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INVESTIGATING METAL/NANOCOLLOID INTERACTIONS IN LANDFILL LEACHATES USING AF4-HR-ICP-MS

by

Yasmin Labibi

Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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Landfill leachates contain a wide range of pollutants including potentially toxic metal(loids) e.g. arsenic. Current landfill risk assessment models predict the fate and transport of these pollutants in the environment, however they consider all species below 0.45 μm to be dissolved, thus the presence of these pollutants in colloidal form is not considered.

In order to investigate the presence and distribution of metal(loids) within the nanocolloidal fraction (<100 nm), AF4 coupled with HR-ICP-MS was selected (alongside AFM and DLS) and optimised for use with landfill leachates. UV_{254} and Fluorescence spectroscopy were also used as detectors for AF4 to detect organic colloids.

AF4-HR-ICP-MS analysis was carried out both offline (fraction collecting and subsequent HR-ICP-MS analysis) and online (interfacing the AF4 output directly with the HR-ICP-MS) with parameters optimised for lower MW particles. Online coupling provided a higher resolution analysis than the offline method. The concentration of elements within the AF4 system was found to be in flux and therefore baseline concentrations were established for each sample injection. Method repeatability and a recovery mass balance of each element were also established.

The method was validated by fractionation of a MSW, an aged MSW and a MBT leachate. All three leachates were found to show the same nanocolloidal distribution with two distinct nanocolloid populations present: a low MW organic rich fraction; and a larger, less organic rich fraction consisting of a mixture of organic and inorganic particles. Metals predominated in the lower MW fraction associated with humic or fulvic-like particles. The similarities between the leachate metal distributions showed that treatment of leachate prior to landfill did not alter the colloidal characteristics.

Preliminary results examining the effects of pH and ionic strength of metal distribution showed that pH had no effect; however the lowering of ionic strength appeared to cause aggregation of colloidal Fe particles, presumably due to the lower organic content, which appears to control the distribution of metals in this size fraction.

This research highlights the importance for landfill risk assessments to be updated to include the presence of colloidal facilitated transport and the necessity for further particle transport studies to be conducted.

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DECLARATION OF AUTHORSHIP

I, Yasmin Labibi

declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

INVESTIGATING METAL/NANOCOLLOID INTERACTIONS IN LANDFILL LEACHATES USING AF4-HR-ICP-MS

I confirm that:

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2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
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7. None of this work has been published before submission

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Definitions and Abbreviations

AF4	Asymmetric-Flow Field Flow Fractionation
AFM	Atomic Force Microscopy
Bioavailability	Defined in ISO 11074 as the degree to which chemicals present in the soil may be absorbed or metabolised by human or ecological receptors or be available for interaction with biological systems
DLS	Dynamic Light Scattering
DERFA	Department for Environmental and Rural Affairs
EC	Electrical Conductivity
EU	European Union
FFF	Field Flow Fractionation
FLU	Fluorescence
Fractogram	A graph of detection signal versus. time, derived from a field-flow fractionation process
HDPE	High-density polyethylene
HNO_3	Nitric Acid
HR-ICP-MS	High Resolution Inductively Coupled Plasma Mass Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
Monodisperse	Particles of uniform size in a dispersed phase
MQ	Milli-Q Water
MW	Molecular Weight
Nanocolloid	Natural colloids in the NP size range (< 100 nm)
Nanoparticle	Particles that can be as small as 1 nm and as large as several tens of nanometres, in at least one dimension
NOM	Natural Organic Matter
NP	Nanoparticle
OM	Organic Matter
PDVF	Polyvinylidene fluoride
PEEK	Polyether ether ketone
PES	Polyethersulfone
Polydisperse	Particles of varying size in a dispersed phase
PSD	Particle Size Distribution

Chapter 1

PSS	Polysulfonate Standards
RC	Regenerated Cellulose
RSD	Relative Standard Deviation
SBS	Sensitivity Baseline Sample
TDS	Total Dissolved Solids
TOC	Total Organic Carbon
UV_{254}	UV absorbance intensity at 254 nm

Chapter 1: Introduction

Prior to the introduction of Landfill Tax in 1996, more than 80% of UK waste was sent to landfill (Eurostat, 2014). The EU Landfill Directive 1999/31/EC set targets for the reduction of landfill with the aim "to prevent or reduce as far as possible negative effects on the environment, in particular the pollution of surface water, groundwater, soil and air, and on the global environment, including the greenhouse effect, as well as any resulting risk to human health, from the landfilling of waste, during the whole lifecycle of the landfill". By 2020, the target is to reduce landfilled waste to 35% of that prior to the Landfill Tax (1996). There are several other regulations which govern landfill such as the Waste Management Licensing Regulations and Water Framework Directive as described in Butt et al.,(2014) (Appendix 1.1).

Despite a reduction of landfilled waste due to increased recycling and less waste generation, it remains an important disposal method for the UK, with approximately 40% of waste currently disposed of in this way (Eurostat, 2014). It is not possible to completely avoid landfill as a disposal method because not all materials can be continually recycled, composted or incinerated (e.g. some incinerator ash is still disposed of in landfill). As a result of the reduction of waste there are now fewer sites currently accepting waste, but sites which are closed (e.g. capped and no longer accepting waste) can still have negative effects on the surrounding environment due to gas emissions and leakage of leachate (Environment Agency, 2010b).

Modern landfills are engineered to restrict outputs of gas and leachate, but total containment within the landfill cannot be guaranteed because the performance of the landfill liners is likely to degrade over time (Christensen et al., 2001). UK landfill liners typically consist of a geological barrier e.g. compacted clay combined with an artificial sealing liner such as high density polyethylene (HDPE) (Environment Agency, 2009a, Environment Agency, 2009b). Once a landfill ceases to receive waste, the risk of pollution does not cease, and the design, the leachate collection and treatment systems, as well as the post closure management, are of major importance in the protection of groundwater resources from future leachate contamination (Quasim and

Chiang, 1994). Although landfill waste, gas and leachate all pose a potential environmental threat, this study focuses on leachate because of its mobility in the subsurface environment and the risk to groundwater resources. These form approximately a third of drinking water supplies in the UK (in some regions this is as high as 80% (Environment Agency, 2014a), and landfill leachates have been identified as a potential environmental threat to this important resource (Christensen et al., 2001).

1.1 Landfill Leachates

Landfill leachates are created from waters that have percolated through wastes buried in the ground. Meteoric water is the main source of the water in the leachate, with lesser contributions from surface water and liquids buried with the waste. As water percolates through the waste, it gains dissolved and suspended components from the degrading waste through several physical, chemical and microbial processes (Abbas et al., 2009, Kjeldsen et al., 2002). Thus, the composition of the landfill leachate is a function of a variety of factors that include the waste type and composition, the age of the landfill site, and the amount and flow rate of water within the site.

1.1.1 Landfill Leachate Composition

As leachates are a function of the wastes from which they are derived, it is important to define the source of landfill waste. Those which receive general household waste are known as Municipal Solid Waste (MSW) sites and thus this waste is known as MSW. Historically, landfills were not regulated and accepted a mix of MSW and potentially hazardous wastes such as those from medical, agricultural, construction and mining industries (Taylor and Allen, 2006). Although MSW waste is thought to be less hazardous, Slack et al.,(2005) found contaminants in MSW from household hazardous waste (HHW) such as paints, detergents, garden pesticides, batteries and electronic equipment. HHW are not covered by regulations, but they are presumed to be a small component of the total waste in the landfill.

In recent years, landfill regulations and government policy has altered the composition of waste sent to landfill, because pre-treatment is often carried out before burial to remove recyclable/easily biodegradable/high calorific materials (depending upon local schemes), thus reducing the volume of buried material (Siddiqui et al., 2013, Robinson et al., 2005). More recently (2011) very low level radioactive wastes (VLLW) have been permitted to be added to landfill (Environment Agency, 2012). All of these actions are likely to affect the composition of the leachates produced in landfills.

MSW waste typically contains plastics, metals and glass, but these are now commonly removed before burial using a mechanical sorting process. Although previously a significant proportion of MSW waste buried consisted of bioreactive wastes (e.g. food wastes), this waste is now biologically treated by composting or anaerobic digestion after the mechanical sorting step. This results in waste with a lower organic matter content and less volume. This treatment is known as mechanical biological treatment (MBT).

1.1.2 Landfill Leachate Composition Phases

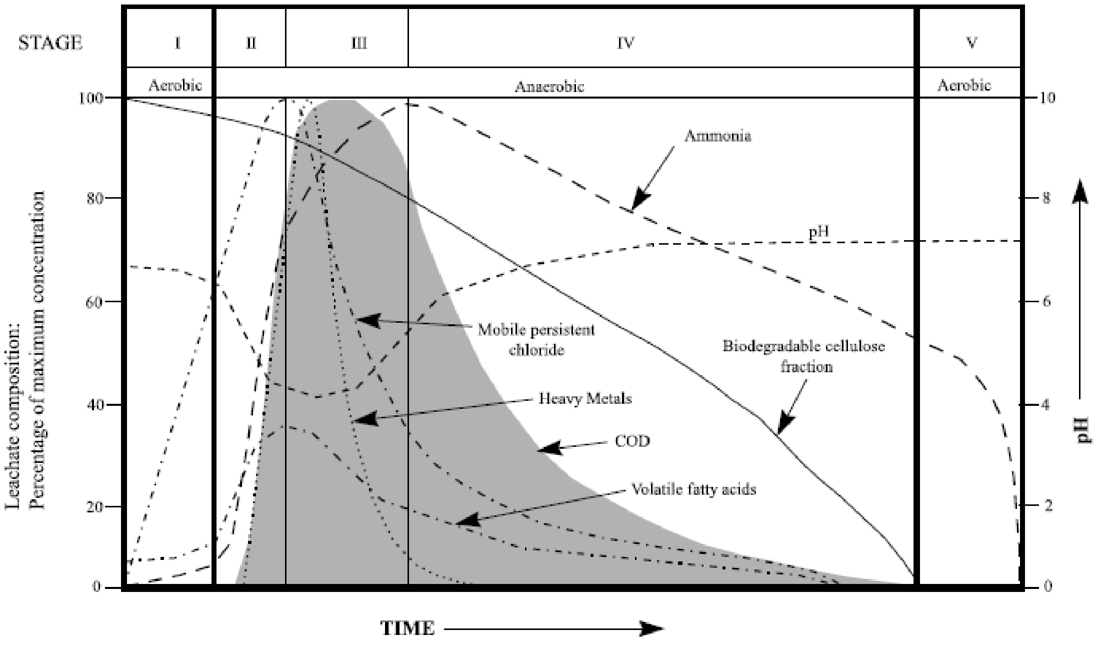
Several studies have described the life of a landfill site in terms of a five stage process related to biodegradation of the waste (Frederick and Stephen, 1985, Pohland and Harper, 1985, Department of the Environment, 1995) : I) aerobic, II) acidogenic, III) acetogenic, IV) methanogenic, and V) aerobic phase. These breakdown processes play a role in defining the composition of leachates throughout the life of the landfill (Figure 1.1).

The aerobic initial stage of waste biodegradation lasts only a few weeks; and is dependent on the availability of oxygen in the waste. Once a landfill is capped (to reduce water entering the site) oxygen is less able to enter the landfill and oxygen levels are depleted by aerobic micro-organisms. This also results in an increase in CO_2 levels and often an increase in temperature (Kjeldsen et al., 2002, Lu et al., 1985).

