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Journal of Chromatography A, 739 (1996) 281–294

JOURNAL OF CHROMATOGRAPHY A

High-performance liquid chromatographic characterization of dissolved organic matter from low-level radioactive waste leachates

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Abstract

Leachates from a waste degradation experiment, containing ~700–3700 mg C/l of dissolved organic matter (DOM), were analyzed by high-performance liquid chromatography (HPLC) and liquid chromatography (LC), using various separation strategies. Scaling up of the separation scheme to a semi-preparative scale, suitable for hyphenated techniques, was also investigated. Separations with reversed-phase columns suggested that ~70–93% of the DOM was hydrophilic, and ion-pair chromatography of this fraction showed the presence of several discrete compounds. Labile and non-labile complexes were formed by adding ^{60}Co radiotracer. Size-exclusion chromatography indicated that the DOM was primarily in the <1000–1500 Da molecular mass range.

Keywords: Radioactive waste; Organic matter, dissolved; Stationary phases, LC; Environmental analysis; Organic acids; Metals; Cobalt

1. Introduction

Low-level radioactive wastes (LLRW) primarily contain refuse consisting of an organic carbon matrix: paper, cardboard, plastics, used clothing, paper wipes, mop heads, etc. In addition, chelating agents such as EDTA, citric acid, etc., could be part of the wastes. Upon burial, microbial degradation of this organic matrix produces volatile fatty acids (VFA) and other degradation compounds of structural polysaccharides.

The chemical and/or microbial degradation of cellulosic material first produces soluble compounds like mono- and disaccharides, degraded sugars, followed by organic acids and ultimately gases, such as CO_2 and CH_4 [1–3]. Inevitably, some of the soluble organic compounds contain functional groups such as aldehydes, ketones, esters, carboxylic acids, etc., which upon stabilization and biodegradation form humic-like materials. In the case of LLRW buried near the surface, these compounds have the potential of complexing cationic nucleides and forming neutral or negatively charged species that would not be retained or retarded by geological material [4,5]. For the safety of waste disposal facilities, it is important to determine the major properties of the dissolved organic matter (DOM) likely to influence radionuclide speciation and mobilization from a LLRW disposal site. In an experiment designed to study these phenomena [6,7], actual LLRW were statically leached with water to promote waste degradation. The leachates were typical of landfill

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0021-9673/96/$15.00
PII S0021-9673(96)00077-5

leachates, with high dissolved salts and metals, some radionuclides and elevated DOM.

Liquid chromatographic techniques have previously been used to monitor changes in the properties of DOM in landfill leachates over time [8,9]. Using gel permeation chromatography, these studies showed variations in the molecular mass of DOM over time, indicating a change in the degree of humification of DOM from both aerobic and anaerobic degradation. In a similar application [10], raw leachates and leachates treated aerobically and anaerobically, were analyzed using reversed-phase HPLC and a combination of ultrafiltration and gel permeation chromatography. At UK Nirex, reversed-phase HPLC was used in an attempt to identify and quantify isosaccharic acid in leachates from low- and intermediate- level wastes [11]. This compound is being investigated extensively, because of its complexation with plutonium and its influence on plutonium sorption on geological material. At Chalk River Laboratories [12], an anion-exchange column was used to preconcentrate and separate anionic forms of radionuclides from a low-level contaminant plume; the study showed an association of several actinides and selected activation products with DOM.

The studies mentioned above demonstrate how difficult it is to obtain specific information from a complex mixture of several unknown compounds. Many organic compounds can be separated (23 peaks or more [9]) with a C_{18} reversed-phase column, and only a crude identification or tentative assignments can be made at best [11]. A combination of several techniques could yield useful information on the properties of the compound classes present [9,13]; therefore, rigorous identification of each component is not necessary. This is especially true if chromatographic techniques are used in hyphenation with other techniques, such as titration, complexing capacity, spectroscopic techniques, etc.

The objectives of the current work are to: (i) use chromatographic techniques to determine the major properties of the DOM present in the leachates of this waste degradation experiment (classes of compounds, molecular size, etc.); (ii) investigate the complexation behaviour of some of these classes of compounds with a radiotracer; and (iii) investigate solid-phase extraction (SPE) as a time-saving alternative to HPLC for isolating hydrophilic compounds, and for characterization of DOM.

2. Methods

2.1. Waste degradation experiment and sampling

Eight compacted LLRW bales (0.31 m^3 each), representing a total of ~320 bags of refuse, were enclosed in separate carbon steel containers (see [7] for a detailed description). Each container was connected to a leachate collection system in a closed loop with daily leachate recirculation. The experiment had been running for approximately one year at the time of sampling. Partial results from four bales (1, 4, 8 and 9) are shown in this work. The water content in bale containers 1 and 9 was ~50% and 70% of the bale mass as received, respectively, whereas the water content in bale containers 4 and 8 was 150% and 170% of the bale mass as received, respectively. As a result, decomposition in bales 4 and 8 was predominantly in the methane-producing stage [14], while bales 1 and 9 were in the acid-producing stage [14]. Both modes are anaerobic. Emphasis will be placed on the two bales in the acid-producing stage, because this is believed to be more representative of landfills and the waste management sites at Chalk River Laboratories.

Approximately 2 l of leachate were sampled from each bale container, using an outlet located in a glove box filled with argon gas [7]. The leachates were characterized for VFA and DOM contents (Table 1). Bale 1 was predominantly used, because of its higher DOM content. A strong cation-exchange resin (Dowex HGR-W2, cross-linked 10% on polystyrene support, Na mode) was added in a batch mode to each leachate solution, to remove excess metals that could precipitate. The amount of resin added was calculated to exceed the cation content in the leachates by a factor of two, which was found to be sufficient to avoid precipitation. Equilibration was allowed to proceed for one week, after which the supernatant solutions were transferred to HPDE Nalgene containers. Portions of these solutions were kept under room conditions for most of the duration of these tests (~14 months). These samples will henceforth be called raw leachates. The solution matrix prior to equilibration with the Dowex resin was dominated by the sodium, calcium and chloride ions, with ~75–600 mg/l Fe, 1–5 mg/l Mn, ~1 mg/l Al. After equilibration, Fe was in the 1 mg/l range (the other ions were not analyzed). The ionic

Table 1
Dissolved organic matter (DOM) and volatile fatty acid (VFA) content of leachates analyzed ~30 days before sampling for this study (291 days after the start of the experiment)

