

Molecular Characterisation of Dissolved Organic Matter (DOM) in Groundwaters from the Äspö Underground Research Laboratory, Sweden: A Novel “Finger Printing” Tool for Palaeohydrological Assessment

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ABSTRACT

The molecular signature of dissolved organic matter (DOM) in groundwaters can be used as a tool when investigating the palaeohydrological response of groundwater systems in relation to changes in recharge environment, and also for examining groundwater compartmentalisation, mixing and transport at underground repositories for radioactive waste. The DOM in groundwaters from two compartmentalised bodies of groundwater of distinctly different origin within the Äspö Underground Research Laboratory (URL), Sweden and in Baltic seawater has been isolated using tangential flow ultrafiltration (TUF) and diafiltration. Recoveries of DOM ranged from 34.7 to 0.1 mg/L with substantial differences in the concentrations of the groundwaters collected only 120 m apart. Analysis by infrared spectroscopy (IR) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) of the isolated DOM revealed that the groundwaters contained abundant alkylphenols which may represent heavily decomposed proteins or lignins originating from biopolymers contained within soils. The difference in the distribution and relative abundance of major pyrolysis products groups such as alkylphenols confirmed that the groundwater and Baltic seawater DOM samples were chemically distinct indicating minimal infiltration of marine groundwater derived by recharge from the Baltic or earlier Littorina Sea within the two compartmentalised groundwater bodies.

INTRODUCTION

Safety considerations for the disposal of radioactive waste and spent fuel in a deep geological repository must take into account the hydrogeological evolution of the site over a timescale of the order of 10^5 to 10^6 years. An important issue considered is the effect of climate change on the deep groundwater system. During the last million years or so (the Quaternary Period), European climate has alternated between extremes of ice ages and conditions much warmer than today. Large areas of northern Europe were covered by ice sheets and experienced extensive permafrost, whilst southern Europe was more arid. The present climate state is not representative of the climate that existed for much of the Quaternary, and it could be argued that the present-day groundwater conditions are not an adequate basis for assessing the long-term safety of a repository. The stability of groundwater conditions is one of the most important safety requirements, because the chemical composition of the water and its flow are key factors that will influence the transport of radionuclides in the geosphere to the surface. The impacts of past Quaternary climate changes on the stability of deep groundwater systems (palaeohydrogeology) are therefore of significant interest to predicting long-term repository safety assessment [1,2].

Understanding of the palaeohydrogeological evolution of groundwater systems is normally elicited from the interpretation of the inorganic chemistry, stable isotopes, fluid inclusion and noble gas composition characteristics of groundwaters and/or mineralogical features formed within groundwater systems [1,3,4]. However, a complementary approach to evaluate flow paths, mixing and compartmentalisation of groundwater masses is to characterise the dissolved organic matter (DOM) contained in the groundwaters. DOM in groundwaters may originate from several sources including: surface soils during infiltration in the recharge area; organic matter contained in the host rocks; or *in situ* production from natural microbial biomass sources. Although the mineralogical record may sometimes preserve a more permanent palaeohydrogeological record compared to the more transient record provided by groundwater

chemistry, the present study sought to look for the presence of organic molecules directly in the groundwaters rather than in the minerals precipitated from the groundwater. This has a number of potential advantages:

- It is easier to collect large volume samples of water;
- Water samples can be relatively easily processed to concentrate biomarkers for analysis, and;
- Water samples are easier to analyse, compared to the careful separation and analysis of individual mineral generations from complex fracture mineralisation.

Operationally defined DOM (0.2 μm - 1000 Da) in fresh and marine waters is generated from the secretion and transformation of biomolecules and is primarily comprised of non-living polymeric materials, which have been selectively preserved as well as viruses and some colloids [6]. The DOM in aquatic environments such as Pacific and Atlantic oceans, rivers, estuaries and freshwater wetlands have been isolated using tangential-flow ultrafiltration (TUF), and characterised at the molecular level using a variety of analytical techniques in order to better understand the global carbon cycle as well as improve knowledge of DOM source, transport and environmental fate [7,8,9]. These studies have shown that DOM from marine, estuarine and river environments can be chemically differentiated since terrestrial biomolecules such as lignin are abundant in stream and river waters but absent or extremely low in open marine waters [10,11]. This study sought to investigate whether the molecular “fingerprints” of DOM from groundwaters could provide complementary information to more traditional geochemical and mineralogical palaeohydrogeological methods.