As oxygen is depleted, the landfill becomes anaerobic and the acidogenic phase begins. In this stage, cellulose and hemicellulose (which comprise 45-60% of the dry weight of waste) are fermented, resulting in the production of acetic acid (Lu et al., 1985, Pohland and Harper, 1985, Kjeldsen et al., 2002) and a lower pH (Figure 1.1).

The acetogenic phase follows, in which acetogenic bacteria convert the long chain fatty acids and alcohols to acetic acid, carbon dioxide and hydrogen. The pH remains lower (< 6) in this stage (Environment Agency, 2003) which favours dissolution/remobilization of inorganic species, such as heavy metals, resulting in a potentially chemically toxic leachate (Christensen et al., 1994).

In the methanogenic phase, the pH rises towards values of at least 8 and methanogenic bacteria convert the products of acetogenesis into more CO_2 and methane (CH_4) (Environment Agency, 2003), leading to lower concentrations of biodegradeable organic compounds. The methanogenic phase may last for decades and it is during this phase that pollution from gases is most damaging to the environment. Reducing the volume of biodegradeable waste to landfill results in lower emissions of CH_4 and this is another reason for the treatment of waste prior to landfilling in recent years.



Source: DOE (UK) (1995)

Figure 1.1 Life stages of landfill and changes in leachate composition

The final phase of the evolution of a landfill is the aerobic stage, in which aerobic conditions are re-established (Environment Agency, 2003). This stage is reached when the organic material in the landfill is largely biodegraded and oxygen can then diffuse through the cap and/or overlying waste at a greater rate than it is utilised (Christensen et al., 1994).

1.1.3 Landfill Leachate Characterisation

MSW leachates may be characterised as “a water-based solution of four main groups of pollutants (dissolved organic matter (DOM), inorganic macro components, heavy metals and xenobiotic organic compounds e.g. pesticides)” (Christensen et al., 1994). Leachates can also contain a variety of other species, such as As, Ba, Li and Hg, at lower concentrations (Christensen et al., 2001). Due to the pretreatment to remove bioreactive wastes and metals, MBT leachates have lower DOM and heavy metal concentrations than MSW wastes (Robinson et al., 2005) however they still contain the same pollutant groups as MSW. Many of the species contained within leachates are toxic, hence pollution of groundwater by landfill leachates is a major potential environmental problem related to landfills and is thus governed by EU legislation Water Framework Directive (2000/60/EC) and the Groundwater Daughter Directive (2006/118/EC). UK MSW landfills are therefore heavily regulated and engineered (as required by the Landfill Directive) in order to reduce the risk of pollution from escaping the landfill. Prior to the Directive, restrictions on what materials could be landfilled were minimal and leachates from older sites that are less well-engineered can contain a wider range of materials that may be more prone to escape into the environment.

1.2 Environmental Monitoring of Landfill Leachates

Environmental monitoring of leachates is carried out regularly at all UK landfills to ensure that regulations are not breached. If leachates are not collected, treated and discharged safely they are a potential pollution source to soil, surface water and groundwater (Fatta et al., 1999). Risks are also posed when liners degrade and fail, or if the landfill was not initially engineered to restrict discharge. Landfills are monitored post closure until the site no longer poses a risk to the environment, and this period is dependent on the site characteristics (Environment Agency, 2010b). Every landfill is unique in terms of both its environmental setting and the nature of its development, hence monitoring programs are tailored to individual sites (Environment Agency, 2014b).

There are parameters, however, that are routinely monitored in landfill leachates (Appendix 1.2 and 1.3); including pH, electrical conductivity as well as several elemental concentrations.

Elevated concentrations of metals/metalloids in the environment can pose risk to human health as well as to the wider ecological environment. Although some pollutants can stem from natural sources, such as the case of As in groundwater in India and Bangladesh (Bhattacharya et al., 2014), often they are the result of anthropogenic activities, such as mining, industry and of course landfill. Commonly occurring heavy metals in landfill leachate include zinc, copper, cadmium, lead, nickel, chromium and mercury (Reinhart, 1993) and in a study by Oman and Junnestadt (2008) 49 metals and other elements were found in 12 Swedish MSW Leachate samples, varying from the ppm level to sub ppb level. None of these concentrations, however, exceeded the drinking water limits. The study also found more than 140 organic and metal-organic and inorganic compounds in the leachate. Table 1.1 sets out the elements which are routinely monitored in landfill leachates and the environment and those which are of interest in this study.

Table 1.1 Elements of environmental interest in landfill leachates

Element	Reason for interest	Elements	Reason for interest
Li	Used as tracer in transport studies[1]	**Zn**	US EPA RECOMMEND[5]
Mg	Landfill Directive[2]	**As**	Drinking Water Directive[3]
Al	Drinking Water Directive[3]	**Br**	Drinking Water Directive[3]
Si	Landfill Directive[2]	**Sr**	Landfill Directive[2]
K	Landfill Directive[2]	**Mo**	Landfill Directive[2]
Ca	Landfill Directive[2]	**Ag**	Used for NP[4]
Ti	Used for NP[4]	**Cd**	Drinking Water Directive[3]
V	US EPA RECOMMEND[5]	**Sn**	WHO Drinking Water Guidelines[6]
Cr	Drinking Water Directive[3]	**Cs**	Environmental Permitting Regulation [7]
Mn	Drinking Water Directive[3]	**Ba**	US EPA RECOMMEND[5]
Fe	Drinking Water Directive[3]	**Au**	Used for NP[4]
Co	US EPA RECOMMEND[6]	**Hg**	Drinking Water Directive[3]
Ni	Drinking Water Directive[3]	**Pb**	Drinking Water Directive[3]
Cu	Drinking Water Directive[3]	**U**	Environmental Permitting Regulation [7]

1. Öman and Rosqvist (1999)
2. Landfill Directive (Environment Agency, 2014b)
3. Drinking Water Directive (Drinking Water Inspectorate, 2010)
4. Manmade NP Report (DEFRA, 2007)
5. US EPA recommend (Environmental Protection Agency, 2012)
6. WHO guidelines (World Health Organisation, 2004)
7. Radioactive substance regulation (DEFRA, 2011)

1.3 Landfill Pollution Models

Landfill systems can be modelled to assess the risk of pollution if leachates escape into the surrounding environment. Risk assessments are imposed by regulators as a tool to meet legal requirements (Butt et al., 2014). Currently, the UK Environment Agency use a model called LandSim to provide quantitative risk assessments of the performance of specific landfill sites in relation to groundwater protection (Drury et al., 2003). The model incorporates the performance of landfill liners, background contaminant concentrations, biodegradation and other information to determine rates of contaminant migration and to predict concentrations of contamination at receptors of varying trophic level. Importantly, however, this and similar models are largely based on solute transport in which species are defined as either dissolved in the liquid phase or contained within solid phases (either sorbed or structurally bound).

Dissolved phases conventionally refer to the < 0.45 μm fraction of a leachate, such that any particles with smaller diameters are modelled as part of the dissolved phase. Thus these models may not provide an accurate assessment of the behaviour of contaminants in the environment if a significant portion of the elements of interest are present within colloids.

1.4 Landfill Leachate Colloids and Nanoparticles

The dissolved phase, (defined here as the <0.45 μm fraction), includes both colloids and nanoparticles (NP) (Figure 1.2). NPs are defined as particles that can be as small as 1 nm and as large as several tens of nanometres, in at least one dimension (Hochella et al., 2008), and they constitute a sub-fraction of the component of leachates defined as colloids by the International Union of Pure and Applied Chemistry (IUPAC).

The term nanoparticle is generally used in the literature to refer to manmade (engineered) NP, while natural particles of NP size are termed colloids. In this thesis, the term nanocolloids will be used to refer to natural colloids in the NP size range (< 100 nm). Although nanocolloids in landfill leachates are a result of anthropogenic processes, these are still considered as natural because they have not been engineered to be a particular size.

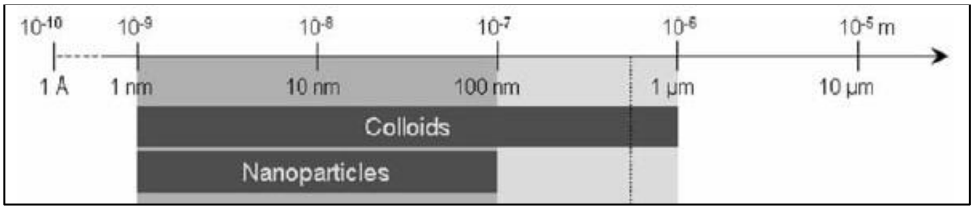


Figure 1.2 Size of colloids and nanoparticles. Figure adapted from Christian et al., (2008)

Nanocolloids are ubiquitous in the natural environment and are of particular interest because of their large surface area to volume ratio compared with the bulk minerals, which can result in increased reactivity (Plathe, 2010). The high surface area to volume ratio which increases as particles get smaller results in higher apparent adsorption coefficients of dissolved metals to colloids, faster diffusion coefficients of colloids relative to larger particles, and thus greater travel distances of metals associated with colloids compared to those present in larger particles or truly dissolved (Baalousha et al., 2011a, Doucet et al., 2006).

The behaviour of colloids is dominated by surface properties, e.g. the presence of functional groups such as carboxylic acids, rather than bulk properties such as the overall chemical composition (Lead and Wilkinson, 2006). Due to their small size, they may stay suspended in solution for extended periods of time because their average Brownian displacement exceeds the settling velocity (Plathe et al., 2013). This means their ultimate removal from solution is governed by aggregation rather than sedimentation.

1.4.1 Nanocolloid Composition

Nanocolloids may be composed of a variety of phases, including natural organic matter (NOM), inorganic mineral compounds such as clays/silicates, poorly characterised metal oxides and a variety of anthropogenic phases (organic, inorganic and mixed) (Filella et al., 2007, Lead and Wilkinson, 2006).

Figure 1.3 indicates typical colloid compositions in environmental systems and shows that there are typical size ranges for particular types of nanocolloid.

Thus , organic colloids e.g. fulvics and humics are expected to dominate the <10 nm fraction and , inorganic colloids e.g. clays and metal oxides will dominate the larger > 10 nm fraction, but both types can be present in each size fraction (Hassellöv et al., 2006, Filella et al., 2007).