Acid	Bale 1 (m*M*)	Bale 4 (m*M*)	Bale 8 (m*M*)	Bale 9 (m*M*)
Acetic	69.9	6.9	28.9	12.0
Propionic	5.5	1.1	1.1	3.0
Isobutyric	1.3	0.7	0.7	0.5
Butyric	19.6	3.3	2.1	1.1
Isovaleric	0.1	N.D.	N.D.	0.1
Valeric	1.3	0.3	0.1	N.D.
Isocaproic	0.1	N.D.	N.D.	0.1
Caproic	5.3	0.7	0.1	N.D.
Heptanoic	0.2	N.D.	N.D.	N.D.
Total VFA (mg C/l)	3375	461	883	483
Total DOM (mg C/l)	3725	715	1076	672
pH	6.83	6.04	6.68	6.07
^{60}Co (Bq/ml)(in untreated raw leachate)	0.058[a]	0.011	0.018	0.043

N.D.= Not detected.
[a] This result was not available at day 291 for bale 1. The result from bale 1 at day 32 is reported instead.

strength was generally ~0.1 *M*. Some radionuclides were also present in trace amounts (only ^{60}Co is shown in Table 1).

2.2. Chromatographic system

A Waters (Milford, MA, USA) HPLC system 3000 was used with a Waters model 490 UV–visible detector. Several columns and separation approaches were used, to gain as much information as possible on the samples (see Table 2). All the raw leachates were filtered with a 4 mm syringe filter, 0.45 μm pore size (Alltech, Deerfield, IL, USA); the injection volume was 600 μl, and the detector wavelength was set at 230 nm, unless otherwise specified.

2.3. Preparations and other sample manipulations

Sulfuric acid, phosphoric acid or NaOH were used to adjust the sample pH as needed. The two acids were found to be interchangeable without noticeable influence on separation efficiency. The sulfate and phosphate anions do not interfere with the detection at the wavelength used. However, samples containing ^{60}Co were acidified with sulfuric acid, to avoid possible precipitation of cobalt phosphate.

Ion-pairing runs were prepared by mixing 10 ml of the sample with 0.010 ml of 1.0 *M* tetrabutylammonium phosphate (TBA-PO_4) at 25°C for 1 min. The TBA moiety is positively charged and forms ion pairs with negatively charged functional groups of the DOM compounds.

The complexing properties of DOM were done by equilibration with a ^{60}Co radiotracer. A small sample (100 μl) of high specific activity ^{60}Co standard ($8.27 \cdot 10^4$ Bq total, 14.9 ng Co as chloride) was evaporated to dryness in a glass scintillation vial. 10 ml of raw leachate (0.45 μm filtered) were poured into the vial to equilibrate for two or four days. Samples of this solution were analyzed for ^{60}Co assay and chromatographic separations.

All the SPE were done using cartridges containing 500 mg of C_{18} reversed-phase solid support (Alltech), unless otherwise specified.

2.4. VFA analysis

VFA analysis was performed on a Varian (Palo Alto, CA, USA) model 8500 gas chromatograph equipped with a flame ionization detector. The raw leachates were extracted with diethyl ether, and a sample of the organic extract was injected for analysis [15].

2.5. Carbon analysis

A Dohrmann DC-80 carbon analyzer (Rosemount Analytical, Santa Clara, CA, USA) was used for dissolved organic carbon (DOC) analysis. The in-

Table 2
Columns, supports and eluents used in this study

Solid support	Column type	Eluent and separation objective
(1) Supelcosil C_{18}, 5 μm (Supelco)	150 mm×4.6 mm I.D.	0.01 *M* Na_2PO_4/NaH_2PO_4 (pH 7.4) buffer (15 min), 30 min gradient with buffer–acetonitrile (25:75), to buffer–acetonitrile (25:75) mixture for 10 min at 2 ml/min (hydrophilic/hydrophobic separation).
(2) Supelcosil C_{18}, 5μm	150 mm×4.6 mm I.D.	Same as above, except that distilled deionized water (DD water) was used instead of phosphate buffer.
(3) Supelcosil C_{18}, 5μm	150 mm×4.6 mm I.D.	0.01 *M* TBA-PO_4 (pH 7.1) (5 min), 45 min gradient with acetonitrile to 50% 0.01 *M* TBA-PO_4 (pH 7.1) – 50% acetonitrile for 10 min with this mixture, at 1 ml/min (ion pairing)
(4) Inertsil ODS-2, 5μm	150 mm×4.6 mm I.D.	Same as in (2) above, except that initial and final periods were 15 min each, for a total separation time of 60 min.
(5) Shodex OHpak KB-802	300 mm×8 mm I.D. (100–1500 M_r separation)	DD water (60°C), at 0.8 ml/min. Wash: acetonitrile–DD water (20:80) (12 h); conditioning: DD water (1 h) (highly polar polyhydroxymetacrylate support excludes large compounds and retards small ones).
(6) Supelcogel C-610H (6–9 μm)	300 mm×7.8 mm I.D. (for M_r below 2000)	pH 2, 0.1% H_3PO_4 isocratic. (ion exclusion, especially designed for organic acids; small compounds are excluded, large ones are delayed by permeation).
(7) Bio-Gel P-2 (100–200 mesh)	300 mm×15 mm I.D. Glass column (exclusion limit M_r 1800)	DD water at 3 ml/min (spherical polyacrylamide polymer support; large compounds are excluded first).

strument was calibrated in the 400 mg C/l range with freshly prepared potassium hydrogenphthalate. The DOC content of standards agreed within 2% up to 500 mg C/l. Above this level, dilution was necessary.

3. Results

3.1. Separation on C_{18} reversed-phase columns

3.1.1. Raw leachates

Separation of the DOM in raw leachates from bales 1, 4, 8 and 9 on a C_{18} reversed-phase column (protocol 1 in Table 2, except that the gradient started at 8 min, instead of 15) yields two general regions: the first one elutes almost unretained, with the signal returning to the baseline after ~8–10 min. The second region begins at ~10 min with acetonitrile, and the signal returns to the baseline 10–15 min later (Fig. 1). The two series of peaks were consistently observed for all the samples, although their shapes were different. For simplification, the first series of peaks will be called hydrophilics (HPI), because they elute in aqueous solvent only, whereas the peaks appearing in the second region will be called hydrophobics (HPO), because they start to elute at the start of the gradient with an organic solvent. As a crude indication of the relative proportion between these two classes of compounds, 70% of the total peak area is in the first region (HPI) and 30% is in the second region (HPO).

