak anion exchange resin, where the humics are adsorbed simply by an anion exchange mechanism. The advantage of this procedure is the fact that the filters may be operated in field work directly on unconditioned waters, while the XAD-technique requires acidified water samples. A draw-back with the DEAE-cellulose is, however, the poor flow characteristics of columns with this material as a filter.

The humic substances may be eluted from the DEAE-filters by 0.3 M NaOH-solution, which transfers the weak anion exchange resin into a non-charged tertiary amine, thereby inhibiting the interactions with the humic substances.

The technique used at our laboratory for concentrating humic substances is a mixture of the two methods presented above. The preconcentration DEAE-technique described by Paxeus and the purification technique adopted by Malcolm are combined. In Fig. 2-2 the two methods are compared.

The preconcentration technique is very convenient, e.g. when humics at low concentration levels are to be isolated from deep groundwaters by pumping from a bore-hole. Since the Malcolm method is the technique most widely used today we adopt the same cleaning procedure, thereby making the humic materials isolated by us comparable with those isolated at other laboratories.

3 SCHEME FOR CHARACTERIZATION OF HUMIC SUBSTANCES

Humic substances comprise chemically a very heterogenous group of compounds. Even when fractionated in humic and fulvic acids they encompass a huge variety of structural elements of different molecular size. They may, however, be described and defined by a number of general parameters. Some of these are chosen for our routine characterisation of humic substances.

A general characterization scheme is presented below:

(1) Quantitative analysis - UV-spectroscopy
(2) Elemental analysis
(3) IR-spectroscopy
(4) ^{1}H-NMR-spectroscopy
(5) Molecular weight
(6) Age
(7) Functionality
(8) Carbohydrate content
(9) Acid-base properties

3.1 QUANTITATIVE ANALYSIS - UV-SPECTROSCOPY

The yellow to brown colour of many natural waters are caused by humic substances. Consequently, they do absorb light in the visible region, usually below 700 nm, as well as in the UV-region of the spectrum. Most well-defined organic compounds with absorption in this region show one or several absorption maxima. Humic compounds, however, have no distinct absorption peaks in their spectra. This of course is due to the heterogeneity of humic compounds. Since there are many different overlapping peaks the individual maxima may not be observed.

A typical UV-spectrum is shown in Fig. 3-1. The increase in absorbance with decreasing wavelength is characteristic for humic compounds. The chromophores responsible for absorption are mainly made up by aromatic rings or condensed rings with phenolic and carboxylic substituents. These substitutents make the absorbance to a large degree dependent on the experimental conditions. The increased electron density of the chromophores at

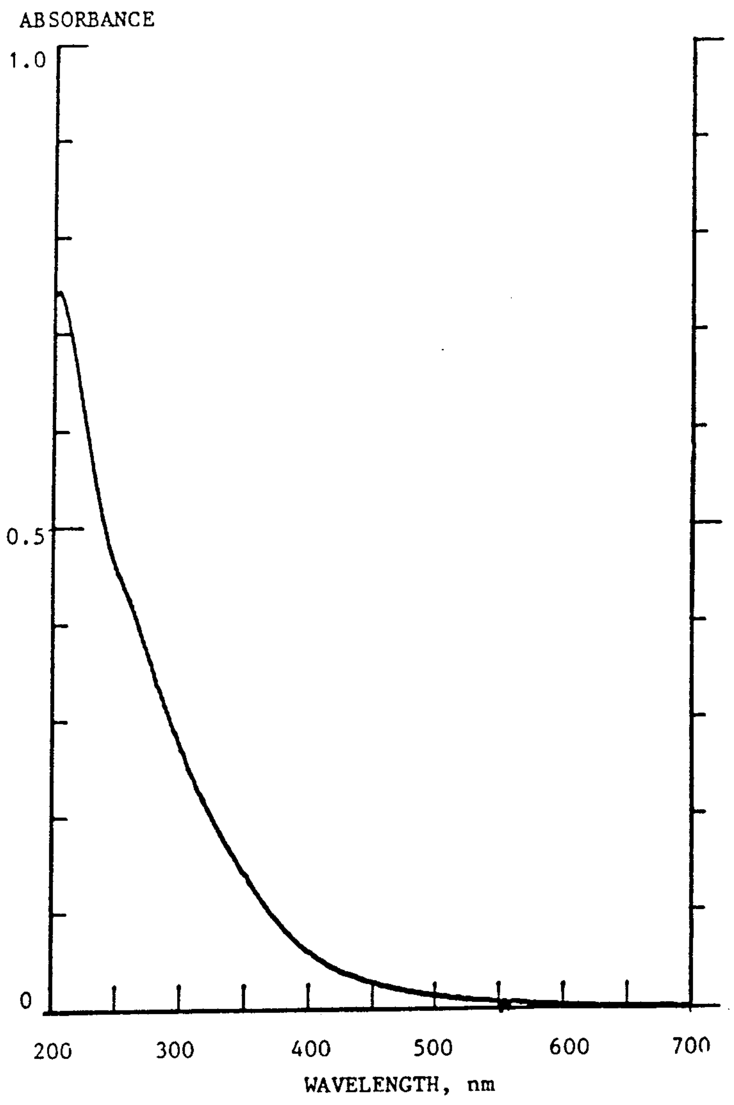

Fig. 3-1 UV-spectrum of an aqueous humic substance (4 mg/l in water)

high pH gives rise to an increased absorbance. Control of pH of the solution is essential since the UV-absorbance decreases from basic via neutral to acidic solution. A pH around 7 is preferred, since humics may be oxidized at high pH, and humic acid may precipitate at low pH.