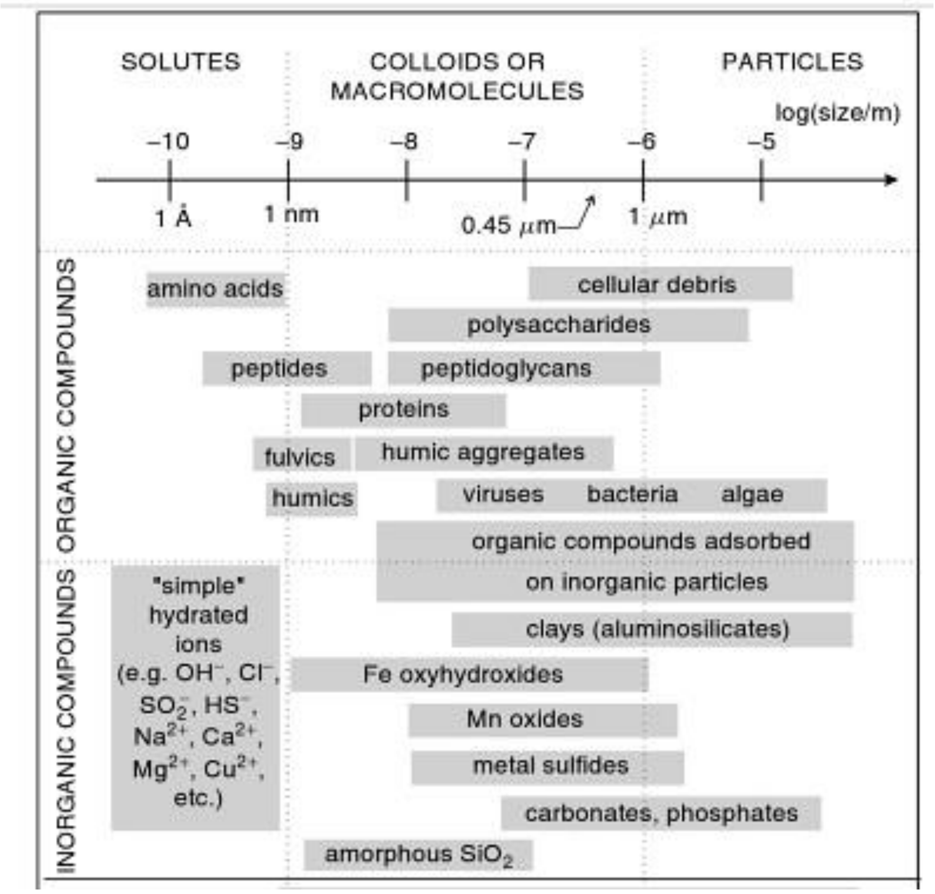


Figure 1.3 Simplified size distribution of various natural organic and inorganic nanocolloids and particles. Adapted from Lead and Wilkinson (2006)

It is difficult, however, to fully categorise nanocolloids because they are rarely found in purified forms in environmental systems and are often part of complex heteroaggregates (Lead and Wilkinson, 2006). For example, although inorganic nanocolloids are typically larger in size, organic particles can also be present in larger size fractions, adsorbed on larger inorganic colloids (Figure 1.3).

Hennebert et al.,(2013) investigated the presence of colloids and NP in leachates from 25 landfills accepting various types of waste and determined that solid waste leachates contain significant amounts of colloids, both organic and inorganic. This study however was not able to identify size distributions or proportions of the types of colloids due to the ultrafiltration method used (see Chapter 2). This will be explored further in Chapter 5.

1.4.1.1 Natural Organic Matter (NOM)

NOM has a heterogeneous nature and comprises a complex mixture of organic macromolecules and refractory organic substances (Neubauer et al., 2011). Dissolved organic matter (DOM) consists of humic and non-humic substances with humic substances forming the majority in environmental samples (Stevenson, 1994). Non-humic substances comprise organic compounds such as amino acids and carbohydrates (Laborda et al., 2008).

Kjeldsen et al.,(2002) reported that dissolved refractory organic matter is generally found in higher concentrations in mature landfill leachates (comprising 30-60% of the total dissolved organic matter (DOM)), whereas DOM in younger leachates is dominated by volatile fatty acids (> 95%). This results from the different leachate life phases (Section 1.1.2), whereby sequential organic substrates (dependent on their biodegradability) are consumed by bacterial processes in the early evolution of the leachates.

Refractory organic matter consists of humic substances (HS), which are made up of fulvic and humic acids and can be derived from any organic source material, including plant and animal debris, food wastes, pesticides (Kang et al., 2002). HS are known to have an important role in the biochemistry of natural waters and soils and in pollutant chemistry (Aiken 1985) the ability to sorb and complex contaminants (Murphy and Zachara, 1995). They are known to be heterogeneous in elemental composition, chemical functionality and molecular size distribution (Kang et al., 2002).

Fulvic and humic compounds are differentiated from one another by their solubility as a function of pH; with fulvic substances being soluble at all pH values, whereas humic compounds are not soluble at pH < 2. Fulvic acids also have a lower molecular weight range than humic acids (Oades, 1989) as shown in Figure 1.3 and therefore may exhibit different transport behaviours due to their size. Weng et al.,(2002) investigated transport behaviours of fulvic and humics in a sandy soil and found that fulvics were more soluble and mobile than humics thus it is important to distinguish between these compounds when investigating fate and transport. When comparing humic and fulvics, the latter have also been shown to be the main fraction of humic substances for reacting with metals (Donisa et al., 2003).

1.4.1.2 Inorganic nanocolloids

Inorganic nanocolloids are commonly occurring mobile phases in the aquatic environment (Filella et al., 2007) and comprise mineral compounds and poorly characterised metal oxides. Baalousha et al.,(2009) stated that in aquatic and terrestrial systems the main types of inorganic nanocolloids were aluminium phyllosilicates, oxides, and hydrous oxides of iron, manganese and silicon, but Hennebert et al.,(2013) suggested that inorganic nanocolloids in waste leachates were different than those in natural environments because in their study, the Al and Si containing particles were not identified as aluminosilicates but were instead found to be associated with Fe, Ca and P. This reiterates the importance of obtaining landfill specific data rather than using other environments to infer processes with a landfill environment.

It is also important to note that the redox conditions of the environment will affect the inorganic nanocolloids present. The reducing environment within a landfill system in the anaerobic stages (Figure 1.1, Stages II-IV) is likely to influence the presence of metal oxides/hydroxides particularly Fe and Mn (Christensen et al., 2001).

Minerals such as aluminosilicates are generally present as clays, and can be distinguished from other colloids using microscopy techniques because of their irregular particle shape and broad size distribution (Plathe et al., 2013). These minerals are of particular interest in landfill studies because landfill liners are commonly made of clay material and it is important to understand the interaction between clays and leachates (Huang, 2014).

Inorganic colloids in the natural environment are often dominated by iron oxides (Worms et al., 2010), but metal oxides such as iron and manganese oxides do not exist as pure oxides in natural waters, and generally contain significant amounts of other elements which makes them harder to characterise (Filella et al., 2007).

1.5 Organic and Inorganic complexes

Organic and inorganic complexes are generally thought to be the major metal-bearing components in landfill leachates (Baun and Christensen, 2004), with a high portion of the metals being complexed by humic substances in the leachate (Gounaris et al., 1993). Despite the focus on organic phases in the literature, inorganic colloids have also been shown to be quantitatively important in trace metal binding with metals bound to larger inorganic particles to a higher extent than humic substances in a groundwater sample (Geckeis et al., 2003). This study also suggested that these inorganic colloids were coated with humic substances and thus organic complexes may also play a role in inorganic colloid binding.

The distribution of metal species between dissolved and colloidal forms may therefore affect the mobility of potentially toxic metals in the environment, because the chemical form of metals (e.g. dissolved, sorbed to particles, organic or inorganic complexes, and/or precipitates) determines their mobility, bioavailability and distribution in the environment (Plathe et al., 2013).

DOM plays an important role in the binding and speciation of metals in the environment, and hence their mobility, toxicity and bioavailability (Kördel. W., 1997). Studies characterising DOM binding behaviour with metals are mostly based on laboratory methods; isolating humic and fulvic substances under well-defined conditions. Tipping et al.,(2011) collated the results of these studies to create a model to predict binding of 40 metals. This theoretical model is, however, based on a simpler environment than that of a landfill. For example, laboratory studies are unlikely to reproduce the full spectrum of organic species present in landfill leachates. Investigating binding in real field samples is not simple, however, and requires utilization of methods for isolating fractions and methods with suitable detection methods, which have previously not been possible.

Humic substances form complexes with metals because they contain a high abundance of oxygen-containing functional groups. Humic substances also form stable complexes with polyvalent cations, with trivalent cations being bound more strongly than divalent cations (Nelson Eby, 2004). In addition to being dependent on the metal in question, the strength of these complexes is controlled by the ionic strength and pH of the solution and the nature of the functional groups of the humic substances, and they play an important role in the mobilisation and transport of metals (Nelson Eby, 2004).

Inorganic colloid-metal complexes were examined in Lofts and Tipping (1998) and they determined that iron, manganese and aluminium oxides and silicates were implicated in metal binding partly due to their large specific surface areas and their binding constants however, organic complexes were dominant. Schmitt et al.,(2002) examined the interaction between metals (Al, Fe, Zn and Pb) and both inorganic (clay) and organic phases (humic substances) and showed that the presence of NOM decreased the adsorption of metals on to clay particles suggesting that organic binding was preferred,

1.6 Distribution of Metal(loids) in Landfill Leachate Nanocolloidal Size Fraction

Thus far, studies of metal associations with different size fractions (including < 0.1μm) in landfill leachates have relied on conventional filtration methods, such as ultrafiltration (Gounaris et al., 1993, Jensen and Christensen, 1999, Jensen et al., 1999, Li et al., 2009b, Matura et al., 2010, Li et al., 2009a, Wu et al., 2012) and centrifugation (Calace et al., 2001).

These studies have all found that different metals are variably bound to nanoparticles (organic and inorganic) within the leachate, present in the truly dissolved fraction and/or present as both organic and inorganic complexes. No clear patterns have emerged, however, of the distribution of metals in different size fractions between these studies or even within the same study (Li et al., 2009b, Jensen and Christensen, 1999) and thus this thesis seeks to investigate these distributions and elucidate patterns. The ambiguity in these studies may partly be due to differences between individual leachate characteristics that result in different metal associations, but it is also a reflection of the different analytical techniques used in the various studies. A more detailed literature review concerning the distribution of elements between these various forms will be presented in Chapter 5.

1.7 Thesis Overview

This thesis aims to investigate the behaviour of metals and metalloids in the nanocolloidal fraction of leachates in order to obtain quantitative data of the partitioning of these species between the various components of the “dissolved fraction” that may be used to enhance landfill risk assessment models by including the influence of colloid facilitated transport.

To undertake this, the research will need to select and optimise nanocolloidal analytical techniques for their use with landfill leachates and apply them to various landfill leachate samples. Thus, the research will address a gap in scientific knowledge which is the optimisation of AF4-HR-ICP-MS to landfill leachate samples to obtain reliable quantitative data of the interactions between metals and nanocolloids.

Chapter 1: Background information about landfill leachates and an introduction to the relevance of the project.

Chapter 2: A review of techniques available to investigate nanocolloids in environmental samples and a justification of the techniques chosen for this project.

Chapter 3: Materials e.g. chemicals and instruments used throughout the thesis are detailed along with method optimisation for the techniques selected in chapter 2.

Chapter 4: A detailed approach to AF4-HR-ICP-MS coupling is presented, specifically that involved in quantifying and assessing the limits of the method. The method is validated using a landfill leachate.

Chapter 5: Application of the method development to three different leachate samples in order to examine the distribution of nanocolloids and associated metallic elements in various leachate compositions and to a leachate sample with altered pH and ionic strength to investigate influences of UK MSW Landfill Leachate intrusion into groundwater on the metal/metalloid distribution in the nanocolloidal fraction.

Chapter 6: Conclusions and future perspectives

Chapter 2: Review of Techniques for the Analysis of Nanocolloids and Associated Elements in Landfill Leachates

2.1 Introduction

In recent years, advances in the technology for NP characterisation and detection have enabled a better understanding of natural and manmade NP. Experimental advances such as the invention of the scanning tunnelling microscope in 1981, followed by the atomic force microscope in 1986 have driven the growth of nanotechnology and more recent advances such as nano-tracking analysis in 2003 are enabling more robust analysis methods. It is important, however, to ensure that the use of these techniques in studying NP is well-documented, so that there is a level of consistency and comparability between different studies (Baalousha and Lead, 2012). In addition, it is also important that the most suitable investigative technique(s) are chosen from the wide range of available methods for the sample type and study in question.

In order to identify the most appropriate analytical techniques for the study of nanocolloids in landfill leachates, the characteristics that need to be analysed must first be defined. This study aims to provide data to investigate the role that nanocolloids play in the transport of toxic metals, metalloids and radionuclides in the subsurface environment. The characteristics of which can then be used to predict and/or enhance appropriate contaminant behavioural transport/risk associated models.