EXPERIMENT

Two ground waters were collected from different boreholes in the tunnel of the Äspö underground research laboratory (URL), namely; KR0012B (redox alcove) representing relatively recent shallow meteoric recharged groundwater, and; KA1755A, which based on its highly depleted ^{18}O isotopic signature is interpreted to represent older groundwater with a possible large component of glacial melt water. An additional sample of modern Baltic seawater from the Northern Misterhult Archipelago Nature Reserve was also collected. A procedural blank (28 L of 18 M Ω distilled water) was treated in an identical manner to the other samples in order to differentiate artefacts from sampling, prefiltration, ultrafiltration, freeze drying and analysis.

Each sample was filtered and collected in two 28 L polypropylene storage barrels (Nalgene). Ultrafiltration was performed using a Millipore TUF system fitted with a 1000 Da cut-off cassette filter. Each sample yielded 1-1.2 L of retentate after 18.5-20 h of processing. The salts were removed from the retentates by diafiltration and the DOM freeze-dried for 48 h.

Infrared Spectra of KR0012B and Baltic Seawater were obtained using a Bio-Rad FTX3000MX series IR. Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) was performed using a platinum resistance heated Chemical Data Systems (CDS) AS2500plus, connected to a Carlo Erba Mega 500 series gas chromatograph (GC). The platinum coil was heated at 610 $^{\circ}\text{C}$ for 10 s. Products were separated using a fused silica Varian Factor 4 VF-1MS column (60 m length \times 0.32 mm i.d. \times 0.25 μm film thickness). The flow rate of helium carrier gas was 1 mL/min. The oven temperature was programmed from 30 $^{\circ}\text{C}$ to 300 $^{\circ}\text{C}$ at 4 $^{\circ}\text{C min}^{-1}$ and held isothermally at 300 $^{\circ}\text{C}$ for 15 min. The GC was directly coupled to a Varian 1200L triple quadrupole GC/MS/MS system operated in EI mode at 70 eV with a mass range 30-550.

Products were identified by comparison of their mass spectra and relative retention times with compounds reported in the literature [12, 13,14] and National Bureau of Standards library.

DISCUSSION

Isolated ultrafiltered dissolved organic matter (DOM)

A comparison of the dry weights of DOM samples showed that KR0012B and Baltic seawater had the highest DOM contents at 970.8 mg and 81.8 mg respectively (Table I). In contrast KA1755A had the lowest DOM value of 2.8 mg and the dH₂O water yielded 1.4 mg (Table I). The presence of DOM in the procedural blank maybe explained by leaching of DOM from pre-filters, TUF filters, tubing or storage barrels. Waters isolated by TUF from the North Sea and Ems-Dollart Estuary have reported concentrations in the range of 6-14 mg/L. In contrast, in this current study, the Baltic seawater had a lower DOM concentration at 2.9 mg/L as compared to the reported North Sea values [15]. One explanation is that the Baltic seawater DOM is diluted with riverine runoff. The large difference in the amounts of DOM isolated from KR0012B and KA1755A is surprising given that the instrumented sites were only around 120 m apart and is entirely consistent with the notion that the two groundwaters have different origins and thus potentially different amounts and sources of organic matter.

Table I. Dissolved organic matter (>1000 Da) isolated from Äspö URL

Sample	Non-purgable organic carbon (mg/L)	Weight DOM powder (mg)	Weight DOM (mg/L)
KR0012B	17.0	970.8	34.7
Misterhult (Baltic seawater)	4.9	81.8	2.9
KA1755A	2.9	2.8	0.1
Procedural blank (distilled H ₂ O)	<0.5	1.4	0.1