The UV absorption may also be used for the quantitative determination of humic substances in water. In Fig. 3-2 concentrations of humics are plotted as a function of UV-absorbance at three different wavelengths. Any wavelength may be chosen as a standard, but a combination of wavelenghts is strongly recommended in order to overcome any isolated absorption that may be caused by some specific compounds.

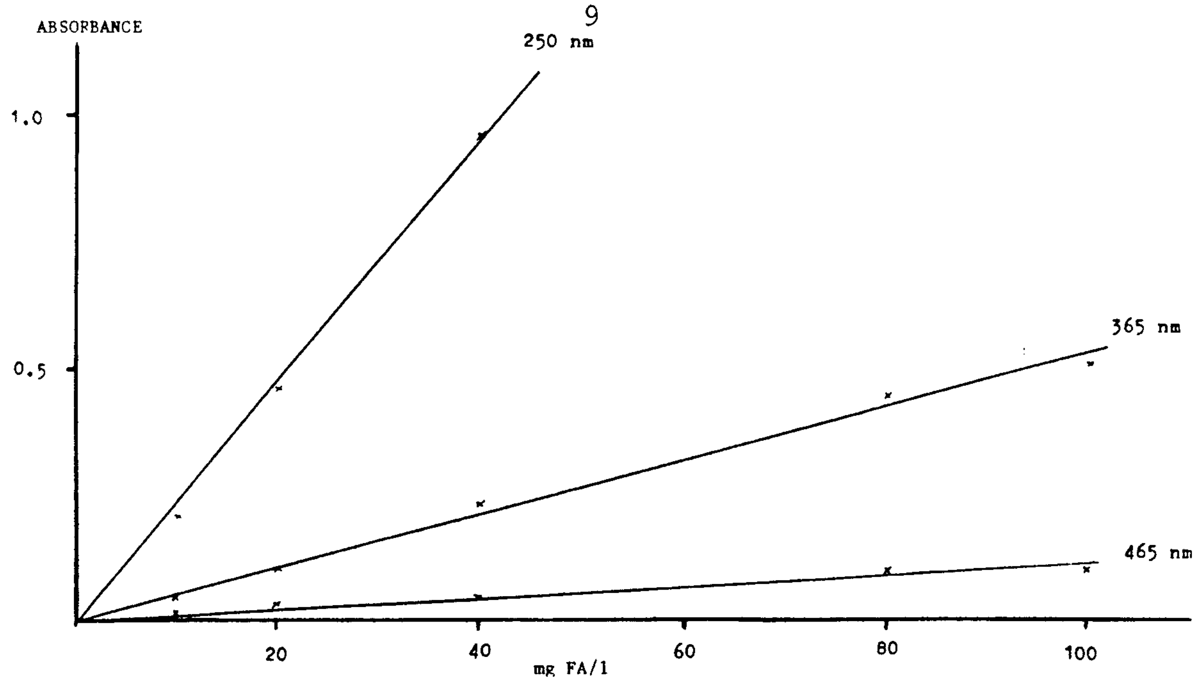

Fig. 3-2 Concentration of a humic acid as a function of the UV-absorbance

3.2 ELEMENTAL ANALYSIS

For well-defined compounds the elemental analysis together with molecular weight often give the exact molecular formula. Although of great importance, the elemental composition of humics do not allow us to deduce the molecular structure. The main reason is the heterogeneity of humic substances. The elemental analysis only gives the average composition of the molecules present in the sample analysed.

There are two main problems that have to be overcome in order to obtain a correct elemental analysis. The sample to be analysed has to be representative of the whole sample. This seems obvious, but it has to be stressed in the case of humic substances. The inhomogeneity of the sample makes it possible to analyse a part that is not representative and the compounds may form different layers in the sample. Therefore the sample should be carefully mixed prior to analysis.

The second problem is associated with the hydroscopic properties of humic materials. The sample has to be carefully dried before analysis, and the weighing should preferably be performed in an inert athmosphere. It is therefore an advantage, if exactly the same procedure is followed each time, thereby assuring that different results observed for different samples do not originate from improper handling.

3.3 IR-SPECTROSCOPY

In order to gain information concerning the functional groups of organic compounds an IR-spectrum is often recorded. The spectrum may also give information about substitution patterns and similar structural details. An IR-spectrum of a surface water fulvic acid is shown in Fig. 3-3.

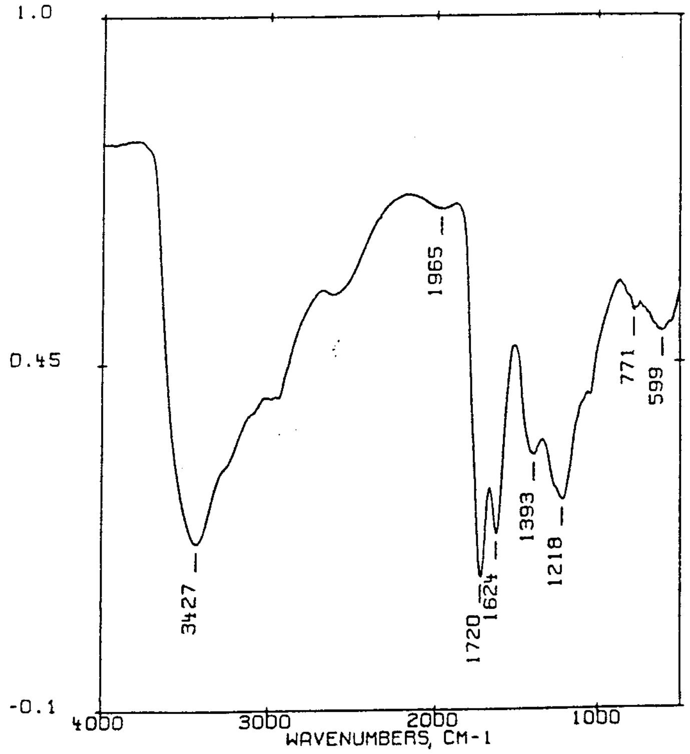

Fig. 3-3 IR-spectrum of a fulvic acid

The spectrum is dominated by absorptions in four different regions:

1. The extremely broad peak ranging from 3700 cm^{-1} to 2500 cm^{-1}. This absorption is caused by -O-H and C-H stretching vibrations. The dominating peak in this sample is the hydroxyl absorption of the carboxylic acid groups. The broadness of the peak has two reasons:

- The heterogeneity of the sample with several different carboxyl groups in most molecular species and mainly the unspecified strength and bond lengths of the O-H groups of carboxylic acids due to hydrogen bonding.

- The carboxylic absorption covers most of the alcoholic and phenolic hydroxyls and almost all of the aromatic C-H (around 3030 cm^{-1}) and the corresponding aliphatic stretching (below 3000 cm^{-1}).

2. The peaks at 1500-1800 cm^{-1}. These absorption peaks are mostly caused by stretching of double bonds. The strongest bands in this region originate from carbonyl groups of carboxylic acids, esters, lactones and ketones. In the 1500 to 1600 cm^{-1} region absorption is mostly caused by vibrations of aromatic rings.

3. The absorption at 1000 to 1200 cm^{-1} are caused by carbon to oxygen single bonds in ethers, esters, alcohols and phenols.

4. Absorptions below 1000 cm^{-1} are mostly caused by skeletal vibrations. These involve many atoms of the different molecular species and are not as easy to interpret as more fundamental absorption bands.

The IR-spectra of humic substances of different origin usually look very similar. The method is consequently not applicable to differentiate between humic samples.

3.4 NMR-SPECTROSCOPY

One of the modern techniques that has spread new light over the chemistry of humic materials is nuclear magnetic resonance spectroscopy. The spectra obtained, whether ^1H or ^{13}C-NMR, are not made up by distinct peaks as are most well-defined compounds. The NMR-spectra of humic materials are rather characterized by bulky absorption bands. Again this reflects the heterogeneity of humic materials. Proton NMR spectra are rather easily obtained for humic materials. The amount of material necessary for "acceptable" spectrum to be recorded in a reasonable time is below 100 mg, an amount that is often available. The demand for material for ^{13}C-nmr spectra is larger. The reason is two-fold. Only about 1% of the natural carbon is the NMR active ^{13}C-isotope and this isotope has a magnetic moment about one fiftieth that of the proton. The large amount of material necessary for carbon-NMR is the reason it is not suggested as a routine characterization method in the present scheme.

A proton NMR spectrum of a fulvic acid, Fig. 3-4, displays the protons along the horizontal axis in accordance with the magnetic fields influencing the atoms. The electron clouds around the protons shield the instrumentally applied magnetic field from the protons. The atoms with the highest ppm-values are surrounded by the weakest electron