The characteristics that are most important for investigating the role of nanocolloids on the fate and behaviour of pollutants are physico-chemical properties, including size, shape, surface charge (Zeta potential), surface coating and composition (e.g., organic or inorganic (Bradford and Bettahar, 2006, McCarthy and McKay, 2004, Flury and Qiu, 2008)). Therefore the techniques chosen for this study must be able to analyse these characteristics, as well as be able to detect and quantify the concentrations of associated pollutants (metals, metalloids and radionuclides).

Size is the defining characteristic of NP and the size distribution of organic matter (OM) in landfill leachates is the primary influence on it’s ultimate biogeochemical cycling fate (Powrie et al., 2010). Knowledge of OM partitioning between different sizes or molecular weight classes will therefore provide insight into understanding its behaviour, as well as that of the elements associated with the various size fractions of OM.

Surface charge is an important control over the stability of NP transport in water and reacts strongly to changes in environmental conditions, e.g. pH, which can affect aggregation, dissolution and sedimentation of particles in solution (Baalousha et al., 2011a). Surface charge is commonly represented by measurements of zeta potential, which is a parameter of the electrochemical potential between particle and dispersion media at the point of the interfacial double layer (Fang et al., 2013). Measurements of zeta potential are complicated in environmental matrices because they are usually based on the assumption of either hard (impermeable) or soft (permeable) spheres in the sample and for samples with non-spherical particles, quoted values of the zeta potential may be misleading. This is particularly evident for landfill leachates which likely contain a range of hard, soft, spherical and non-spherical spheres, hence zeta potential measurements are not considered further in this study.

Shape can be used to classify natural NP and is important because it influences aggregation and charge interactions, but it is rarely characterised in experiments (Baalousha et al., 2011a). Shape has, however, been used to infer particle composition and Buffle et al.,(1998) suggested that humic substances formed irregular shapes while inorganic colloids had clear, angular edges. The shape of NP can, however, be altered by surface coatings and aggregation, making this parameter difficult to define and quantify and therefore to incorporate into pollution prediction models.

Surface coating and particle composition can be measured using similar analytical techniques. These characteristics are important for understanding the type of nanocolloids present in samples and explaining how they interact with pollutants e.g. humic-metal complexes (Worms et al., 2010).

2.2 Techniques

Environmental samples present special challenges for analysis because environmentally relevant particle concentrations often require low detection limits which cannot be achieved with all techniques (Hassellov et al., 2008).

Lopez-Serrano et al.,(2014) divided analytical techniques for NP analysis into three types: characterisation (to analyse the properties mentioned above), fractionation (to extract, separate or fractionate NPs from the sample) and quantification (generally coupled with fractionation to validate and quantify NP standards and provide further NP information).

Table 2.1 summarises the techniques available for particle analysis. While many techniques may be applied to analyse particles in the colloidal size range, it is important for this study that they are able to analyse particles and associated elemental concentrations in the nanocolloidal range (< 100 nm). The focus of this study is on nanocolloidal fractions because previous studies (Bolea et al., 2006, Bolea et al., 2010, Stolpe et al., 2005, Stolpe et al., 2014) suggest that a significant proportion of toxic metals and OM that are traditionally classified as “dissolved” are likely to be present in this fraction.

Not all the techniques described in previous studies are suitable for use in this study, but those that are will be discussed in more detail. It is important to note, however, that while it would be beneficial to combine many techniques to ensure that all possible characteristics are analysed, limited financial and time resources restrict the number that can be actually used.

Table 2.1 Techniques for nanocolloid analysis in landfill leachates. Adapted from Hassellov et al.,(2008) and Lopez-Serrano et al.,(2014)

	Technique	NP Info Provided	Size range(nm) / Detection Limits
Characterisation	Atomic Force Microscopy (AFM)	Size, Shape Surface texture, ,	0.5 - <1000
	Scanning Electron Microscopy (SEM)	Size, Shape, Surface, crystallographic composition	10 - <1000
	Transmission Electron Microscopy (TEM)	Size, Shape, elemental composition(if coupled)	1 - <1000
	X-Ray Fluorescence (XRF)	Surface, crystallographic and elemental composition	0.5- < 1000
	Dynamic Light Scattering (DLS)	Size	3 - <1000
	Ultra Violet Visible Spectroscopy (UV-VIS)	Size, structure, surface chemistry	NA
	Nanoparticle Tracking Analysis (NTA)	Size	30- <1000
	Multi Angle Light Scattering (MALS)	Size, Shape	10-1000
Fractionation	Cross Flow Filtration/Ultrafiltration (CFF)	-	1-30
	Size Exclusion Chromatography (SEC)	-	0.5-10
	Field Flow Fractionation (FFF)	-	1-1000
	Ultracentrifugation	-	10 - < 1000
Quantification	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Elemental Concentration	ppb
	Inductively coupled plasma mass spectroscopy (ICP-MS)	Elemental Concentration	ppb
	Laser Induced Breakdown Detection(LIBD)	Elemental Concentration	-
	Ultra Violet diode array detector (UV-DAD)	Structure, surface chemistry	-
	Fluorescence (FLU)	Organic Substance Type	-

2.2.1 Characterisation Techniques

The size range of particles that can be quantified using the techniques described in Table 2.1 is the main criteria for their selection in this study. SEM, NTA and MALS are only able to detect particles >10 nm, and so are not the preferred options for this study. XRF and UV techniques are able to detect smaller particle sizes, but they are not able to provide size or shape information which is vital for this study. AFM, TEM and DLS are techniques that have potential for characterisation of nanocolloids in landfill leachates and will be explored further.

2.2.1.1 Characterisation: Atomic Force Microscopy (AFM)

AFM is a type of scanning probe microscope that can be used to image the size and shape of NP in samples and to gain information on particle size diameter (PSD). The sample is immobilised on an atomically flat surface, often made of gold or mica (Dufrêne, 2009) and the topography of the sample surface is then determined by measuring the force between the sample and a cantilever-tip system. Knowledge of the force between the tip and the sample is required for sensible imaging (Baalousha et al., 2011b). Interactions between the cantilever tip and the sample surface are detected by a laser and transferred to a photodetector for imaging which can measure either particle height or the diameter of spherical particles (Baalousha et al., 2011a).

AFM allows NP to be measured under environmentally-relevant, aqueous conditions and therefore the sample does not need to be extensively processed prior to analysis, which might otherwise lead to measurement artefacts. It may be necessary, however, for the sample to be filtered and diluted prior to analysis because large particles and high concentrations of particles can mask smaller NP during imaging.

AFM has been used to image environmental NPs in several studies (Baalousha and Lead, 2007, Lead et al., 2005), but it has not yet been applied to landfill leachates. Lapworth et al.,(2013) did, however, employ AFM to investigate nanocolloids in groundwaters polluted by a landfill leachate plume. In this case an anaerobic chamber was used to keep the samples in the relevant environmental conditions during the analyses; hence this application may be useful for landfill leachates.

2.2.1.2 Characterisation: Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is a microscopy technique that produces high resolution two dimensional images by transmitting an electron beam through an ultrathin sample on a conducting grid (often composed of Cu). Samples must be ultrathin because it is important that electrons are not absorbed by the material. The electrons that are transported through the sample are then focused onto an imaging detector (Hassellov et al., 2008).

TEM is commonly used to measure NP >10 nm (Ebenstein et al., 2002) and it can be used to provide an average NP size as well as PSD. This technique can, however, cause sample degradation through the interaction with the electron beam and during sample preparation. Sample preparation for TEM can be time-consuming because of the thin samples required and a rigorous sampling and handling procedure needs to be followed to obtain accurate results (Baalousha et al., 2011b). In addition, OM samples may need to be stained in order to be imaged (Hassellov, 2008). The use of copper grids also hampers the detection of copper in the sample if TEM is coupled with elemental detection such as Energy Dispersive Spectrometry (EDS)

TEM has previously been used to investigate nanocolloids in landfill leachates in Matura et al.,(2012) and more recently in Hennebert et al.,(2013) (which employed the TEM-EDS technique). In both of these studies, TEM was used alongside filtration methods but these studies only identified inorganic colloids in the sample therefore the ability of this technique to identify organic colloids in landfill leachates is unknown.

2.2.1.3 Characterisation: Dynamic Light Scattering (DLS)

Dynamic light scattering is another measure of NP size or PSD and is often used in conjunction with other techniques to confirm size measurements. It is a non-invasive technique that allows the measurement of size and PSD without any sample alteration. By focusing a laser on a sample in a cuvette, the light scattered by NP due to Brownian motion can be detected and measured, and the different intensities of the scattered light can be used to calculate particle size using the Stokes-Einstein relationship (Malvern, 2014). It is important to note, however, that the size calculated is the hydrodynamic diameter and thus presumes that the particle is spherical.

Although DLS is a quick and easy technique for measurement of PSD due to the minimal sample preparation required (filtration or centrifugation only), there are drawbacks. Samples which have high polydispersity can give misleading DLS results because larger particles scatter more light and associated smaller NP in the same sample are not then detected (Baalousha and Lead, 2012). Multi-modal populations are particularly problematic because intensity-normalised results are disproportionately skewed to larger particles/aggregates in suspension even if smaller particles are predominant (Bednar et al., 2013). DLS is therefore more suited to monodispersive samples or used as a method in conjunction with other techniques.

Although DLS has been used extensively for characterising manmade NPs, it is yet to be used to characterise landfill leachates.

2.2.2 Fractionation Techniques

Fractionation is the most important technique for this study because it allows for information on size fractions to be obtained rather than information solely related to the bulk sample. For example, obtaining detailed information on the associations of elements across the nanocolloid size range is vital for developing risk assessment models. Ultracentrifugation is not able to fractionate particles with sizes below 10 nm and so it is not a preferred technique for this study. All other fractionation methods in Table 2.1 are discussed in sections below.

2.2.2.1 Fractionation: Cross Flow Filtration/Ultrafiltration

Cross flow filtration is the most common technique for investigating colloids in landfill leachates. The technique is able to process large volumes of water with minimal alteration of colloidal particles. Filtration/ultrafiltration membranes are used to separate particles from solution by recirculating or stirring a solution over a membrane with particles smaller than the cut off diameter passing through the membrane due to a pressure drop. Although this technique only allows for two fractions to be obtained, sequential filtrations can produce a range of different size fractions.

Campagna et al.,(2013) used ultrafiltration membranes from 100 kDa - 500 Da to investigate the MW distribution of landfill leachates throughout the treatment process and found that almost half of OM was present below 500 Da. Several studies have also used ultrafiltration coupled with an element detection method to identify metal associations with different size fractions in landfill leachates (Matura et al., 2010, Li et al., 2009b, Gounaris et al., 1993, Jensen and Christensen, 1999, Jensen et al., 1999, Wu et al., 2012).These studies will be discussed further in Chapter 5. An inter- calibration study of CFF by Buesseler et al.,(1996) found, however, that there were variations in MW cut off and that sample alteration can occur due to aggregation and adsorption to the membrane. The method is also time-consuming, large volumes of sample are required and size fractions are limited to available membrane sizes.