Infrared (IR) spectroscopy

Both IR spectra of DOM from KR0012B and Baltic seawater showed an intense, broad signal centred at 3440 cm⁻¹, the absorption band can be assigned to a number of localised vibrations including O-H stretching from alcohols and phenols as well as N-H stretching from amines and or amide groups (Figure 1). However, the relative abundance of moieties with O-H as compared to those with stretching N-H bonds cannot be determined due to the weaker absorption of the latter [16]. The appearance of a shoulder in both spectra at 2957 cm⁻¹ is probably due to CH stretching in aliphatic moieties. In the case of KR0012B a slight shoulder centered at 1740 cm⁻¹ was observed, this could be C=O of H-bonded carboxyl groups and C=O of ketonic carbonyl groups which are not conjugated to the aromatic ring [17]. Previous IR studies of peat, river and soil humic acids have reported that the most prominent feature of the

spectra was a peak at $\sim 1725\text{ cm}^{-1}$ thus the functional group chemistry of DOM in this current work does not correspond to that of humic acids [18]. Comparison of the spectra in the region of 1600 cm^{-1} revealed intense signals indicative of C=C in plane aromatic and/or asymmetric –COO⁻ stretch in both KR0012B and Baltic seawater which suggested a significant aromatic component (Figure 1). IR spectra for KA17755A were not obtained due to the low recovery of DOM.

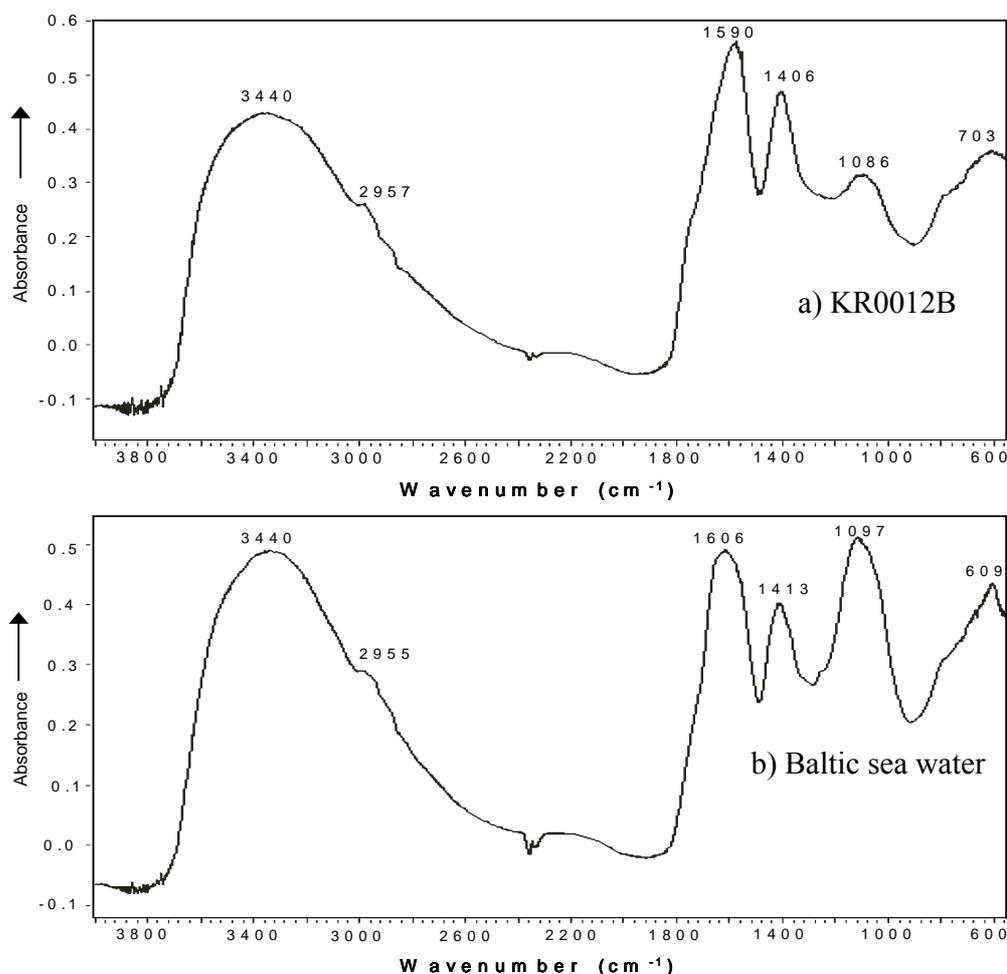


Figure 1. Infrared spectra of dissolved organic matter isolated from groundwater and seawater.