Fig. 2 shows the separation of bale 1 raw leachate using a different gradient elution (protocol 2, Table 2). The use of distilled deionized (DD) water as the first eluent, instead of phosphate buffer, seems to separate some individual components in the HPO region (Fig. 2). A second reversed-phase column,

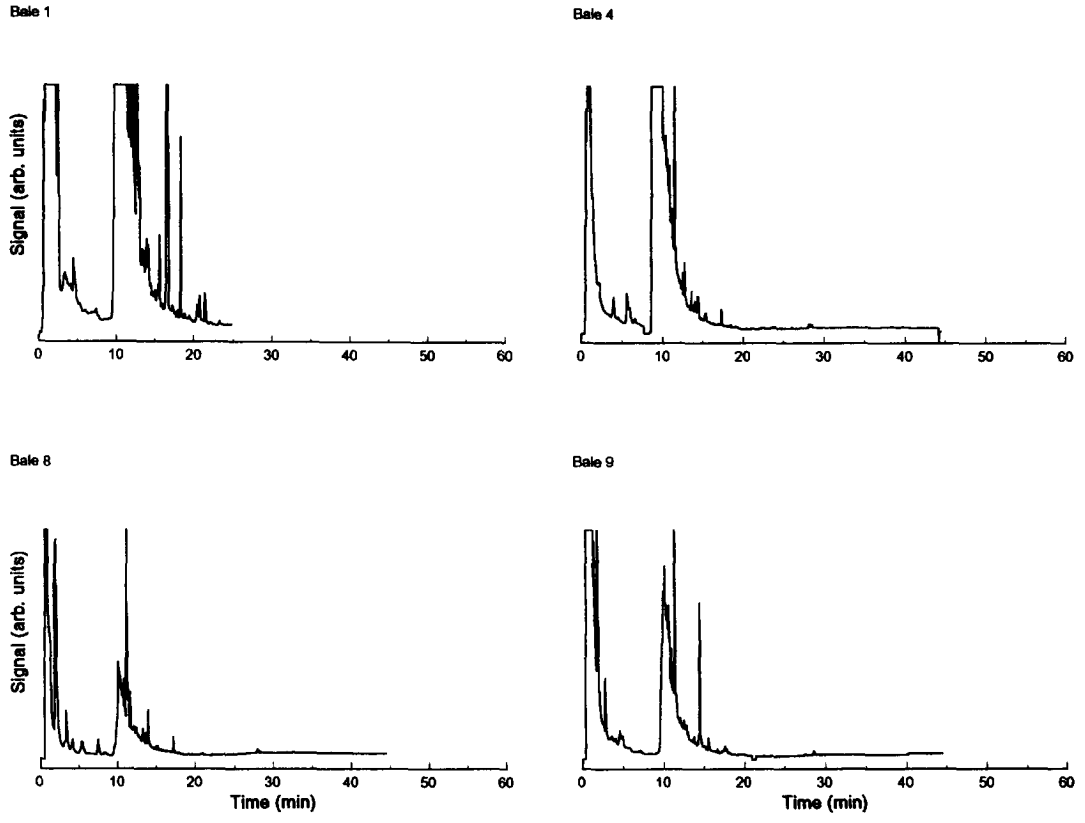

Fig. 1. Chromatograms of raw leachates from bales 1, 4, 8 and 9 on C_{18} reversed-phase column (protocol 1 in Table 2).

Inertsil ODS-2 (GL Sciences) (protocol 4 in Table 2), yields a more efficient separation of the HPI compounds, with the resolution of at least five components (Fig. 3). HPO compounds were also retained more strongly. Both reversed-phase columns were able to separate HPO components as several sharp peaks were observed, each of those peaks possibly representing a discrete compound.

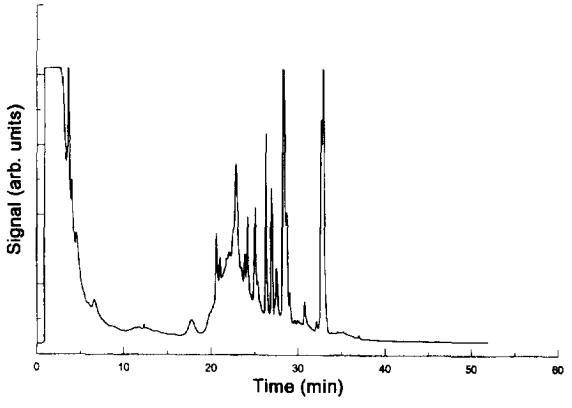

Fig. 2. Chromatogram of raw leachate from bale 1 on C_{18} reversed-phase support (protocol 2 in Table 2).

The total DOM in the HPI fraction isolated from the Inertsil column was determined in the five series of peaks (Fig. 3). The results suggest that the HPI contained 89% of the DOM in this sample, as opposed to ~70% (obtained from peak areas) using the Supelcosil column.

3.1.2. Ion-pairing separations

Ion-pairing separations of raw leachates and HPI fractions (protocol 3 in Table 2) are shown in Fig. 4. The chromatograms for both raw leachate and the HPI fraction show a complex mixture of sharp peaks, which indicates the presence of several major and minor discrete compounds. The two chromatograms

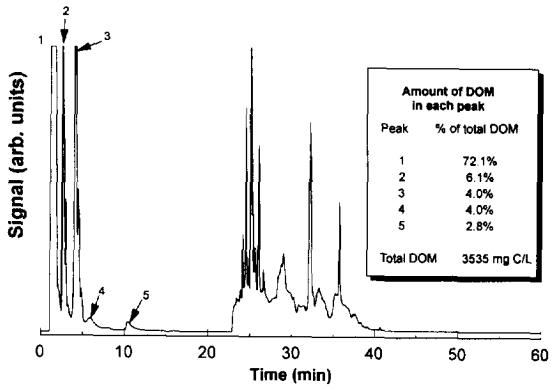

Fig. 3. Chromatogram of raw leachate from bale 1 on C_{18} reversed-phase support (protocol 4 in Table 2). The inset refers to the percentage of each peak relative to the total DOM after the ~14-month stabilization period. The HPI accounts for 89% of the total DOM.

are nearly undistinguishable from each other, which suggests that the compounds containing most, if not all of the negative functional groups ionized at pH 7.1, are contained in the HPI fraction.

3.1.3. Separations after equilibration with ^{60}Co

Complexation with a ^{60}Co radiotracer was investigated using protocols 2 and 3 (Table 2) after equilibration. The first equilibration was done in December 1993 (~11-month old sample), and the second one in March 1994, using different samples of the same unspiked leachate, but older in the latter case. Equilibrations were allowed to proceed for two and four days, respectively, after which each sample was immediately analyzed (radioassay for total ^{60}Co and HPLC). The difference in equilibration times is not believed to be significant. Fractions were collected every 7.5 min (Fig. 5) and measured for their ^{60}Co content (Table 3).