2.2.2.2 Fractionation: Size Exclusion Chromatography (SEC)

SEC fractionation is carried out by passing a solution through a column packed with porous material with a distribution of pore sizes in the range of the particles to be fractionated (Barth and Boyes, 1992). Separation of particles occurs by the ability of particles to enter the pores in the packing material and is thus carried out according to their hydrodynamic volume (size and shape). Smaller particles enter pores more freely than larger particles, thus extending their elution time from the column. As a result of the pore sizes used, a SEC column has an operating molar mass range and particles outside the range elute first from the column. Fractionated particles then elute from the column depending on their hydrodynamic volume, with particles that enter the same pore size range eluting at the same time (Hassellov et al., 2008).

The method has limitations in that care must be taken to ensure that the sample does not interact with the column and packing material. There is also a limit to the number of pore sizes that can be used because enough difference between the pores sizes is needed to generate sufficient difference for elution times of the particles. The method, however, can be coupled with quantifying detectors, such as MALS, DLS and UV-VIS, which can be used to detect particle size and composition.

SEC has been previously used for fractionation of landfill leachates. Persson et al.,(2006) used the method to study transformations in DOM MW along a groundwater gradient and found that DOM MW decreased as distance from the landfill increased. Lu et al.,(2009) collected fractions from SEC and analysed them using fluorescence to investigate metal binding properties of DOM. This study found that Cd preferentially bound to fulvic-like components wheras Cu complexed with humic like components.

2.2.2.3 Fractionation: Asymmetric-flow Field Flow Fractionation (AF4)

Asymmetric flow field flow fractionation (AF4) is a one phase technique first introduced by Giddings (1966) (Figure 2.1) which allows for continuous size separation of particles in a solution. AF4 is part of a family of field flow fractionation devices, but it is the only technique suitable for samples of this size range (<100 nm). Fractionation occurs when particles are separated in a channel by an asymmetric cross flow which forces particles towards a membrane after which they are then eluted according to their diffusion coefficient.

During the injection step (2.1a), the sample is injected into the channel and held in place by opposing focussed flows. This flow can be applied for a chosen length of time, immobilising particles in a position in the channel and forcing them towards the membrane. The focusing step follows (2.1b); smaller particles in the sample have higher diffusion coefficients which means they diffuse away from the membrane towards the middle of the channel more quickly. Ordering of particles in the channel depending on diffusion coefficints takes place during focusing and once focusing is completed, the focus flow is removed so that only the flow across the channel and the perpendicular cross flow remain. The final step is the elution step (2.1c).

Due to the parabolic flow profile, the solution in the middle of the channel flows fastest. This causes the smaller particles which diffuse further towards the middle of the channel to elute first.

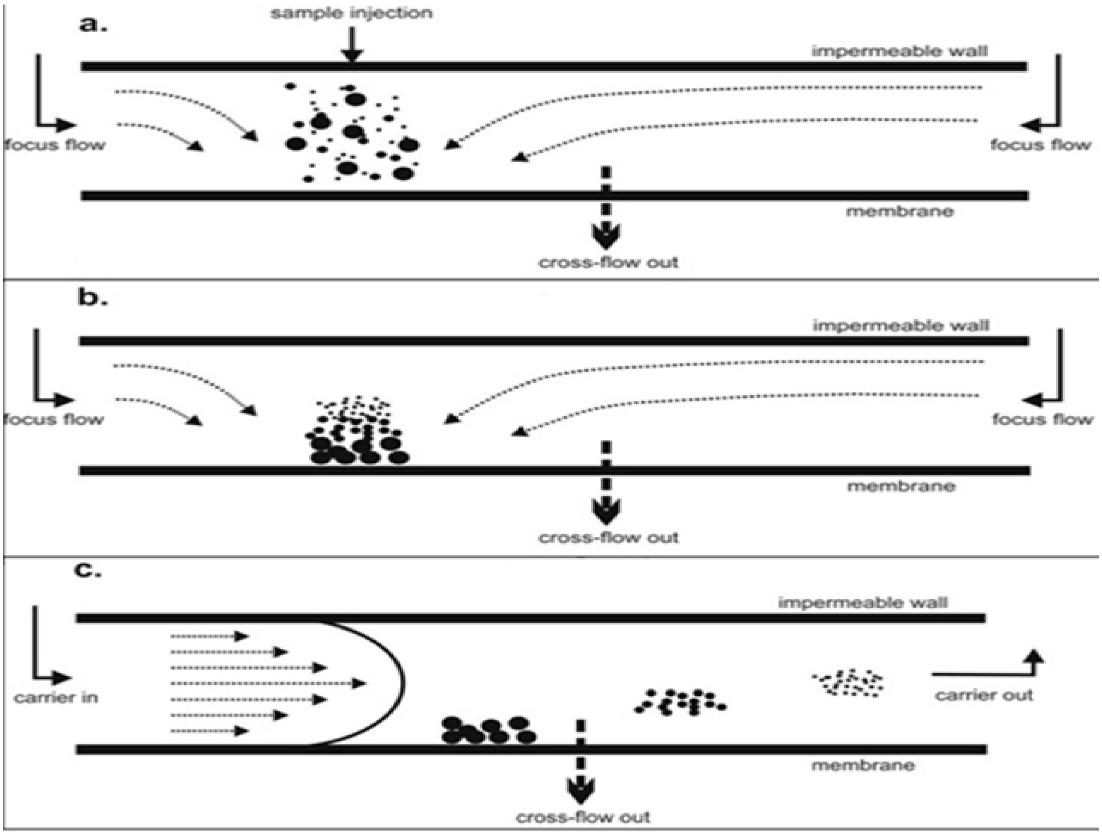


Figure 2.1 Diagram illustrating the stages of AF4 a. injection, b. separation, c. Elution. Figure from Plathe (2010)

The particles then flow out of the channel, in order of their diffusion coefficients , to the detectors and/or collectors, creating a graph known as a fractogram (retention time V signal intensity). The retention time of particles in the channel relates to the diffusion coefficient and can thus be used to calculate the hydrodynamic diameter. The relationship between retention time and relative molar mass can also be determined using standards of known molar mass (Beckett, 1987).

AF4 can be used to fractionate manmade and natural NP. Several parameters must be optimised for fractionation, but this means that the method can be adapted to suit particular samples.

The AF4 technique not only removes the time consuming nature of multiple filtration steps, but analytical devices for quantification, such as ICP-MS, can be coupled online so as to measure parameters as the sample is separated. AF4 detectors are versatile and there is the possibility for both online coupling to detectors as well as fraction collecting for offline analyses (Bednar et al., 2013).

AF4 has been used in many studies to fractionate nanocolloids (Bolea et al., 2006, Jimenez et al., 2011, Laborda et al., 2011, Worms et al., 2010) and has been used in one landfill leachate study (Dubascoux et al., 2008a) however this study examined the speciation of organo-tins rather than conducting a multi-element investigation. More details of these AF4 studies will be discussed in subsequent chapters.

2.2.3 Quantification Techniques

Quantification techniques are required to analyse elemental concentrations associated with nanocolloids and particles. When coupled with fractionation these tools can be part of a powerful method for nanocolloid analysis. ICP-AES and ICP-MS are similar techniques for measuring elemental concentrations but due to the lower detection limits in ICP-MS, this is the preferred technique for this study.

2.2.3.1 Quantification: UV-DAD

UV-DAD is a spectroscopy technique that measures absorption across the complete wavelength spectrum between 190 and 800 nm. It is a common nondestructive technique for on-line analyses and has been coupled with AF4 and SEC to detect elution of particles and identify organic compounds (Bolea et al., 2006). Due to the limited sensitivity and lack of quantitative standards, UV detectors lack specificity to identify organic compounds even when multiple wavelengths are analysed (Bednar et al., 2013). Specific wavelengths can be chosen and the signal at 254 nm is a widely accepted wavelength for detecting NOM (Coble, 1996).

UV detectors have been used for online measurements in conjunction with AF4 in many environmental studies (Gueguen and Cuss, 2011, Cuss and Guéguen, 2012, Stolpe et al., 2012) in order to analyse organic-particle associations as a function of size and all showed that this technique is suitable for detecting OM in environmental samples.

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2.2.3.2 Quantification: Fluorescence (FLU)

Fluorescence (FLU) is a spectroscopy technique which measures the light emission as a result of the return of electrons from a singlet excited state to a singlet ground state (Baalousha et al., 2011b). The sample is irradiated with light at a specific excitation wavelength and the intensity of emitted light is then measured at a designated emission wavelength. The technique is highly specific for organic substances and is able to distinguish between humic and fulvic- like substances by focusing on specific excitation and emission wavelengths (Coble, 1996) .

The technique can be coupled online with fractionation so that the size distribution of organic substances can be determined as used in Stolpe et al., (2010b). When coupled online to a fractionation method, the detector is only able to measure at the designated excitation and emission wavelengths, but it does provide a continuous record during the fractionation period. When fractions are collected and analysed off-line, FLU can be used to provide an Excitation Emission Matrix (EEM) which can be used to investigate the particle size distribution of different organic substances (e.g. protein-like or fulvic-like substances) within leachates (Wu et al., 2012a).

2.2.3.3 Quantification: Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is used for quantitative multi-element analysis of a solution and is capable of measuring most elements in the periodic table with low detection limits. As a consequence, it has become the method of choice for low-level quantification of metals in environmental matrices (Lesher et al., 2012). When coupled with AF4, it enables the continuous determination of elements within different fractions so as to examine metal associations with colloidal size distributions. ICP-MS can also be used to measure bulk concentrations of elements in samples.

By ionising a sample with the inductively coupled plasma, ions are directed into a mass spectrometer which separates the ions according to their mass to charge ratio. The number of ions detected at a selected mass is proportional to the concentration of a particular element in the sample (Wolf, 2005). Element concentrations can be quantified by measuring standards of known element concentrations and calibrating the response.

Due to isobaric and spectral interferences caused by mass overlap, some elements require higher mass resolution detectors. Although measuring elements in high resolution reduces measurement sensitivity, for elements such as As (important in terms of pollution) high resolution is necessary to allow for spectral separation of the interference of $^{40}Ar^{35}Cl$ (amu 74.9312) from ^{75}As (amu 74.9216). High Resolution (HR)-ICP-MS has the inbuilt capability to switch between different mass resolutions during a measurement and, therefore, different elements can be measured in different resolutions so that the sensitivity is not compromised. This enables a variety of elements to be quantified simultaneously in one solution. A conventional quadropole ICP-MS may be able to resolve this interference but would not achieve equivalent detection limits therefore a HR-ICP-MS is preferred for study of landfill leachates.

Since the initial study of Hassellov (1999), coupling of AF4 with ICP-MS has been increasingly used (Siripinyanond and Barnes, 1999, Worms et al., 2010, Bolea et al., 2010, Bolea et al., 2006, Jimenez et al., 2011, Laborda et al.,

2011) to investigate elemental concentrations with size fractions in a variety of environmental samples. Stolpe et al.,(2005) was the first to couple AF4 with HR-ICP-MS, which enabled a higher number of elements (45) to be analysed online; 15 more than with previous studies using a conventional quadropole ICP-MS (Lyven et al., 2003). With a high number of potential elements in landfill leachates, coupling of the AF4 with HR-ICP-MS has the potential to enable a comprehensive determination of metal associations with size fractions in this environment.

2.3 Discussion of Techniques

It is important that characterisation methods are able to determine the size and shape of particles, therefore AFM and TEM are both suitable for this study. The size detection limits for both techniques are similar, but there are differences in sample preparation techniques. AFM sample preparation is less time consuming than that for TEM, particularly when imaging organic nanocolloids, as will be necessary in this research. Although TEM has the advantage that it can be coupled with techniques to allow for individual particle element detection, this information can be provided by other tecnhiques. The ability for AFM to measure under environmentally relevant conditions is ultimately what makes AFM more suitable for the study of landfill leachate nanocolloids.