The slightly stronger and sharper form of the peak at $\sim 1600\text{ cm}^{-1}$ in KR0012B may therefore indicate that the groundwater sample has a higher aromatic content than the Baltic seawater. The KR0012B and Baltic seawater DOM exhibited strong signals centered at 1413 and 1406 cm^{-1} respectively, which can be assigned in part to aromatic ring stretching vibrations and associated C-H in plane deformation (Figure 1). The appearance of the peak at $\sim 1090\text{ cm}^{-1}$ could be due to C-O deformation in secondary alcohols and aliphatic ethers. Previous studies of polysaccharides from wood and microbial cell walls have reported intense absorptions in the range 1050-1170 cm^{-1} thus the peak at $\sim 1090\text{ cm}^{-1}$ may be due to cellulose or xylans. The lower intensity of the peak at $\sim 1090\text{ cm}^{-1}$ in DOM from KR0012B as compared to Baltic seawater suggests that the groundwater could have a lower polysaccharide content than the seawater.

Overall, analysis by IR confirmed that the groundwaters and Baltic seawater DOM samples were chemically distinct thus supporting the notion that DOM of KR0012B had not been significantly modified by infiltration of DOM sourced from the Baltic Sea which lies in close proximity to the Äspö URL.

Pyrolysis-gas chromatography-mass spectrometry

Analytical pyrolysis (Py-GC-MS) was performed on DOM preparations of Baltic seawater and Äspö groundwaters from boreholes KR0012B and KA1755A (Figure 2).