Most of the ^{60}Co in the December 1993 run eluted in the first fraction, either bound to HPI compounds or free. Cobalt-60 alone in DOM-free water eluted within 2 min (not shown). The HPI region of the December 1993 run showed a few minor peaks in the 5–15 min region, which were not observed in the other two runs. The major sharp peaks eluting early in the HPO region appear to be repeated between these runs, although with shifted retention times in the March 1994 run. The centre of mass of the HPO region has a different shape, with less minor components, or possibly noise, in this run, compared to the December 1993 run, suggesting that transformations of the DOM components may have occurred.

The location of the ^{60}Co label in the chromatograms was markedly different for both dates (Table 3). In the December 1993 run, most of the ^{60}Co

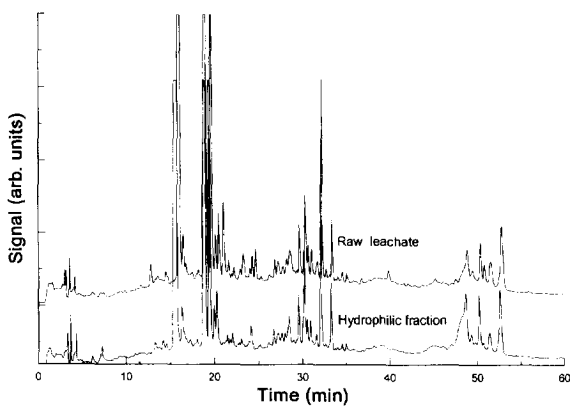

Fig. 4. Separation of raw leachate from bale 9 on C_{18} column, after reaction with TBA-PO_4 (protocol 3 in Table 2); raw leachate (top) and HPI fraction (bottom). The peaks at ~50 min are impurities in the mobile phase and do not interfere under 45 min.

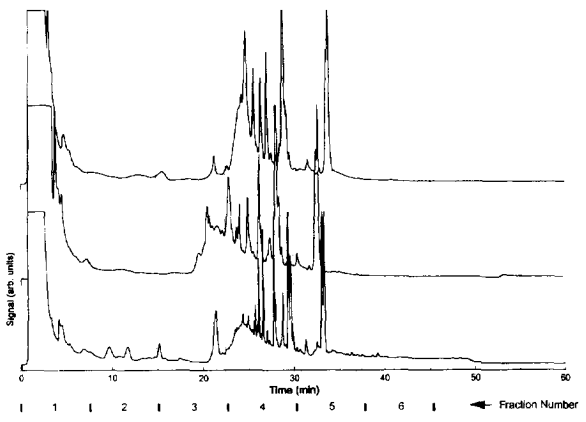

Fig. 5. Differences in DOM separations, after ageing and heating (raw leachate from bale 1); equilibration dates: December 1993 (bottom), March 1994 (middle), and March 1994 after heating at 80°C (top). The fraction numbers refer to Table 3 for ^{60}Co analysis (also see text).

Table 3
Amounts of ^{60}Co in the different fractions associated with the elution of leachates from bale 1 (protocol 2 in Table 2)

Fraction No.	Amount of ^{60}Co (Bq) for equilibration done on		
	1 December 1993	8 March 1994	8 March 1994, afterheating at 80°C
1	5629	962	2417
2	77	1018	31
3	54	1772	19
4	273	799	102
5	53	22	32
6	12	6	8
Total	6098	4579	2609
Solution	4686	4428	3898
% recovery	130	103	67

The same leachate was used, except that the March 1994 leachate had been sitting under ambient room conditions for an additional 3 months.

eluted almost unretained, with a significant signal in fraction 4, which corresponded to the bulk of the HPO region. Conversely, in the March 1994 run, the ^{60}Co content was significantly smaller in the first fraction, but relatively high in fractions 2, 3 and 4. These observations are a strong indication that the capacity of the DOM to complex cobalt has changed between the runs. Further to this, the March 1994 sample was heated at 80°C for 30 min. The ^{60}Co distribution in the resulting separation was very close to the one observed for the December 1993 run. This consistency is remarkable, and it suggests that both labile and non-labile complexes were formed in the sample (see fraction 4). Heat broke down the most labile ^{60}Co complexes formed with DOM components whereas the more stable ones were still present. After heating, the general features in the HPO region more closely resembled those in the December 1993 run.

A portion of the HPI fraction from both samples (December 1993, March 1994) was further separated by ion-pairing chromatography (protocol 3 in Table 2). Both chromatograms were nearly identical (only the HPI from the December 1993 run is shown in Fig. 6), which suggests that the compounds in the HPI fractions did not change significantly in the three-month ageing period. However, the ^{60}Co elution profiles were quite different between the runs (Table 4), suggesting that labile complexes, which could dissociate during elution, may have formed with HPI components.

3.2. Molecular size determination

3.2.1. Ion pairing (protocol 3 in Table 2)

A VFA standard and a mixture of complexing agents (oxalic acid, citric acid and EDTA), whose presence is suspected in these leachates, were separated by ion-pairing chromatography, and compared with raw leachate from bale 9 (Fig. 7). The VFA separation was not very good for the C_2 and C_3 compounds, but the isomeric species of C_4 to C_7 VFA were separated. Although there could be a shift in retention times between the chromatograms (see the impurity peaks at ~50 min), the molecular mass (or chain length) of the compounds in the leachates

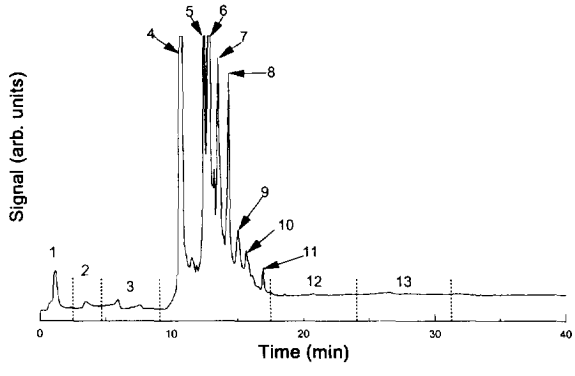

Fig. 6. Analysis of the HPI fraction from bale 1 (Fig. 5; December 1993 separation), previously equilibrated with ^{60}Co. The fraction numbers correspond to those in Table 4 (protocol 3 in Table 2).