DLS also measures size, and the minimal sample preparation required and fast analysis time make it ideal to use as a preliminary characterisation method in conjunction with AFM. Although the method is known to be limited when used with polydispersed samples, analysis of a sample with DLS prior to AFM and fractionation enables these techniques to be optimised for a more detailed analysis of polydispersed samples.

All of the fractionation techniques presented here can, potentially alter samples during fractionation, and because it is easier from a financial and logistical point of view to apply the same storage and preparation methods to all the analytical techniques, careful consideration must be given to choosing an appropriate technique. AF4 provides continuous fractionation of particle sizes in one phase, and is therefore is less time consuming, resource intensive and provides higher resolution results then CFF or SEC making it the most suitable technique for producing the large amounts of data required for this study. The ability of AF4 to either fraction collect or to analyse online is also another positive attribute of this technique.

UV-DAD and Fluorescence provide complimentary information on organic nanocolloids. Both techniques can easily be coupled with AF4, but they are not able to detect inorganic nanocolloids or provide elemental concentrations, hence HR-ICP-MS analyses are also required and can be coupled with the AF4-UV-FLU system.

2.4 Selected techniques for Landfill Leachate Analysis

AFM is the most suitable technique for imaging nanocolloids in landfill leachates due to the simple preparation steps and its ability to carry out the measurements under environmentally relevant conditions.

DLS can provide quick and easy analysis of the size and distribution of nanocolloids. Although this technique may provide ambiguous results for polydisperse samples, it is valuable as a preliminary step to confirm the nanocolloid size range before using complementary fractionation and quantification techniques.

AF4 is the best fractionation technique available for landfill leachates because it provides continuous separation in one phase. In order to obtain information about organic and inorganic particles and elemental concentrations, AF4 will be coupled with UV-DAD, Fluorescence and HR-ICP-MS.

Chapter 3: Materials and Method Optimisation for the Characterisation of Nanocolloids in Landfill Leachates

3.1 Introduction

The aim of this chapter is to determine the optimal parameters for identifying, separating and characterising NP in landfill leachate using a multi-technique approach. The materials used throughout this thesis will also be reported here. To the author's knowledge, this study is the first to use AF4, DLS and AFM together to characterise the nanocolloid fraction of landfill leachates and associated elemental composition.

By optimising a method to analyse landfill leachate nanocolloids and associated element concentrations in an untreated landfill leachate from a UK municipal solid waste (MSW) site, this chapter aims to provide a standard method which can be applied to landfill leachates of different types. This will enable data to be obtained which can contribute to current landfill risk assessment models, thus enhancing their accuracy.

The characteristics to be determined and the techniques to be optimised will include: Particle size distribution (AF4), particle composition (UV, fluorescence) and particle size and shape (DLS and AFM). These physicochemical characteristics are important in predicting the mobility of contaminants in the subsurface environment.

3.2 Materials

The following section will detail the materials used throughout this thesis.

3.2.1 Chemicals

Milli-Q water refers to 18.2 MΩ ultrapure water produced from a Milli-Q system through a filter size of 0.22 µm (Millipore, Merck, Germany). All Milli-Q was vacuum filtered through a 0.1 µm polyvinyl difluoride (PVDF) 47 mm diameter filter containing nitrocellulose (Millipore, Merck, Germany) using a HPLC

Solvent Vacuum Filtration and Clarification Kit with a 5-Litre Glass Flask (Millipore, Merck, Germany). This extra filtration step was used to eliminate any large particles which may interfere with the characterisation results and also to reduce the risk of blocking in the AF4 system as recommended by the manufacturer. All reagents used were of high purity grade and purchased from Fisher Scientific (UK) unless stated. Ultra high purity NaCl suprapur (VWR) was used for analysis involving HR-ICP-MS. Working solutions were prepared by dissolving the corresponding mass of reagent in Milli-Q.

Working elemental standard mixtures were prepared by dilution of single element 1000 mg/L synthetic standards (Inorganic Ventures). All sample ICP-MS preparation was carried out in a clean laboratory. All polypropylene vials were washed with 10% HNO_3 prior to use. All HNO_3 used was sub-boiled (Savillex dst-1000).

3.2.2 Polystyrene Sulfonate Standards

Polystyrene sulfonate standards (PSS) (Polysciences, Inc.) of varying specific molecular mass fractions (1 -400 kDa) were used for molecular weight calibration. These were prepared at 20 mg/L in the appropriate carrier solution (section 3.3.4.1).

3.2.3 Samples (Landfill Leachates)

The Environment Agency (2009) defined 4 broad classes of leachate in terms of the waste composition from which they are derived:

1) conventional municipal solid waste (MSW)
2) mechanically separated and biologically treated municipal solid waste (MBP)
3) bottom ash from incineration of municipal solid waste (MSWI)
4) treated hazardous wastes.

This study will focus on leachates from classes 1 and 2. Leachates from classes 3 and 4 are both hazardous and thus have associated handling risks so were not available for use in this study. Furthermore, it can be difficult to obtain landfill leachate samples for research purposes due to concerns with anonymity of results in case of breach of landfill regulations. While this study aims to develop a method that is suitable for analyses of MSW and MBP leachates, it is important to recognise that there is no such thing as a “standard” leachate sample, because of the wide range in waste composition at landfill sites. Other factors such as rainfall and landfill engineering also affect waste characteristics. Nevertheless, a landfill leachate that was previously well characterised was required in order to develop the methods describe herein. Also, the leachate had to be easily accessible and available in large volumes.

Given the scope of this study, for the purpose of method development, representative leachate material was required that ideally contained readily measureable concentrations of the elements of interest without using multiple samples or the addition of synthetic standards. Creating a synthetic leachate, such as that used in Environment Agency (2003) was considered, but it was decided to explore the use of a real leachate in order to obtain results representative of an actual environment and to create a method that was able to overcome the challenges likely to be posed by analyses of real samples.

The material chosen for development work was a MSW leachate (from here on referred to as MSW) collected from a leachate well by an on-site sampling technician. The site was in Essex, UK and currently accepts municipal solid waste (although it had previously accepted a variety of waste types). At the time of sampling, the landfill was approximately 5 years old and the waste was expected to be in the methanogenic stage (Figure 1.1). The sample was collected in a 20 litre plastic container which was transported and stored at 4 °C.

This material was selected because it was available in large volumes due to longstanding collaborations between our research group and the site. An additional benefit of this collaboration was that previous characterisation work has been completed and complimentary research was being conducted (Dalton, 2014). The leachate was known to contain a diverse elemental composition and also have conductivity higher than a typical leachate (Table 3.1). High conductivity/ionic strength can be a challenge to the characterisation of nanocolloids and detection of elements because dilution of high TDS samples is required for ICP-MS analysis but thus reduces the concentrations in the sample and also because changes in ionic strength during particle analysis can cause particle aggregation (see Section 3.3.4.1). Hence, by developing a method able to tackle an analytically challenging leachate it was hoped that other less problematic leachate types could be handled with relative ease.

In order to apply and more widely validate any developed method, it was recognised that leachates with different compositions and characteristics were also needed (see Chapter 5). Due to the difficulties of obtaining leachate samples mentioned earlier, two additional synthetic leachates were used. These were a mechanically biologically treated (MBT) waste (class 2) and an aged municipal solid waste (AMSW) (class 1) leachate created from real waste. Both leachates have been previously characterised (Table 3.1) (Dalton, 2014).

The MBT leachate was created synthetically from waste from a MBT plant in Dorset, UK. The aged municipal solid waste (AMSW) leachate was created synthetically using aged waste (> 10 years buried) from a cell (same landfill site in Essex, UK as MSW) which was leached to create the AMSW leachate. Further details on the creation method of these leachates can be found in Dalton (2014).

Table 3.1 Leachate Characteristics from Dalton (2014)

Sample	MSW	MBT	AMSW	MSW Range (Christensen et al., 2001)	MBT Range (Robinson et al., 2005**)**
pH	8.32	7.29	7.21	4.5-9	7.5-8.5
Conductivity (µS/cm)	41	7.7	2.9	2.5-35	10-20
TOC (mg/L)	2389	1080	139	30-2900	500-2000

Table 3.1 shows that the MSW leachate has a pH and TOC content towards the higher end of values recorded in the literature. The high TOC value is representative of a leachate at the earlier stages of the methanogenic stage. In contrast, the low TOC value of the AMSW reflects that fact that the leachate was derived from older waste in which more biodegradation of organic matter has occurred. The pH of AMSW is representative of a typical methanogenic leachate. Limited data is available for MBT leachate in the literature because

this treatment method is relatively new, but characteristics listed in Table 3.1 are in the range recorded for MBT and also for MSW leachates suggesting that MBT does not differ significantly from MSW. The MBT values are representative of a leachate which is in a later period of the methanogenic stage, as would be expected after the treatment process. Sample characteristics will be discussed further in Chapter 5.

3.2.4 Instrumentation

The various instruments used to carry out the experiments described in this thesis will briefly be described here with more details of their use in the relevant method development sections.

3.2.4.1 Atomic Force Microscopy (AFM)

AFM images of the sample particle shapes and sizes were obtained using a MAC Mode III, 5500 Scanning Probe Microscopy (Agilent Technologies, US) with standard silicone AFM probes (Nanoworld, Windsor Scientific, UK). Images were acquired in non-contact tapping mode with a nominal cantilever force constant of 42 N/m, resonance frequency of 300 kHz and a tip radius of < 10 nm. Images were recorded in both the topography and imaging modes. PicoView 1.1 and PicoImage (Agilent Technologies, US) software were used for data acquisition and image analysis. All the scans were performed at ambient conditions.

The samples were deposited on a freshly cleaved mica sheet (11 mm x 11 mm x 0.15 mm (Agar Scientific, UK). The AFM sample preparation will be discussed further in Section 3.3.2.

3.2.4.2 Dynamic Light Scattering (DLS)

A Malvern Zetasizer S (Malvern, UK) was used to provide particle size and distribution measurements. All measurements were made at room temperature using disposable plastic cuvettes.

3.2.4.3 Asymmetric-Flow Field Flow Fractionation (AF4)

The AF4 system used for sample fractionation was an AF2000 (Post Nova Analytics, Landsberg, Germany). The system was as standard, with the following changes: The metal needle injection port was replaced with a PEEK

Loop Filler Port (P/N 9012, Rheodyne, Upchurch, UK) so that the system was metal free. All tubing was PEEK and all glass bottles were replaced with HDPE bottles to reduce system metal contamination. Further materials used as part of the AF4 system will be discussed in 3.3.4.

3.2.4.4 UV-VIS Photo Diode Array (UV-DAD)

The AF4 system was coupled with a UV-DAD (SPD-M20 UV-DAD, Shimadzu, Germany) detector to detect particles eluting from the AF4 channel (Section 2.2.3.1) The UV-DAD was connected in-line with the AF4 so that elutant from the AF4 flowed from the channel outlet and then through a flow cell in the UV-DAD. This provided a continuous signal by taking measurements every 0.01 seconds during a fractionation run. In order to measure NOM, the signal at 254 nm was selected (Coble, 1996).

3.2.4.5 Fluorescence (FLU)

A fluorescence detector (RF-20A, Shimadzu, Germany) (Section 2.2.3.2) was connected in-line with the AF4 and UV-DAD detector. The detector allowed measurement of two fluorescence channels by specifying excitation and emission wavelengths for each channel. In order to measure humic and fulvic-like substances, the channels were set at Excitation:260 nm, Emission:450 nm and Excitation:335 nm, Emission:450 nm, respectively (Coble,1996).