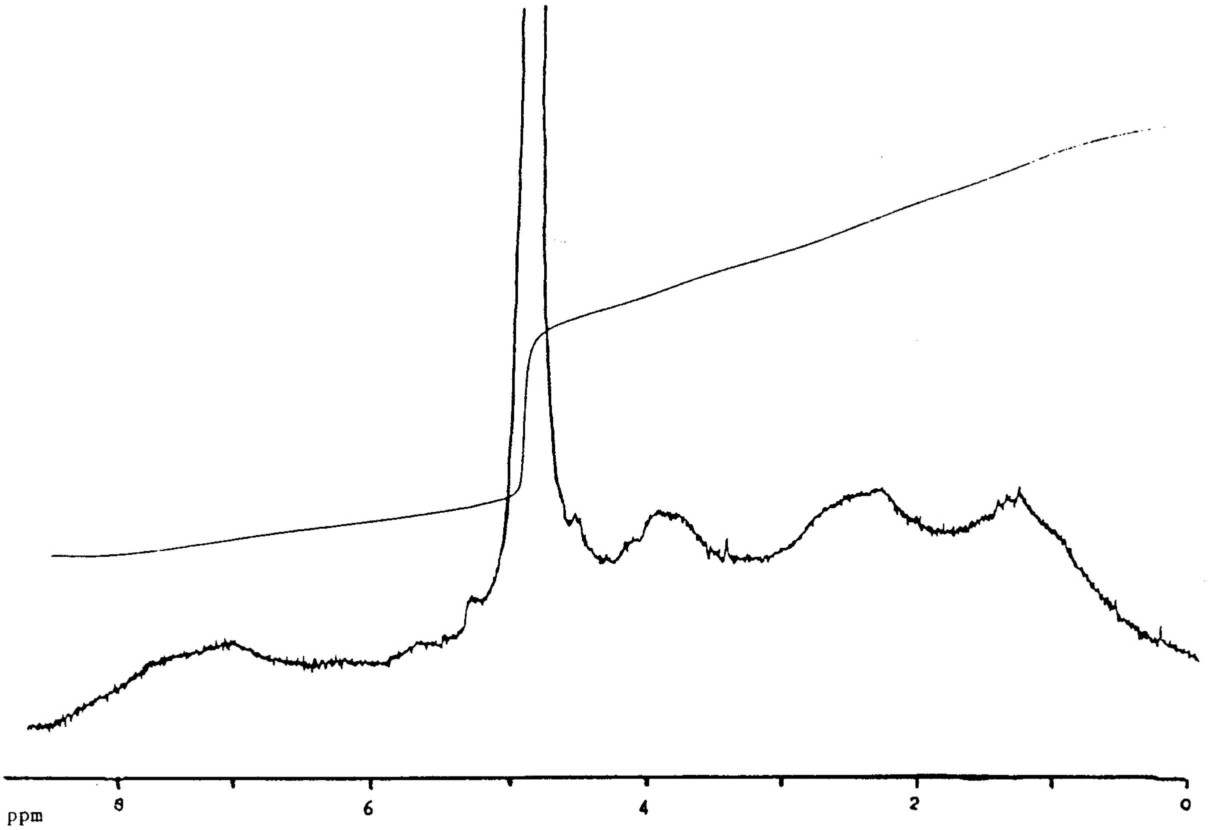

Fig. 3-4 ^{1}H-NMR spectrum of a fulvic acid (in D_2O/OD^-)

densities, as very acidic protons. The protons with the highest electron densities are found at the extreme right in the spectrum since their electron clouds shield their nucleus from the magnetic field of the instrument.

The electron densities may vary around the protons because of the difference in electronegativity of the surrounding elements or because of the electron distribution caused by the mesomeric effects of the molecules. The magnetic field that affects a proton may also be influenced by local magnetic fields within the molecule. This effect is most distinct for aromatic protons which are found between 6 and 8 ppm despite their high electron density.

The obvious information obtained from a ^{1}H-NMR spectrum is the aliphatic to aromatic proton ratio. Within these groups the exact position of a signal is a consequence of the electronic surroundings. Methoxy groups for example will be observed at a higher ppm-value than the corresponding methyl groups bound to carbon. By this kind of reasoning direct information about the chemical character of humic substances may be obtained.

3.5 MOLECULAR WEIGHT DISTRIBUTION

The molecular weight of humic substances reported vary from a few hundreds to several hundred thousands. There are several methods available for the determination of molecular weights. The results obtained vary with the definition of molecular weight, i.e. number average or weight average molecular weight, but also with the methods used, with the isolation procedure and with the purification technique utilized.

It has been argued that techniques like ultrafiltration and gel permeation chromatography (GPC) do not measure the weights of the actual molecules but rather of aggregates of several molecules. The results obtained by these methods appear, however, to represent appropriate relative molecular weights.

The technique utilized in our laboratory is GPC using an HPLC-system. The column is a silica based material modifed by a vinylic alcohol. The mobile phase is a 50 mM phosphate buffer, pH 6.9.

In Fig. 3-5 two chromatograms are given using this system. One is a fulvic acid isolated from a surface water and the other is a fulvic acid from a deep groundwater. The difference in retention times indicates a lower molecular weight for the fulvic acid originating from groundwater than for the one from a surface water.

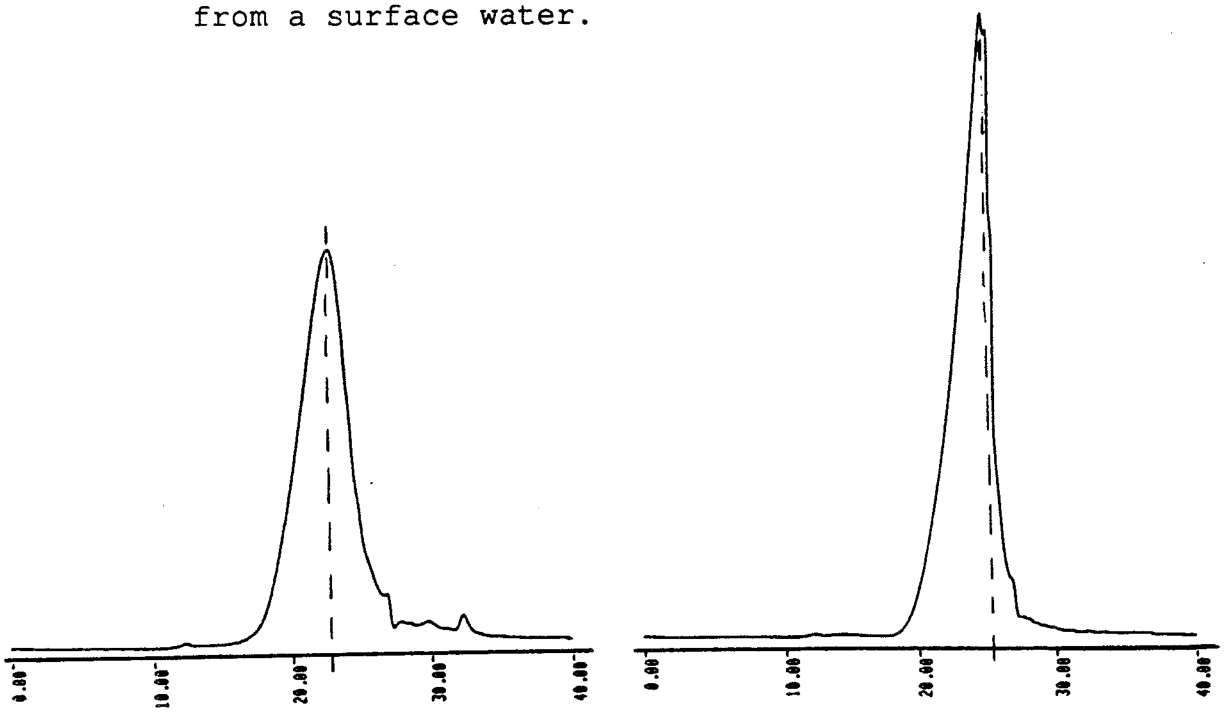

Fig. 3-5 Gel permeation chromatograms. Fulvic acid from surface water (left) and deep groundwater (right)