Table 4
Amounts of ^{60}Co associated with different fractions from the hydrophilic fraction of Fig. 5

Fraction No.	Amount of ^{60}Co (Bq) in each fraction equilibrated on	
	1 December 1993	8 March 1994
1	11	24
2	8	612
3	17	23
4	17	9
5	93	8
6	17	6
7	15	8
8	5	6
9	4	3
10	3	2
11	0.4	2
12	1	1.7
13	0.6	0.6
Total	192	705
Solution	694	636
% recovery	28	111

Fraction numbers refer to Fig. 6.

does not seem to be much higher than the C_7 VFA. The complexing agents, if present, are likely to be in the HPI fraction separated earlier with the reversed-phase column. Here again, their presence cannot be confirmed based on their retention times. It is also likely that these complexing agents are already present as metal complexes in the leachates, with retention times that differ from what was observed here.

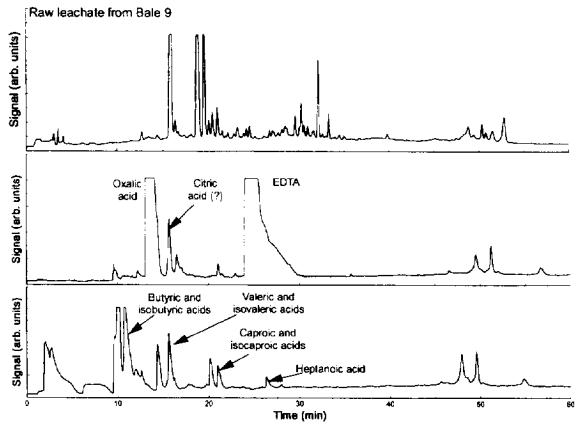

Fig. 7. Ion-pairing separations of Bale 9 raw leachate (top), complexing agents standard (middle), and VFA standard (bottom) using protocol 3 in Table 2. Peaks at ~50 min are impurities in the mobile phase. Detector wavelength: 215 nm for complexing agents, 254 nm for VFA. The top chromatogram is the same as "raw leachate" in Fig. 4, on a compressed scale.

3.2.2. Shodex OHpak KB-802 (protocol 5 in Table 2)

Fig. 8 shows the size-permeation separation of the HPI fraction of bale 1 raw leachate after removal of the HPO fraction with a C_{18} SPE cartridge. The chromatograms showed seven or more relatively sharp peaks, and some of them were likely a combination of unresolved peaks. Comparison with the VFA standard confirmed again that the molecular size is relatively small (the exclusion size is M_r 1500). Reproducibility was very difficult to obtain, however, even though the column was thoroughly washed between runs and conditioned overnight. Only the first daily injection was reproducible on a day-to-day or run-to-run basis. Both chromatograms in Fig. 8 represent the first daily injection of two different days. It is suspected that the SPE may not remove HPO compounds efficiently, which could cause the reproducibility problem by adsorption to the stationary phase.

3.2.3. Supelcogel C-610 H (protocol 6 in Table 2)

The HPI fraction from bale 1 raw leachate, previously isolated from a run with the Inertsil C_{18} column, was compared with a standard VFA mixture with this column (Fig. 9). The low molecular weight range of the components in the HPI fraction was confirmed again with this separation approach. The peaks between 12 and 22 min corresponded reasonably well to the C_2–C_4 VFA, and their relative ratios are somewhat consistent with the results in Table 1 for bale 1, except for one of the C_4 VFA. This peak assignment, however, is only tentative. The peak at ~30 min in the leachate cannot be assigned to VFA compounds, but its closeness to the C_6 acids suggests the presence of other compounds of similar molecular size.

3.2.4. Bio-Gel P-2 (protocol 7 in Table 2)

This scheme uses conventional low-pressure LC, with a column packed manually in our laboratory. The efficiency and resolution of this column, however, are much lower than for the previous ones, because of the larger particle size of the solid support. The information obtained from the chro-

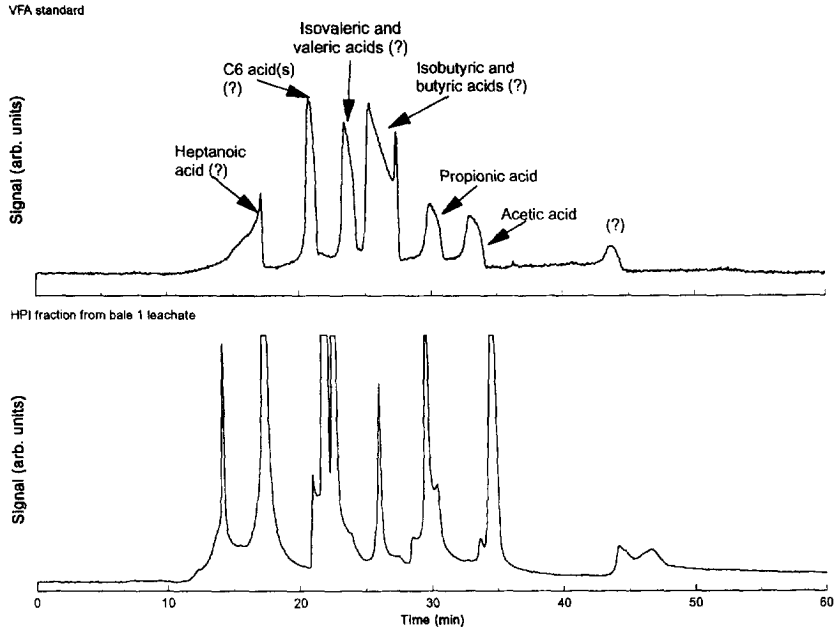

Fig. 8. Size-exclusion separation of HPI fraction of bale 1 raw leachate on Shodex OHpak KB-802 column (protocol 5 in Table 2). VFA standard (top) and HPI fraction (bottom).

matograms (Fig. 10) is consistent with what was obtained with the other columns. The bulk of the molecular masses of the compounds was fairly low, as only a small area of the peaks recorded was totally excluded (exclusion limit: M_r 1800).

3.3. DOM analysis and SPE

The separation between HPI and HPO could depend upon the type of C_{18} solid support, the solution pH, and the compounds present in each fraction. The validity and the completeness of the HPI/HPO separation was tested using two different approaches.