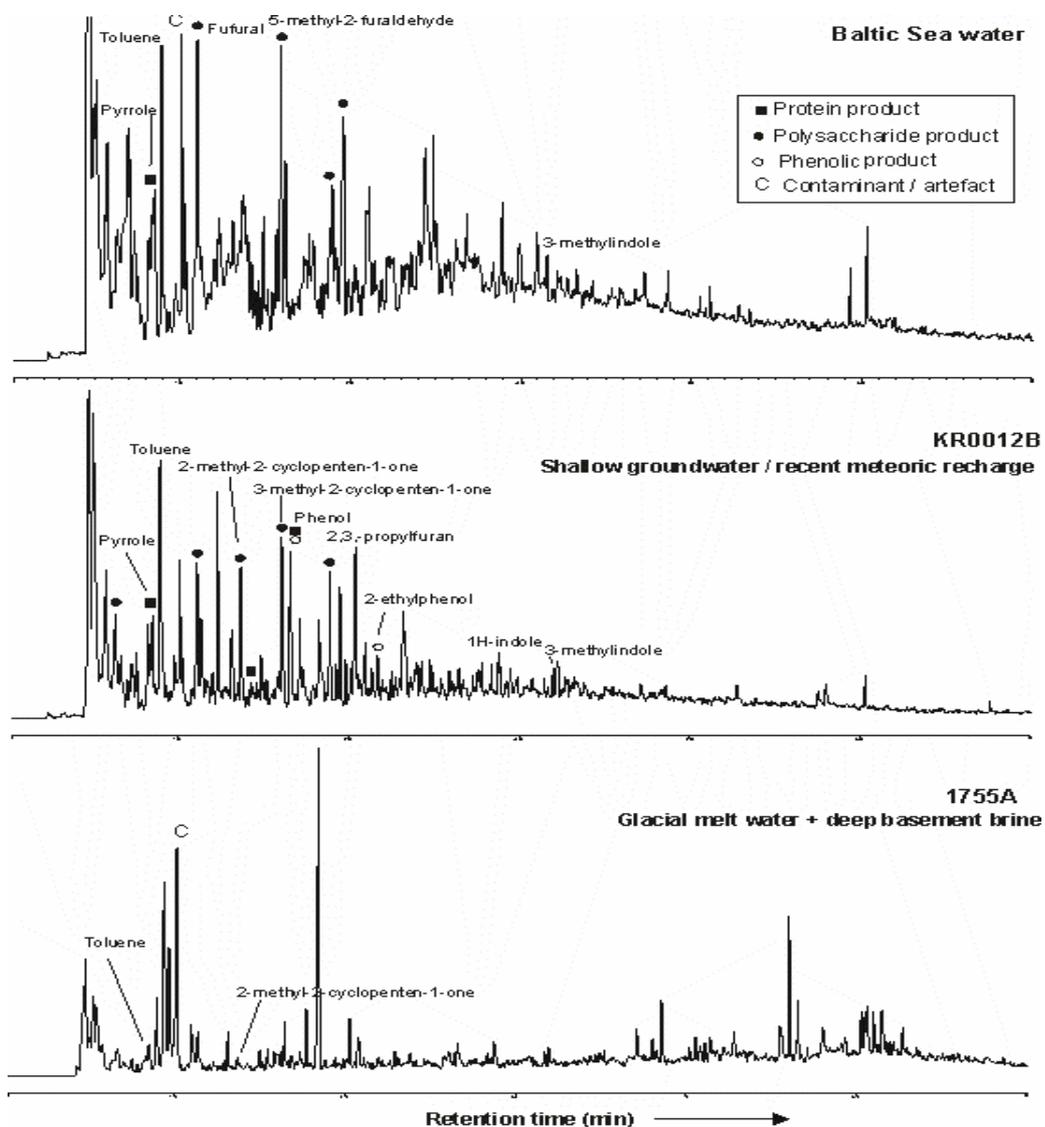


Figure 2. Chromatogram of the total ion current of the pyrolysis products of ultrafiltered DOM from Baltic seawater and groundwaters from boreholes KR0012B and KA1755A.

A comparison of the distribution of DOM products showed that both KR0012B groundwater and Baltic seawater contained a mixture of monomers, which contrasts with the limited distribution of monomers from KA1755A (Figure 2). The simple aromatic compound toluene was encountered in all three waters. However, this product has a number of possible origins including phenylalanine-containing proteins, protein derivatives, or can be produced as a secondary reaction product during pyrolysis of the polysaccharide cellulose [14]. Similarly, N-containing molecules with multiple origins including proteinaceous organic matter such as pyrrole and methylpyrrole were observed in KR0012B and Baltic seawater. Other compounds common to all DOM preparations included the N-containing compounds indole and 3-methylindole (m/z 117+131) the latter of which originates in part from the amino acid moiety tryptophan, a major component of algal proteins (Figure 2) [19].

A variety of different polysaccharide products were observed in relatively high abundance as compared to other protein or phenolic products in the two Äspö URL groundwaters and Baltic seawater (Figure 2). Pyrolysis of KR0012B gave polysaccharides tentatively identified as cyclohexy-1,3diene, 2-methylcyclopenten-1-one and 3-methylcyclopenten-1-one, as well as 2,3-propylfuran (Figure 2). Pyrolysis of cellulose and xylans (e.g. plant derived polysaccharides) also yield similar products, however thermal depolymerisation of cellulose generally yields large amounts of 1,6-anhydro- β -D-glucopyranose [20]. The absence of 1,6-anhydro- β -D-glucopyranose in KR0012B does not necessarily exclude a plant origin for the polysaccharide products since other studies have shown that 1,6-anhydro- β -D-glucopyranose can decompose at elevated pyrolysis temperatures [14]. The polysaccharides from Baltic seawater DOM included those tentatively identified as furfural, 2-methyl-5-ethylfuran, 5-methylfuraldehyde, which probably originate from multiple sources such as plants (cellulose), marine humic substances and algae. Aromatic products including methylbenzenes, xylenes and a variety of phenolic molecules were encountered in KR0012B, in contrast only phenolic products were observed in KA1755A and Baltic seawater (Figures 2). Mass chromatograms reflecting alkylphenols are shown for the DOM preparations in Figure 3. Alkylphenols originate from a number of biopolymers including decayed lignins, polyphenols and proteins as well as being pyrolysis products of green, red and brown algae [13]. One plausible explanation for the broader distribution of alkylphenols in KR0012B as compared to the other DOM samples could be that KR0012B contains alkylphenols from multiple sources including those derived on land from woody plant matter in soils and peat. Such an assumption appears reasonable given that combined the inorganic hydrogeochemical and stable isotope data suggested relatively recent shallow meteoric recharge. However, it must be noted that the absence of methoxyphenolic products - the main marker for lignin - either excludes a land plant origin or alternatively indicates that the original lignin monomers have undergone extensive degradation [12] in the soil.