The exact molecular weights of the materials are difficult to assess since adequate references are not available. With proteins as references the weight average molecular weights correspond to 8000 and 6000 respectively. The actual molecular weights however are much lower since the proteins used as references are globular and consequently have much larger molecular weights in relation to their sizes. By using sulphonated polystyrenes as references the weight average molecular weights are 2700 and 1600, respectively, and the corresponding number average weights some 25-35% lower. These values may be more relevant, considering the similarities in structure between the humics and the reference substances.

3.6 AGE (^{14}C)

It was previously assumed that humic substances of all origins were of significant age. Lately, however, it has been shown that humic compounds in surface water are formed quite recently. This may also be seen from the seasonal variations when the highest concentrations of humic materials in surface water are found in late spring and early summer. These humic substances are to a great extent formed from mouldered plants.

Groundwater humic substances, on the other hand, may be of substantial age. This age can be assessed from determinations of the ^{14}C-content. The age determination based on ^{14}C in the organic matter of the water is, in fact, a better indicator of the residence time of the corresponding water sample in the ground than data for the carbonate, which has been subject to exchange and substitution reactions with mineralogic carbonates /Pettersson *et al.*, 1989/. One has to keep in mind, however, that minute contaminations may have a considerable impact on the results in terms of calculated "ages".

3.7 FUNCTIONALITY

Organic compounds are primarily characterized by their functional groups. These groups are most important for the chemical as well as the physical properties of the compounds. It is well known that humic substances to a large extent are made up by carboxylic and phenolic groups. These groups are qualitatively analyzed by IR-spectroscopy. The amount of acidic groups may be determined by titrations. Other techniques that have been used for quantitative analysis of functional groups for humic substances involve NMR-spectroscopy. The NMR spectra of the native materials do not give substantial information about functionality, but the different functional groups may be derivatized by an NMR-active group. By integrating the derivatized and the native substances the difference, as compared to an internal standard, gives the amount of the corresponding functional group. The demand for material for the method, however, is large.

Integration of peaks in NMR-spectra gives low accuracy and the method is complicated.

The method being developed at our laboratory is based on derivatization. By this approach the humic material is derivatized by some more or less specific agent. The material is isolated, the derivative is hydrolysed, and the agent is quantitatively analysed by gas chromatography. The advantage of this method is that very small amounts of humic material are needed. The quantitative analysis is easily performed by high resolution gas chromatography with good accuracy. Two ways to approach this idea are outlined below.

$$(1)\quad \mathrm{Hu\text{-}OH} + (\mathrm{CH_3CO})_2\mathrm{O} \rightarrow \mathrm{Hu\text{-}O\text{-}CO\text{-}CH_3} + \mathrm{CH_3CO\text{-}OH}$$

$$\mathrm{Hu\text{-}O\text{-}CO\text{-}CH_3} + \mathrm{C_4H_9OH} \xrightarrow{H^+} \mathrm{Hu\text{-}OH} + \mathrm{C_4H_9\text{-}O\text{-}CO\text{-}CH_3}$$

$$(2)\quad \mathrm{Hu\text{-}CO\text{-}OH} + \mathrm{CH_2N_2} \rightarrow \mathrm{Hu\text{-}CO\text{-}OCH_3} + \mathrm{N_2}$$

$$\mathrm{Hu\text{-}CO\text{-}OCH_3} + \mathrm{C_4H_9OH} \xrightarrow{H^+} \mathrm{Hu\text{-}CO\text{-}O\text{-}C_4H_9} + \mathrm{CH_3OH}$$

$$\mathrm{CH_3OH} + \mathrm{C_2H_5\text{-}COCl} \rightarrow \mathrm{C_2H_5\text{-}CO\text{-}OCH_3} + \mathrm{HCl}$$

By the first method (1) the humic material is acetylated. The reagent is removed, the acetylated material is treated with butanol in acidic solution and the butyl acetate formed is analysed after separation from the humic material. Thus, all hydroxy groups can be determined quantitatively.

By the second method (2) the acidic groups of the humic material are methylated by diazomethane, the derivatized humic material is treated with butanol and the free methanol is analysed as a propionate ester. By this method only the carboxylic groups in the humic samples are determined. Phenolic groups are also derivatised by diazomethane, but the methyl ethers are not hydrolyzed at the conditions used.

Each separate step of the two techniques outlined above seem to work, although the reproducibility of the methods must be improved.

3.8 CARBOHYDRATE CONTENTS

Carbohydrates constitute a few percent of the total mass of humic materials. The carbohydrate content is easily determined by a classical colour reaction. 1-Naphtol is dissolved in concentrated sulphuric acid to be sulphonated. The reagent is added to the humic material to be analysed. The carbohydrate hexoses are first converted into 5-hydroxymethylfurfural, and the corresponding pentoses are converted into furfural. These derivatives are complexing with the sulphonated naphtol to form a chromophore which is determined spectrophotometrically.