In the first test, two 10 ml samples of bale 1 raw leachate were adjusted to pH 3 and pH 8, and passed through two different SPE columns (containing 1 ml of C_{18} support each). The DOM content of the raw sample was 2952 mg C/l, whereas the eluates from the columns loaded at pH 3 and pH 8 had DOM of 2039 and 2643 mg C/l, respectively. The HPI contents corresponded to 69% of the total DOM at pH 3, and 89% at pH 8. This illustrates that pH control is important, as some DOM compounds protonate between pH 8 and 3, and may become uncharged, to display a hydrophobic behaviour. Chromatographic separation of these eluates (not shown) indicated the presence of HPO compounds in both cases.

In the second test, bale 1 raw leachate (adjusted to pH 6.8) was passed through a SPE cartridge, and the breakthrough solutions were analyzed after set volumes (Table 5, Fig. 11). Based on the DOM analysis, breakthrough occurred after only 1 ml of leachate had passed through the cartridge, and little adsorption was noticeable after 4 ml. HPLC separation showed that HPI compounds were breaking through early, and the HPO region started to become significant after 8 ml. Assuming that all HPO compounds were adsorbed from solution for the first 4 ml, the relative proportion of HPI in the sample would be ~93%.

These two tests, combined with other HPLC analyses, suggest that the HPI fraction in the leachates from bale 1 constitutes approximately 70–93% of the total DOM. The true relative abundance of HPI vs. HPO depends upon the separation technique used and the solution pH. Leachates from other

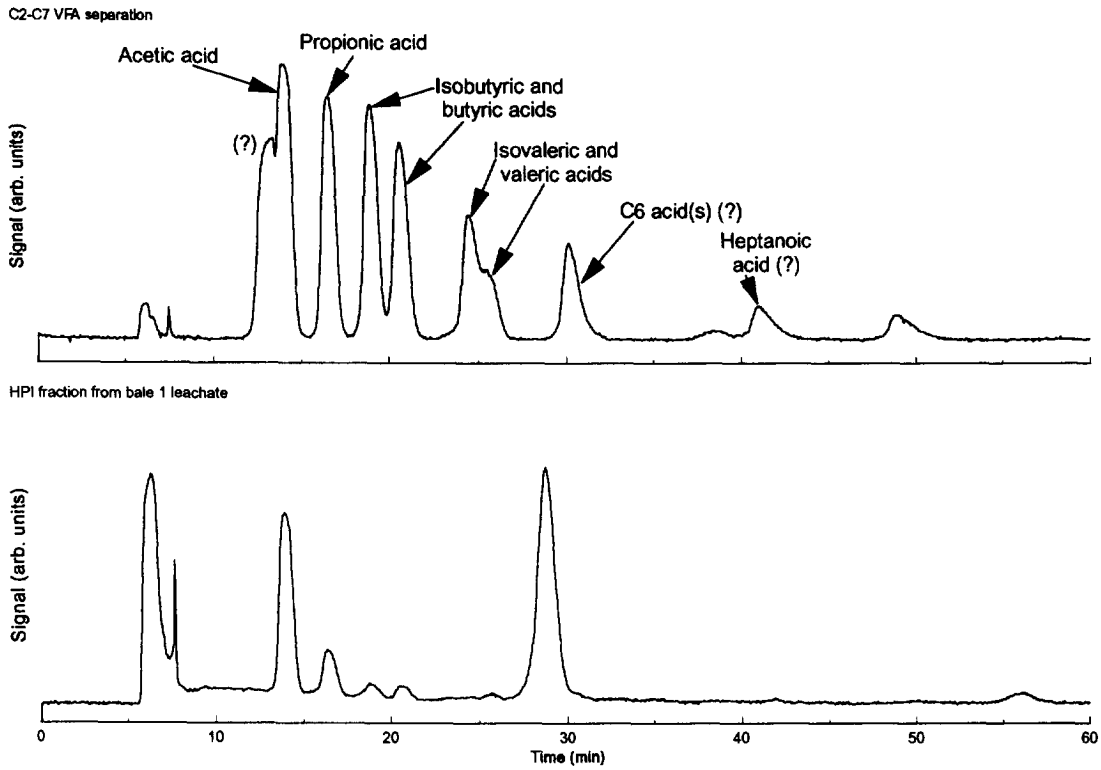

Fig. 9. Ion-exclusion separation of HPI fration of bale 1 raw leachate on Supelcogel C-610H column. VFA standard (top), and HPI fraction (bottom), 0–0.1 AU scale.

bales, although not tested as intensively as the one from bale 1, are likely to be distributed in a similar ratio.

4. Discussion

4.1. Features of the chromatograms and the samples

Separations of DOM using C_{18} reversed-phase columns consistently showed two distinct regions with all bale leachates. Differences were found between types of C_{18} columns; for example, see Fig. 2 and Fig. 3. Ten peaks or more can be seen in the HPO region. In the case of the Inertsil column, the HPI fraction eluted in three major peaks and two minor ones before the start of the gradient. A more gradual gradient may lead to a better separation of the individual peaks, especially in the HPO region, because this column appears to provide a stronger hydrophobic interaction with the analytes. Chromatograms using the Inertsil column were quite reproducible, especially when the sample pH was pre-adjusted to ~6.8 before injection. This column was preferred over the Supelcosil, for our applications.

Other studies using C_{18} reversed-phase columns have reported similar separation trends into HPI and HPO components [10,13]. In a study on the separation of Suwanee fulvic acid [13], ~40% of the total DOM was found in the HPI fraction, which showed at least six separate peaks. In a different study [10], raw landfill leachates were separated into HPI and HPO regions, with two major peaks and three minor ones in the HPI region, and up to 18 distinct peaks in the HPO region. These examples also show that the HPI fraction of aqueous DOM can be separated into several individual compounds using only water, or a