After exiting the UV-DAD flow cell the elutant flowed in to the FLU flow cell and detector producing a continuous signal, by measurements being recorded every 0.008 seconds.

3.2.4.6 Fraction Collector

A fraction collector FRC-10A (Shimadzu, Germany) was used to collect elutant leaving the FLU Detector in time specified fractions using the LC solutions software (Shimadzu, Germany). Further details will be given in Chapter 4.

3.2.4.7 High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS)

The HR-ICP-MS used was an Element 2 XR (Thermo Scientific, Germany). Specific HR-ICP-MS operating procedures will be detailed in Chapter 4.

3.2.4.8 Electrical conductivity, pH and TOC

Electrical Conductivity and pH were measured using a handheld probe (Hanna Instruments, UK). TOC was measured using a multi N/C 3100 (Analytik-Jena, Germany).

3.3 Methods and Method Optimisation

A number of the techniques used in this study for investigating nanocolloids had not previously been applied to landfill leachates, so no established methods were available. Hence, instrument parameters and sample preparation methods needed to be optimised in order to harmonise the logistics of the various measurement techniques and to provide reliable data. For example, the AF4 parameters needed to be optimised to ensure that high quality elemental concentration data would be obtained when coupled with HR-ICP-MS. Note that all method optimisation was carried out using the MSW sample.

3.3.1 Sample Preparation

For the techniques being used in this study (AFM, DLS, AF4, HR-ICP-MS), a raw leachate was not suitable for use without some sample preparation because of the large particles that it was expected to contain (section 1.4). Samples were therefore vacuum filtered using polyvinyl diflouride (PVDF) 47 mm diameter nitrocellulose filters (Millipore, Merck, Germany) mounted on a Nalgene Polysulfone Graduated Filter Holder with Receiver, 47mm Membrane Diameter, 500mL Capacity (Fisher Scientific, UK). The samples were filtered first at 0.45 µm and then at 0.1 µm to reduce filter clogging from large particles. Filters were changed when flow rates visibly lowered due to clogging. Samples were refrigerated after filtration but removed prior to analysis and allowed to reach room temperature.

3.3.2 Atomic Force Microscopy (AFM)

Atomic force microscopy was used to confirm PSD obtained by the DLS and AF4 techniques, and this also provided information regarding the size and shape of the NP in the sample. AFM was selected in preference to transmission electron microscopy (TEM) because it allows for the NP to be measured under environmentally-relevant, aqueous conditions, (section 2.2.1.1). It was necessary, however, for the sample to be filtered and diluted prior to analysis because large particles > 0.45 μm and high concentrations of particles can mask the smaller NP during imaging.

3.3.2.1 AFM Method Optimisation

There is no standard AFM sample preparation method in common use so multiple preparations were required to evaluate any artefacts on the analyses and to select the most suitable method for a leachate sample. 3 different methods were assessed; adsorption from solution (immersion), sorption from a thin layer (dried then rinsed) and drop deposition (dried) (Baalousha and Lead, 2012). Samples prepared using these methods were imaged and the results evaluated.

Successful analysis of nanocolloids with AFM would be expected to show shapes and sizes of individual NP, with minimal artefacts on the mica sheet. Indistinct images (such as those showing particle aggregation or salt deposition) will result in unreliable results when they are analysed using the PicoImage software and may require more dilution or a different preparation method to improve the image.

Three aliquots of 0.45 μm filtered MSW were diluted 10x using 0.02 M NaCl. Dilutions with higher NaCl concentrations (more similar to the ionic strength of the samples) were initially trialled but these resulted in drying artefacts because the salt particles attached to the substrate and made imaging of the NP difficult. In order to compare the preparation methods, the influence of ionic strength on PSD was not considered at this stage. Without dilution the concentration of particles was too high resulting in overloading of particles on the mica sheet and poor imaging.

3.3.2.2 AFM Method Development Results

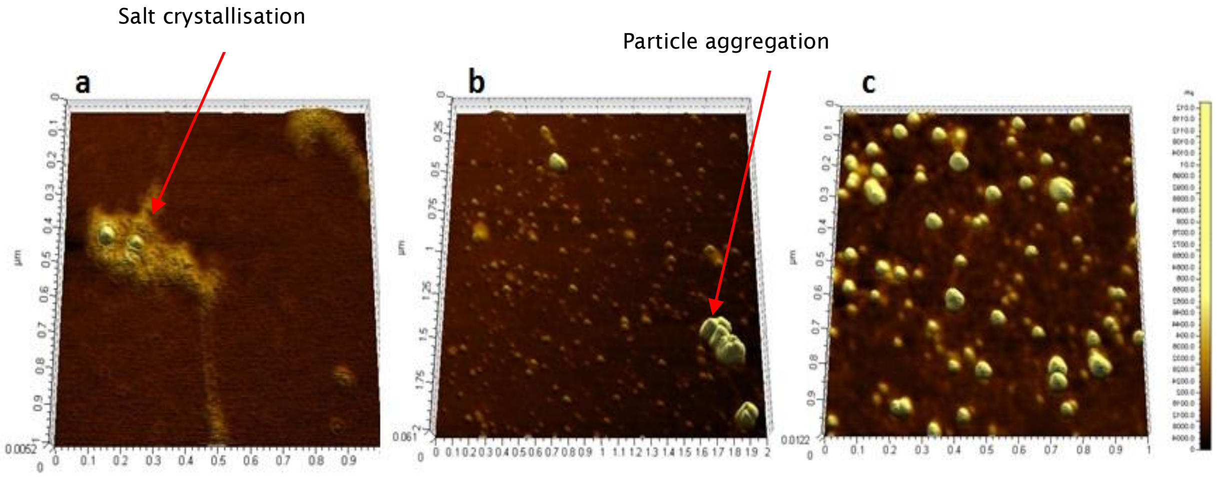


Figure 3.1AFM Method comparisons a: adsorption from solution, b: drop deposition, c: sorption from a thin layer

Figure 3.1 shows the results of the AFM imaging of the samples produced from the 3 methods (Note that Figure 3.1b has a different scale). The adsorption from solution method (Figure 3.1a) was not successful because there was little adsorption of particles on to the mica and salt crystallisation was visible. Although particles can be imaged using drop deposition (Figure 3.1b), salt crystallisation can be seen to alter the images with large aggregated particles visible. The PicoImage software was used with the intention of obtaining characteristics of particles present (e.g. mean diameter), hence any salt crystallisation or aggregation would skew these results. Sorption from a thin layer (Figure 3.1c) appeared to provide the clearest image, on the basis that individual particles were imaged and no salt crystallisation was visible, most likely because the rinsing steps dissolved away excess salts that might otherwise have been deposited on the mica. Hence, sorption from a thin layer was the favoured method for further analyses in this study.

Although 0.02 M NaCl solution was suitable to assess the AFM method when evaluating adsorption of particles on the mica sheet, there were concerns about PSD perturbation due to the ionic strength alteration of the solution.

Therefore, the PSD values and images were evaluated carefully when alternative solution concentrations were used. Effects of ionic strength on PSD will be examined further in section 3.3.4.1.

3.3.2.3 AFM Method Summary- Sorption from a thin layer

- Sample diluted 10x with NaCl solution of appropriate strength (0.1 µm filtered) in 20 ml vial and left overnight at ambient conditions.
- 20 µl of solution deposited on to mica sheet and left at room temperature (~1 hour).
- Mica sheet gently rinsed by immersion in 0.1 µm filtered Milli-Q (3x in different Milli-Q aliquots) to rinse excess salts and particles.
- Mica sheet lightly shaken and placed in covered Petri dish to dry overnight at room temperature.
- Sample analysed within 72 hours.

3.3.3 Dynamic Light Scattering (DLS)

The relative capabilities and uncertainties of the DLS technique were discussed in section 2.2.1.3 and largely relate to analyses of samples containing a wide range of particle sizes. DLS was used alongside AFM to confirm PSD.

3.3.3.1 Dynamic Light Scattering Method Optimisation

A Malvern Zetasizer S (Malvern, UK) was used to provide particle size and size distribution measurements. An aliquot of the 0.1 µm filtered fraction was pipetted in to a disposable plastic cuvette at the recommended volume. The cuvette was placed in the measurement cell and the measurement started using the Zetasizer software in batch mode. All measurements were made at room temperature with a 120 second temperature equilibration time.
Each measurement consisted of between 10-25 runs (as determined by the DLS software) which were then used for the final measurement calculation. In order to obtain more precise data, a minimum of 6 repeat measurements were taken for each sample in order to calculate a mean PSD.

3.3.3.2 Dynamic Light Scattering Results

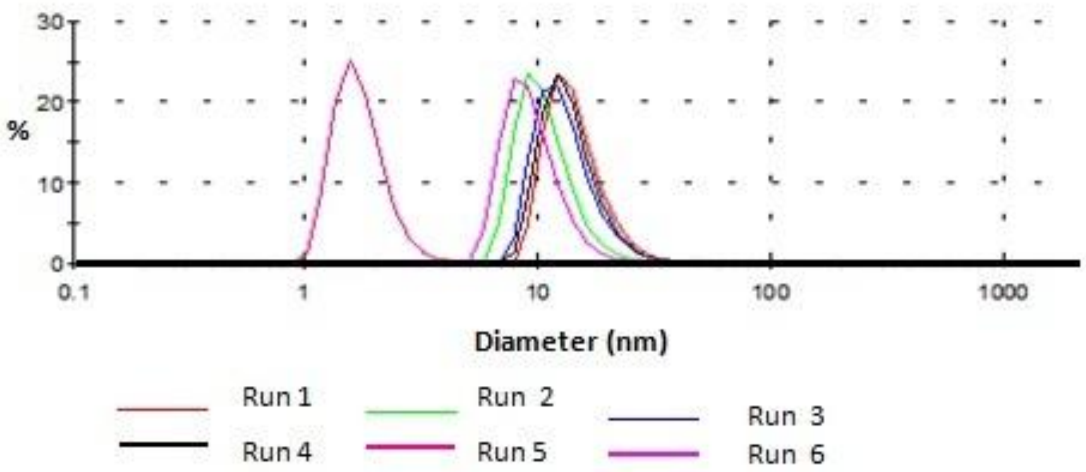


Figure 3.2 Preliminary PSD analysis of MSW using DLS (6 runs)

Figure 3.2 shows that different PSD results were obtained from 6 runs performed under identical conditions. In particular, Run 5 shows the particles to be much smaller than indicated by the other runs, with an average peak particle size of ~2 nm. Runs 2 and 6 show a peak between 9-10 nm and runs 1 and 3 have very similar PSD, with a peak around 13 nm. DLS is known to favour larger particles in polydisperse samples because the larger particles scatter more light than smaller particles resulting in less small particles being detected. It is possible that larger particles may have masked smaller particles in all runs but run 5. The difference between the runs may be due to the different optimal laser position assumed in each run.

It is therefore important to carry out DLS analysis multiple times in order to obtain representative data. The software calculates a polydispersity index (ranging from 0-1); with values greater than 1 indicating that the sample may not be suitable for use with DLS and thus, this value can be used to assess the quality of the data. The variability of results with polydisperse samples such as leachates suggests that DLS must be used in conjunction with other techniques in order to obtain accurate and precise data.

3.3.3.3 Dynamic Light Scattering Method Summary

- Transfer sample aliquot into plastic cuvette to reach recommended volume line
- Place cuvette in cell and start measurement
- Repeat measurement at least 6 times (reviewing data and polydispersity index)
- Use mean of measurements (removing outliers) for results

3.3.4 Asymmetric Flow Field Flow fractionation (AF4) Parameter Optimisation

AF4 was used to fractionate the leachate sample and thus assess the PSD of the nanocolloidal size fraction. The technique allows for desired size fractions to be collected and used for further analysis.