The pattern of pyrolysis products from all three waters was different confirming the notion that the three selected end-member waters (Baltic seawater, old groundwater with a possible high glacial melt water content and relatively recent shallow meteoric recharged groundwater) can be differentiated using DOM signatures. Furthermore, the clear difference between KA1755A and KR0012B suggests that the two groundwaters are isolated and compartmentalised. Overall the simple assemblage of pyrolysis products in KA1755A is considered to be consistent with this groundwater being sourced from deep basement brine mixed with a large component of glacial meltwater in that the biological productivity, and thus generation of DOM precursor molecules, would be restricted in these environments.

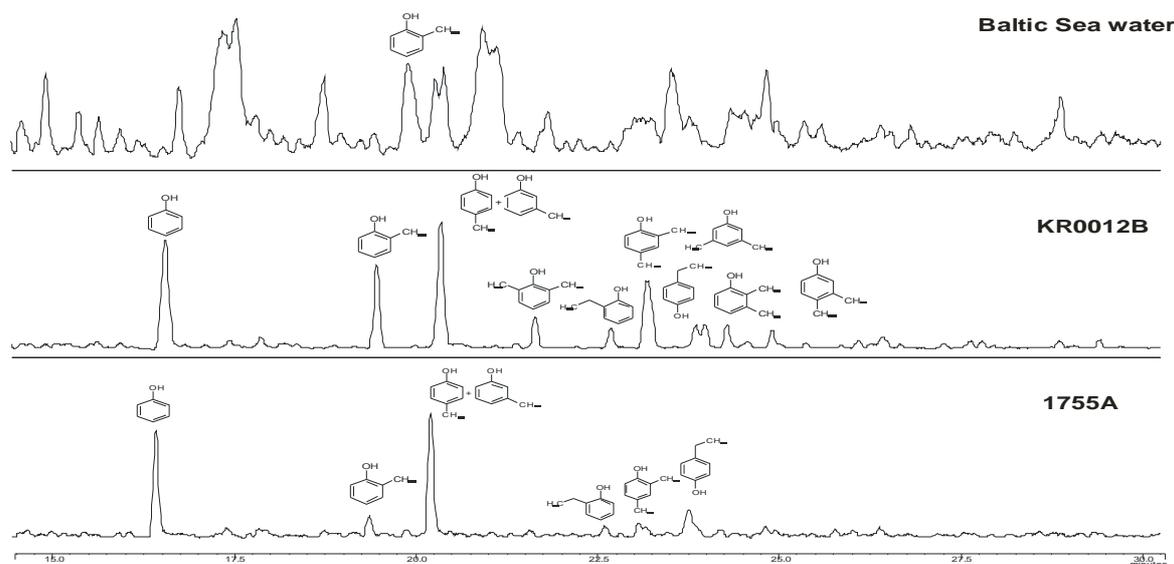


Figure 3. Partial mass chromatograms (m/z 94, 107, 108, 121, 122) of C_0 - C_2 alkylphenols from Baltic seawater, and groundwaters from boreholes KR0012B and KA1755A. Note: only peaks with chemical structures are alkylphenols.

CONCLUSIONS

This pilot study has demonstrated that the chemical composition of DOM can potentially provide a new tool to evaluate palaeohydrological evolution of groundwater systems in relation to changes in recharge environment, mixing and transport of groundwaters at underground repositories for radioactive waste. Molecular level characterisation of DOM extracts from two Äspö groundwaters and modern Baltic seawater revealed different distributions of polysaccharide, protein and alkylphenols as well as other unidentified moieties reflecting the palaeoenvironment and transport history. DOM in both Äspö groundwater samples was distinct from that in modern Baltic seawater indicating little evidence of mixing with Baltic or earlier Littorina Sea waters. The extraction and analysis of DOM in groundwater provides palaeohydrogeological information that complements and supplements traditional hydrogeochemical and mineralogical approaches, in that it appears to have some utility in identifying compartmentalised waters and may potentially be used to infer the palaeoenvironment at recharge and the extent of biological activity.

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