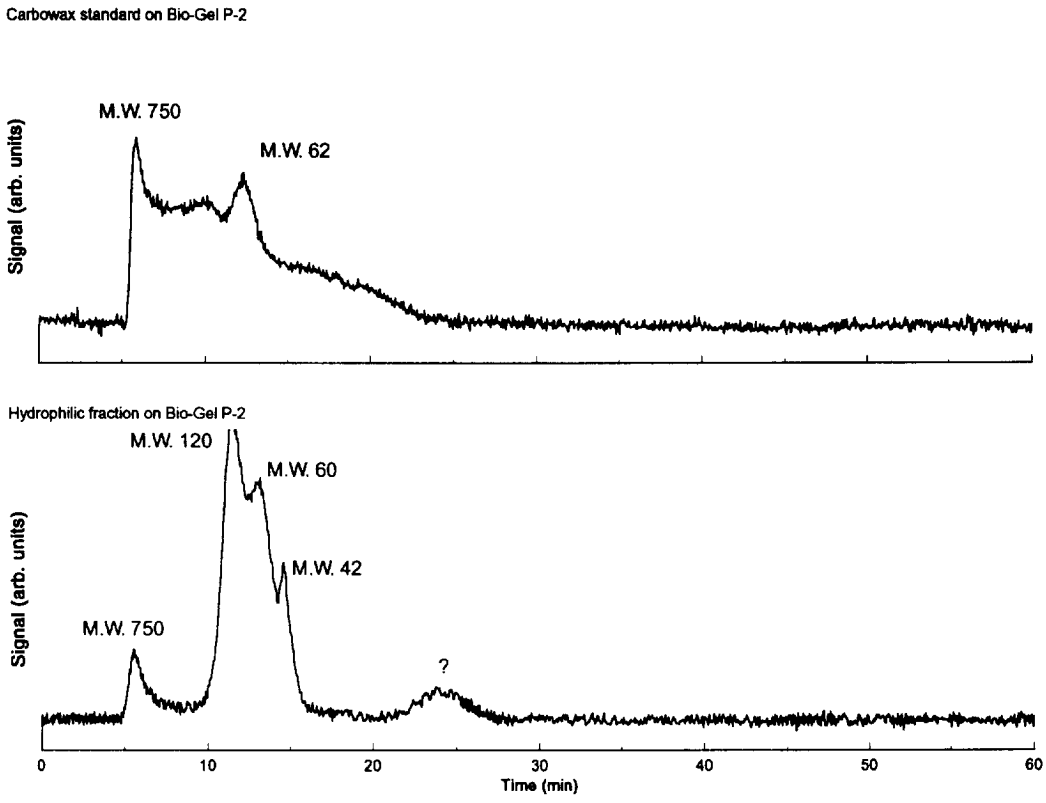

Fig. 10. Size-exclusion separation of HPI fration of bale 1 (previously isolated with Inertsil column) on Bio-Gel P-2 column. Carbowax VFA standard (top) and HPI fraction (bottom).

weak buffer as an eluent on a reversed-phase column.

Ion-pairing separations of the HPI fraction on a C_{18} column revealed complex chromatograms of 20 or more discrete peaks (Fig. 4 and Fig. 6). This separation approach has also been applied to fulvic acid extracted from groundwater [16]. Their chromatograms were quite different from the ones reported in this work, as four broad peaks eluted between ~5 and ~18 min, which is apparently typical of fulvic acids. In our work, we have attempted to identify individual compounds by comparing the retention times with those of known standards, but this was tentative at best. The presence of the VFA compounds known to be present, or suspected complexing agents (oxalic acid, citric acid and EDTA), could not be confirmed. If present in the leachates, EDTA is likely to be complexed with metals, even after sample pre-treatment with the ion-exchange resin, giving different retention times with different metals [17]. Therefore, identification of strong com-

Table 5
DOM analysis of the effluent from a C_{18} SPE cartridge (bale 1, raw leachate)

Sample volume (ml)	DOM (mg C/l)	% of total
1	141	4
2	2030	57
4	3274	93
8	3380	96
13	3429	97
18	3462	98
Total DOM in sample*	3535	100

* Note: this analysis was done after the ~14 month stabilization at room temperature, and it may differ from the result in Table 1.

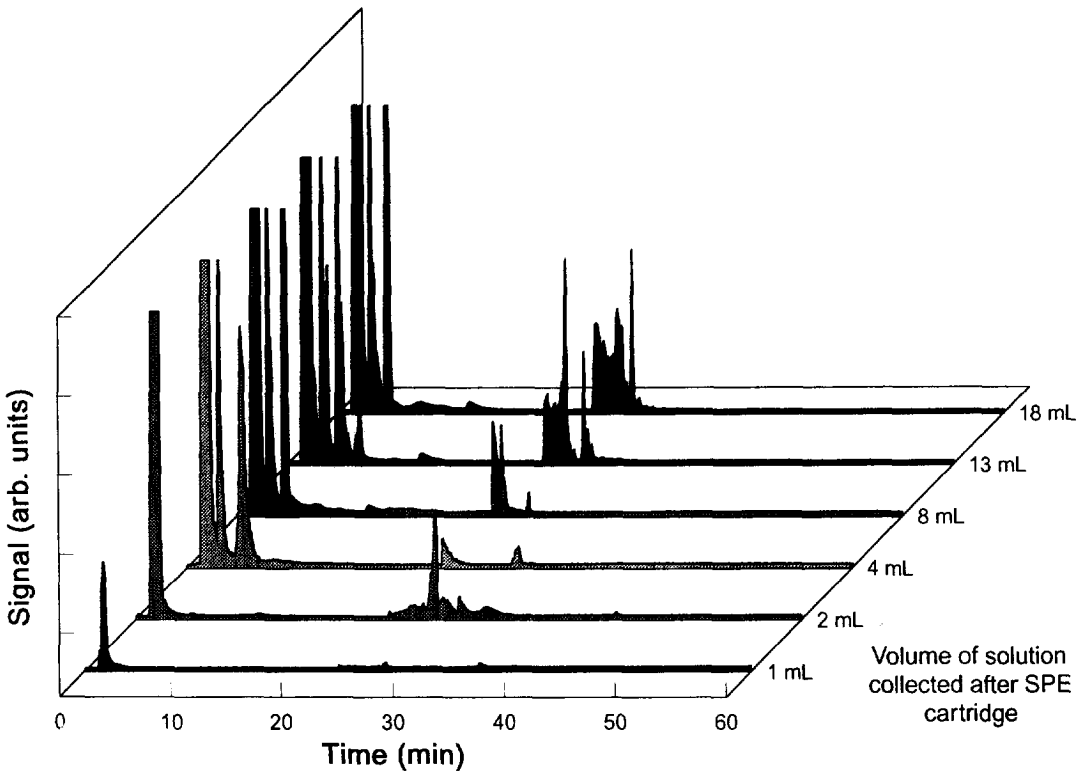

Fig. 11. Analysis of the breakthrough of DOM after the sample has passed through a C_{18} solid support SPE column (separation using protocol 4 in Table 2).

plexing agents based on retention times would be difficult. Size permeation and/or exclusion HPLC, using a Shodex or a Supelcogel column (protocols 5 and 6, respectively, in Table 2), were not sufficient in assigning individual peaks to known compounds. The Bio-Gel separation (protocol 7 in Table 2) did not yield much specific information. On the other hand, all of these schemes indicate consistently that the molecular mass of the hydrophilic components is low, probably 1000–1500 (nominal) or less.