3.9 ACID-BASE PROPERTIES

Studies of the potentiometric properties of aquatic humic substances are conducted and interpreted using a unified physicochemical approach /Marinsky and Ephraim, 1986/. This approach has the ability to differentiate electrostatic effects from heterogeneity factors in the acid-base titrations of the organic macro molecules in aqueous medium.

Potentiometric acid-base titrations are conducted under the following conditions, in order to get information on total titrable acid capacity as well as acid constants and functionality:

(1) Aqueous systems; three different electrolyte concentrations (0.100 M, 0.010 M and 0.001 M $NaClO_4$)

(2) Non-aqueous systems; N,N-dimethylformamide as non-aqueous medium and tetrabutylammonium hydroxide in 2-propanol as titrant.

(3) Aqueous systems; titration in the presence of Cu^{2+} and La^{3+}/Eu^{3+}.

Fig 3-6 show the apparent pK_a-values as a function of α, pH + $pClO_4$ and pH, respectively, where $pK_a = pH - \log \alpha / (1-\alpha)$ and α = the degree of neutralization.

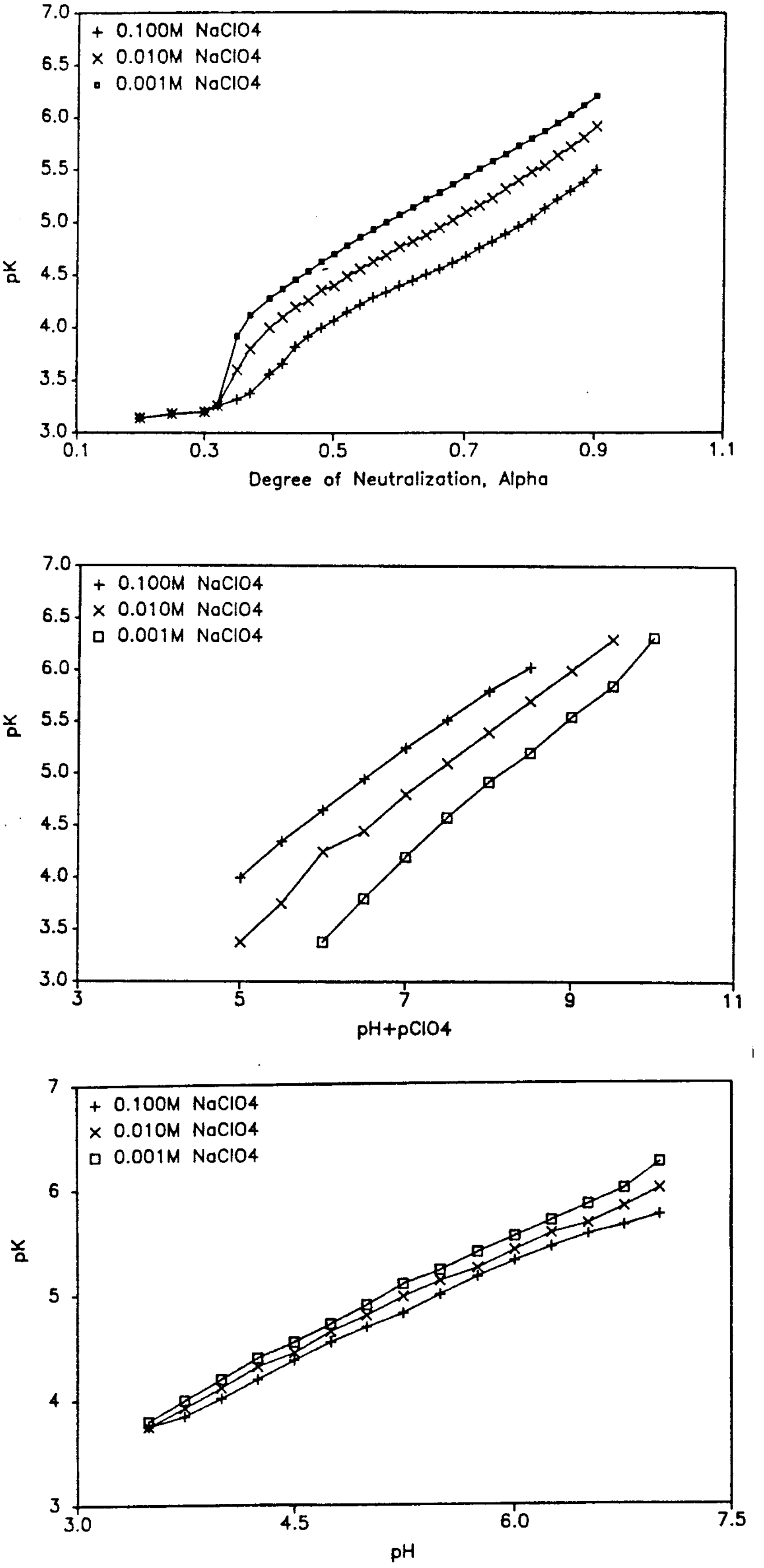

Fig. 3-6 Potentiometric titrations of a fulvic acid (from Bersbo). Apparent pK$_a$ as a function of α, pH + ClO$_4$ and pH for different ionic strengths

The ionic strength dependence gives information on the polyelectrolyte behaviour of the humic substance (molecular weight, linearity, hydrophobic character, etc.). From the results of titrations in aqueous systems and in the non-aqueous system the total capacity due to caboxylic groups and phenolic groups can be assessed, as well as the nature of the various acid sites. An example of the distribution of pK_a-values, corresponding to various sites, are given in Fig. 3-7. These calculated pK_a-values could correspond to carboxyl acids in ortho position (pK_a=1.7 and 5.0), carboxylic acid next to an OH (pK_a=3.3), acidic hydroxyl next to a weaker hydroxyl (pK_a=6.5) and an acidic hydroxyl next to an acetyl group (pK_a=7.0), respectively.