4.2. Eluent

Analysis of the samples on C_{18} reversed-phase columns (protocols 1, 2 and 4 in Table 2) was done with two different eluents: DD water and phosphate buffer. Both approaches have advantages and shortcomings. The phosphate buffer gives a good pH control. Peak elution was fast, but resolution was somewhat sacrificed, especially in the HPO region (compare Fig. 1 and Fig. 2). Problems of high and continuous ^{60}Co bleeding were observed when running samples with ^{60}Co, and this was attributed to precipitation of cobalt phosphate in the column guard. On the other hand, DD water decreased the potential matrix effect due to high salt content, and more peaks were separated than with the buffer. We did not observe bleeding problems with ^{60}Co, which substantiates the possible formation of phosphate precipitate. In some instances, however, there were reproducibility problems, as successive injections of the same sample produced chromatograms with differing peak shapes and/or retention times. Reproducibility was improved, especially with the Inertsil column, when the pH of the samples was readjusted to ~6.8 prior to injection. Separation of the HPI components previously pH adjusted and separated with the Inertsil column was also improved with the Shodex column.

4.3. Complex formation with ^{60}Co

It was not possible to determine definitely whether the early ^{60}Co elution was for the metal co-eluting free and unretained along with the HPI compounds, or whether it was complexed with unretained DOM species. Subsequent ion-pairing separations suggested that complex formation with HPI compounds was possible, and that those complexes were probably labile.

Co-elution of the tracer with hydrophobic components is an indication that Co can make complexes with some of these compounds. A combination of labile and non-labile complexes was formed with HPO compounds, and this complexation potential of the DOM changed with ageing. It was interesting to observe that heat destroyed labile complexes in the aged samples, leaving behind a label distribution similar to the previous equilibration done in December 1993. The numbers obtained after heating (Table 3) were well above the background ^{60}Co contamination of ~0.06 Bq/ml in the leachates.

Cobalt tracer association with DOM peaks or regions, separated chromatographically, has also been observed with fulvic acid [16] and in groundwaters from contaminated sites [4,12]. Similarly to our study, Cooper et al. [12] have reported the formation of labile and non-labile complexes upon addition of ^{60}Co. This was somewhat in contrast with one of their earlier studies [4], where such complexes were not necessarily readily exchangeable, especially if the samples were shielded from light. The Co exchange experiment done with the bale leachates suggested that stable complexes may form with Co, but the environmental significance of this is unknown at the moment. Labile complexes would probably be broken up by exchange with soil components and have no noticeable impact on ^{60}Co mobility, but the migration potential of stable complexes with HPO components may be different form this. Independent evidence using ion-exchange resin equilibration [18] suggested that ~4000 mg C/l of DOM in these leachates could complex ~50 μg/l of Co. This amount could be significant for this metal; however, this ion-exchange technique does not yield information about the stability of these complexes.

It is also likely that all of the DOM components prone to form strong and stable complexes are already complexed with a metal present in the leachates. These sites might not be readily exchangeable, because exchange depends on the relative thermodynamic stability of the complexes and the kinetics of the exchange reactions.

4.4. Separations using SPE

Results presented here indicate that this approach should be used carefully. Firstly, both analytical reversed-phase columns have more material of a much smaller particle size, hence a much higher separation efficiency and sample capacity, than the SPE cartridges. A larger-capacity SPE cartridge may be able to compensate for this shortcoming. Secondly, some HPO components co-elute along with HPI, indicating that some elution control or monitoring would be needed to effectively separate HPI from HPO components. Thirdly, pH control is important, as some HPI compounds could be labelled as being HPO if the pH is too low (protonation).

It remains that the separation between HPI and HPO compounds is defined operationally, whether SPE or column separation is used.

4.5. Sample stability

Leachates contain a large amount of DOM of various molecular sizes. The redox potential was reducing at the time of sampling, and bacteria and/or fungi were undoubtedly present in the samples. Occasionally, a film of detrital material was observed at the bottom of the sample vials, accompanied by a rise in pH (some samples had a pH of up to 9.3). Some variability in the chromatograms was observed; for example, see Fig. 2 (fresh sample) and the bottom two chromatograms in Fig. 5 (older samples).

Microbial activity in samples would result in assimilation of the lighter DOM species, and the production of heavier species. For example, in a separate study involving this experiment on waste degradation [19], a higher proportion of organic colloidal material was observed in bale 4 leachate, and this was attributed to bacterial assimilation of VFA in the leachate during an 18-month stabilization

period under ambient room conditions. Evidence of changes in fresh landfill leachates has been shown elsewhere [8,10]. The general trend in both of these studies was that a higher proportion of higher-molecular-mass HPO (vs. HPI) was formed over time, with anaerobic or aerobic treatment of the leachates.

5. Conclusions

Separation of DOM in waste leachates using reversed-phase HPLC has revealed the presence of two general types of compounds, HPI and HPO, with ~70–93% of the total DOM in the HPI fraction. Ten or more discrete peaks could be separated in the HPO fraction. Up to 20 or more discrete HPI compounds were separated using ion-pairing chromatography on a C_{18} reversed-phase column. The molecular size of the compounds contained in the DOM was fairly small (M_r<1000–1500), based on several types of molecular-size-based separations, and this is consistent with other studies [8–10]. A preliminary identification of some individual compounds was done, but it was tentative at best. It was also shown that both labile and non-labile complexes can form with Co radiotracer, but only the non-labile complexes with HPO are believed to have a potential impact on subsurface migration. Finally, SPE could be used as a time-saving alternative to clean up and isolate HPI compounds, but it is limited to small samples, or large volumes of solid-phase should be used.

Future work to isolate larger volumes of solution, which is necessary for some specific analysis of the separated fractions (titration, complexing capacity, ion-exchange, etc.) seems promising. For that prospect, a 5 ml sample was injected into a larger C_{18} column (similar to protocol 3 in Table 2, except that the column diameter was 10 mm), and the chromatogram (not shown) corresponded closely with those in Fig. 4, without sacrificing much separation and peak resolution.

Acknowledgments

The authors wish to thank J. Gulens, E.L. Cooper and R.R. Rao at Chalk River Laboratories (CRL) and the two journal reviewers for offering suggestions that helped improve this manuscript. M.K. Haas (CRL) and G. Manni (AECL Engineering Technologies, Mississauga, Canada) have helped in sampling and performing the DOM analysis. G.F. Keenleyside (The Definite Article, Nepean, Canada) has provided helpful editorial comments.

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