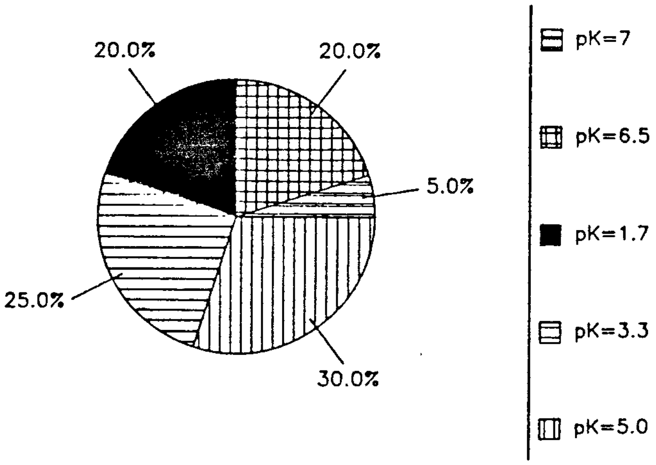

Fig. 3-7 Assignment of acidic sites for a fulvic acid (from Bersbo)

The titration in the presence of Cu^{2+} gives information on the chelating properties of the humic substance, expressed as "extra" acidity.

The titration procedures and evaluation of potentiometric data are discussed in detail elsewhere /Ephraim, 1989; Ephraim *et al.*, 1989a; Ephraim *et al.*, 1989b/.

4 CONCLUSIONS AND FUTURE WORK

The complete separation and characterization scheme can be summarized as follows:

(1) Isolation from the natural water by adsorption on DEAE-cellulose (without pH-adjustment). Efficiency: More than 90%

(2) Quantitative analysis in original water by measurement of the UV-absorbance; comparison with calibration curves. (Preconcentration of the natural water may be required)

Sensitivity: Concentrations above 0.1 mg/l can be estimated. The presence of large amounts of iron as well as other organics interfere.

If the recovered amount of humics is large enough, an UV-spectrum can be recorded on the pure substance to verify the calibration (or for the creation of a calibration curve specific for the particular substance).

(3) Recovery and purification of a solid humic and fulvic acid fraction

(4) Characterization steps:

a. Elemental analysis	(ca 20 mg required)
b. IR-spectrum	(ca 1 mg required)
c. ^{1}H-NMR	(ca 40 mg required)
d. Molecular weight	(ca 1 mg required)
e. Functionality	(ca 10 mg required)
f. Carbohydrates	(ca 1 mg required)
g. ^{14}C-analysis	(ca 10 mg required)

(5) Acid-base titrations:

a. Aqueous I=0.100, 0.010, 0.001	(ca 20 mg required)
b. Non-aqueous	(ca 10 mg required)
c. In the presence of Cu^{2+}	(ca 20 mg required)

The chemical information on the humic substance will allow a discussion on potential metal complexing properties as well as origin and degree of degradation (possibly related to the water composition).

Reference material of various origin (deep groundwater, shallow groundwater, surface water) representing various aqueous environments will be used for complexation and sorption/mobility studies of relevant trace elements.

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APPENDIX 1

AVAILABLE HUMIC AND FULVIC ACIDS FROM SURFACE AND GROUNDWATERS

Humic substances that have been isolated so far within the project are given below.

Place	Name [a]	Water[b]	Amount, mg
Aldrich	HA-A	c	1000
Amazonas	FA-Am	SW	50
Bersbo	FA-B	SW	5800
	HA-B	SW	1200
Denmark	HA-D	GW	250
Fanay-Auger[d]	FA-FA	GW	200
	HA-FA	GW	100
Finnsjön	FA-Fi	GW	650
	HA-Fi	GW	1 [e]
Fjällveden	FA-Fj	GW	300
Forsmark	FA-Fo	GW	10 [e]
Gideå	FA-Gi	GW	220
Gorleben [f]	HA-Go	GW	100
Lake Bradford [g]	HA-LB	h	200
Lansjärv	FA-La	GW	40
Stripa	FA-S	GW	94
Tiveden	FA-T	GW	50
Vadstena	FA-V	GW	50
Äspö	FA-Ä	GW	10 [e]

[a] HA=humic acid, FA=fulvic acid
[b] SW=surface water, GW=groundwater
[c] Extracted from soil
[d] Obtained from V. Moulin
[e] Not purified
[f] Obtained from J. I. Kim
[g] Obtained from G. Choppin
[h] Extracted from lake sediment

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[1] Starprog AB
[2] SKB AB
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Rolf Hesböl, Ignasi Puigdomenech, Sverker Evans
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Statistical estimation and stochastic simulation using PROPER
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[1] Starprog AB
[2] SKB AB
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Karsten Pedersen
University of Gothenburg, Department of General and Marine Microbiology, Gothenburg
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Yngve Albinsson, Birgit Sätmark,
Ingemar Engkvist, W. Johansson
Department of Nuclear Chemistry,
Chalmers University of Technology, Gothenburg
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R S Forsyth, T J Jonsson, O Mattsson
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Lars Werme[1], Patrik Sellin[1], Roy Forsyth[2]
[1] Swedish Nuclear Fuel and waste Management Co (SKB)
[2] Studsvik Nuclear
May 1990

TR 90-09

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Ulla Bergström, Sture Nordlinder
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Outline of regional geology, mineralogy and geochemistry, Poços de Caldas, Minas Gerais, Brazil

H D Schorscher[1], M E Shea[2]
[1] University of Sao Paulo
[2] Battelle, Chicago
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Mineralogy, petrology and geochemistry of the Poços de Caldas analogue study sites, Minas Gerais, Brazil.
I: Osamu Utsumi uranium mine

N Waber[1], H D Schorscher[2], A B MacKenzie[3], T Peters[1]
[1] University of Bern
[2] University of Sao Paulo
[3] Scottish Universities Research & Reactor Centre (SURRC), Glasgow
December 1990

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M E Shea
Battelle, Chicago
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Geomorphological and hydrogeological features of the Poços de Caldas caldera, and the Osamu Utsumi mine and Morro do Ferro analogue study sites, Brazil

D C Holmes[1], A E Pitty[2], R Noy[1]
[1] British Geological Survey, Keyworth
[2] INTERRA/ECL, Leicestershire, UK
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Chemical and isotopic composition of groundwaters and their seasonal variability at the Osamu Utsumi and Morro do Ferro analogue study sites, Poços de Caldas, Brazil

D K Nordstrom[1], J A T Smellie[2], M Wolf[3]
[1] US Geological Survey, Menlo Park
[2] Conterra AB, Uppsala
[3] Gesellschaft für Strahlen- und Umweltforschung (GSF), Munich
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A B MacKenzie[1], P Linsalata[2], N Miekeley[3],
J K Osmond[4], D B Curtis[5]
[1] Scottis Universities Research & Reactor Centre (SURRC), Glasgow
[2] New York Medical Centre
[3] Catholic University of Rio de Janeiro (PUC)
[4] Florida State University
[5] Los Alamos National Laboratory
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Natural series nuclide and rare earth element geochemistry of waters from the Osamu Utsumi mine and Morro do Ferro analogue study sites, Poços de Caldas, Brazil

N Miekeley[1], O Coutinho de Jesus[1],
C-L Porto da Silveira[1], P Linsalata[2], J N Andrews[3],
J K Osmond[4]
[1] Catholic University of Rio de Janeiro (PUC)
[2] New York Medical Centre
[3] University of Bath
[4] Florida State University
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Chemical and physical characterisation of suspended particles and colloids in waters from the Osamu Utsumi mine and Morro do Ferro analogue study sites, Poços de Caldas, Brazil

N Miekeley[1], O Coutinho de Jesus[1],
C-L Porto da Silveira[1], C Degueldre[2]
[1] Catholic University of Rio de Janeiro (PUC)
[2] PSI, Villingen, Switzerland
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Microbiological analysis at the Osamu Utsumi mine and Morro do Ferro analogue study sites, Poços de Caldas, Brazil

J West[1], A Vialta[2], I G McKinley[3]
[1] British Geological Survey, Keyworth
[2] Uranio do Brasil, Poços de Caldas
[3] NAGRA, Baden, Sitzerland
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J Bruno[1], J E Cross[2], J Eikenberg[3], I G McKinley[4],
D Read[5], A Sandino[1], P Sellin[6]
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[2] AERE, Harwell, UK
[3] PSI, Villingen, Switzerland
[4] NAGRA, Baden, Switzerland
[5] Atkins ES, Epsom, UK
[6] Swedish Nuclear and Waste Management Co (SKB), Stockholm
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Testing models of redox front migration and geochemistry at the Osamu Utsumi mine and Morro do Ferro analogue sites, Poços de Caldas, Brazil

J Cross[1], A Haworth[1], P C Lichtner[2],
A B MacKenzi[3], L Moreno[4], I Neretnieks[4],
D K Nordstrom[5], D Read[6], L Romero[4],
S M Sharland[1], C J Tweed[1]
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[5] US Geological Survey, Menlo Park
[6] Atkins ES, Epsom, UK
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L M Cathles[1], M E Shea[2]
[1] University of Cornell, New York
[2] Battelle, Chicago
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Geochemical modelling of water-rock interactions at the Osamu Utsumi mine and Morro do Ferro analogue sites, Poços de Caldas, Brazil

D K Nordstrom[1], I Puigdomenech[2], R H McNutt[3]
[1] US Geological Survey, Menlo Park
[2] Studsvik Nuclear, Sweden
[3] McMaster University, Ontario, Canada
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N A Chapman[1], I G McKinley[2], M E Shea[3], J A T Smellie[4]

[1] INTERRA/ECL, Leicestershire, UK
[2] NAGRA, Baden, Switzerland
[3] Battelle, Chicago
[4] Conterra AB, Uppsala

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Kinetics of $UO_2(s)$ dissolution reducing conditions: numerical modelling

I Puigdomenech[1], I Cass[2], J Bruno[3]

[1] Studsvik AB, Nyköping, Sweden
[2] Department of Chemical Engineering, E.T.S.E.I.B. (U.P.C.), Barcelona, Spain
[3] Department of Inorganic Chemistry, The Royal Institute of Technology, Stockholm, Sweden

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Karsten Pedersen[1], Yngve Albinsson[2]

[1] University of Göteborg, Department of General and Marine Microbiology, Gothenburg, Sweden
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