In order for fractionation to be successful several parameters affecting separation efficiency had to be optimised. Table 3.2 shows the parameters that can be optimised and the conditions used in published studies investigating natural nanoparticles. An additional consideration was the compatibility of the parameters chosen with the techniques used for further particle analysis (UV, FLU and ICP-MS). Baalousha et al.,(2011b) stated that method optimisation should account for; the choice of carrier solution, the membrane pore size and composition and the strength of the applied cross flow required in order to obtain good particle size separation. Therefore, all of these parameters are investigated further in this study.

Table 3.2 Carrier Solution and other parameters from natural NP studies

Carrier Solution	Membrane	Detector Flow (ml/min)	Cross flow (ml/min)	Tip flow (ml/min)	Focus flow (ml/min)	Injection volume (µl)	focus time (min)	Spacer (µm)	Sample	Authors
10 mM $NaNO_3$ pH 5.7	1 kDa/ 10 kDa	1	3.09/4.09	0.09	4	-	14	-	Colloidal organic matter rivers	(Alasonati et al., 2010)
Milli-Q containing 0.025% SDS and 0.02% NaN_3	10 kDa	1	0.3	-	-	-	-	254	Soil Waters	(Baalousha et al., 2005)
0.025% SDS + 0.02% NaN_3 Sodium Azide	1 kDa RC	1	0.05-0.1 for 50 minutes	-	-	-	-	-	River	(Baalousha et al., 2006a)
1 mM $NaNO_3$	1 kDa RC	1	3	-	-	-	-	-	River	(Baalousha and Lead, 2007)
0.01 M $NaHCO_3$	1kDa	-	3	0.5	-	20	-	-	River water	(Benedetti et al., 2003)
Ultrapure water to pH 9.3 with NaOH 1M	5 kDa RC	-	0-900 Seconds 3.4 1500 = 0 2700 =0	-	-	100	-	500	Compost leachate	(Bolea et al., 2006)
Ultrapure water pH 8 with NaOH 1M	1 kDa PES	0.8	Variable	-	-	100	-	350	Compost leachates	(Bolea et al., 2010)
Ultrapure water to pH 9.3 with NaOH 1M	5 kDa RC	-	2.4-0 over 30mins linear 15mins 0	-	-	100	-	500	Groundwater	(Bouby et al., 2008)
Ultrapure water to pH 9.3 with NaOH 1M pH checked every 8 hours	5 kDa RC	0.8	2.4-0 over 30mins linear 15mins 0	-	-	100	-	500	Bentonite colloids	(Bouby et al., 2011)
1-10mM NH_4NO_3	1 kDa RC	1	0.3/0.5/0.8	-	-	100/400	-	-	Landfill Leachate	(Dubascoux et al., 2008a)

Carrier Solution	Membrane	Detector Flow (ml/min)	Cross flow (ml/min)	Tip flow (ml/min)	Focus flow (ml/min)	Injection volume (µl)	focus time (min)	Spacer	Sample	Authors
1 mM NH_4NO_3 + O.1 mM SDS	10 kDa RC	1.4	0.4		0.75		5		River	(Dubascoux et al., 2008b)
Milli-Q with ionic strength adjusted with sodium chloride 58ppm	300 Da PES	-	3.5	0.33	3.3	2000	-	500	Rivers	(Gueguen and Cuss, 2011)
25 mM NaCl	1 kDa RC	5	5 for 25 minutes	-	-	100	-	500	River	(Krachler et al., 2010)
Ultrapure water pH 8 with KOH 1 M for colloids , NH_4NO_3 0.02 M for macromolecules <50 kDa	1 kDa PES	Variable	-	-	0.8	100	-	350	Compost leachates	(Laborda et al., 2011)
0.5 M $Na_4P_20_7$ Na+ molarity of 2 Mm, 0.5 mM NaCl, 0.5 mM $Na_4P_20_7$ Na+ molarity of 2 mM		1.4/ 1	0.4/0.6	-	0.05	100/50	20/15	-	River Water	(Plathe et al., 2010)
0.001 M NaCl +0.003 M NaN_3	1 kDa	0.9	4.8	-	0.1	100		-	Groundwater	(Ranville et al., 2007)
30 mM Tris buffer at pH 7.5	1 kDa	1	2	-	-	20	2	254	River And Estuarine	(Siripinyanond and Barnes, 2002)
15 mM NH_4CL pH 8 river, 55 mM NH_4CL pH seawater	1 kDa PES	0.5	3	-	4.5	-	30	-	River/Seawater	(Stolpe et al., 2010b)
10 mM $NaNO_3$ pH 5.4	1 kDa RC	1	3 for LMM 0.25 for HMM	-	4	1000	-	-	Wastewater	(Worms et al., 2010)

"- " means that data was not reported, LMM=low molar mass, HMM= high molar mass, RC =Regenerated Cellulose, PES = Polyethersulfone

3.3.4.1 Carrier Solution

The first parameter considered was the carrier solution because it is integral to the AF4 system and must be optimised to ensure efficient particle separation (Baalousha et al., 2011). An ideal carrier solution should not alter the aggregation state of the particles (Bolea et al., 2010). The solution chosen should therefore mimic the chemical characteristics of the sample's matrix as closely as possible, so that it's characteristics are not altered. Dubascoux (2008c) found that although the chemical composition of carrier solutions did not influence the fractionation and recovery of a sample, the ionic strength did. A simple solution of electrolytes with ionic strength and pH conditions close to the sample being investigated is therefore most often used (Baalousha et al., 2011). It should be noted that these published studies did not consider coupling of the AF4 system with ICP-MS for the determination of the elemental concentrations of the particles, so the effects of the choice of carrier solution on the chemical composition of the particles was not considered whereas in this research it was an important consideration.

HR-ICP-MS analysis adds an extra complication to the selection of a suitable carrier solution, because it needs to both closely mimic the nature of the leachate sample and be compatible with post-separation analytical techniques. In particular, it needs to contain low levels of the elements of interest so as not to overprint the signature of the sample and lead to high background levels in the system.

A selection of carrier solutions (see Table 3.2) were reviewed and a list of potential candidates selected on the basis that they would not alter sample characteristics and would be compatible for analysis using HR-ICP-MS (Table 3.3). Chemicals and reagents not available in high purity grade were ruled out due to possible elemental contamination of the sample from the carrier solution. Milli-Q water was initially the preferred carrier solution due to its availability and compatibility with HR-ICP-MS (after acidification) as well as to avoid interactions between the sample and the carrier solution.

Unfortunately, initial fractionation runs with Milli-Q did not provide sufficient separation of the size fractions. In a preliminary fractionation run of the MSW sample using a 2 ml/min cross flow of Milli-Q for 60 minutes (parameters in

Table 3.6), no particles were detected suggesting that the dilution of the sample and lowering of ionic strength may have promoted particle aggregation.

Table 3.3 Potential Carrier Solutions selected from Table 3.2

Potential Carrier	Concentrations in literature
Sodium Nitrate $NaNO_3$	1-10 mM
Ammonium Chloride NH_4Cl	5-55 mM
Sodium Chloride NaCl	1-25 mM
Ammonium Nitrate NH_4NO_3	1-15 mM
Tris Buffer	5-100 mM

In order to assess the blank level element concentrations of the potential carrier solutions, 100 mL of each solution in Table 3.3 were prepared at 0.1 M concentrations using Milli-Q and analysed using the HR-ICP-MS. This screening found that some potential carrier solutions contained elemental levels higher than the MSW concentration if diluted 10-fold (considered the minimum dilution required for ICP-MS) (Table 3.4).

Previous work has shown that the MSW selected in this study has higher elemental concentrations than typical leachates (Dalton, 2014) hence high background concentrations of elements of interest in the carrier solution would cause difficulties when measuring other leachates. NaCl Suprapur (VWR) was found to contain the lowest concentrations of most of the elements of interest (Table 3.4), so it was chosen as the carrier solution.

Once the carrier solution was selected, the optimum molar concentration had to be determined. The molar concentration affects the sample separation because changes in the ionic strength of the solution can induce particle aggregation or break up of pre-existing particle aggregates (Bolea et al., 2010). Electrical conductivity was used as an indicator of the ionic strength of the leachate, which yielded a value of 41 mS. A 1 M NaCl solution has a conductivity of 85 mS, hence a ~ 0.5 M NaCl carrier solution was required to match the conductivity of the leachate. This concentration of carrier solution would be higher than values reported in the literature (Table 3.3), but this reflects the high ionic strength of the MSW leachate used in this study and the concern that a lower ionic strength of carrier solution would affect the PSD.

High ionic strength carrier solutions have, however, been reported to reduce recovery of the sample because of increased membrane interaction e.g. adsorption (Neubauer et al., 2011), and may also cause challenges when conducting HR-ICP-MS analyses because the high salt content can cause blockages in the nebuliser, and a build-up of salt deposits on the ICP-MS interface resulting in excessive drift in the signal intensity. A compromise therefore, had to be found.

Table 3.4 Concentrations of elements in potential carriers (ppb) obtained using HR-ICP-MS analysis

	MSW	**NH_4Cl**	**NaCl**	**NH_4NO_3**	**NaOH**	**Tris**	**NH_4Cl SP**	**NaCl SP**	**KCl SP**
Li	557	0	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Mg	52098	4.5	24	29.9	0	9.7	0.8	0	0
Al	1333	0	0.6	0	14.5	1.5	1	0	0
Si	53986	0	29.8	0	31.2	0	0	28.4	21.1
K	1028	23.7	61.1	83.7	573	22.9	3.2	25.2	NA
Ca	15097	21.6	171	350	73.8	88.1	124.9	1.4	26.2
Ti	457	0	0	0	0	0	0	0	0
V	103	0	0	0	0	0	0	0	0
Cr	570	0	0	0	2.4	0.2	0	0.2	0
Mn	26.6	0	0.1	0.1	0.3	0	0	0	0
Fe	2689	1	1.8	0	12.6	0.7	0.2	0.4	0
Co	69.8	0	0	0	0	0	0	0	0
Ni	262	0	0.2	0	0	2.9	0	0	0
Cu	21.1	0.3	3.8	2.9	1.2	1.5	0	0	0
Zn	BD	3	8.8	34.9	5.4	10.1	15.1	0	0.8
As	2.5	0	0	0	0	0	0	0	0
Br	219	20.1	237	0	0.1	11.2	3.9	2.9	6.5
Sr	836	0.1	0.8	1.4	0.6	0.4	0.1	0	0
Mo	24.0	0	0	0	0	0	0	0	1355
Ag	BD	0.3	0.1	0	0	0	0	0	0
Cd	12.1	0	0	0	0	0	0	0	0
Sn	108	0.1	0	0	0	0.1	0	0	0
Cs	23.5	0	0	0	0	0	0	0	0
Ba	791	0.1	0.4	0.3	0.3	0.1	0	0	0.1
Au	0	1	0.1	0	0	0	0	0	0
Hg	0	0.9	0	0	0	0	0	0	0
Pb	43.6	0	0.1	0.4	0	0.3	0	0	0
U	0.49	0	0	0	0	0	0	0	0

Although both recovery rates and preservation of sample characteristics are important for this study, the high conductivity of the sample (as is typical of landfill leachates), means that the effects of ionic strength on the particle size distribution were determined to be more important than optimising the recovery rates. Recovery rates will be discussed further in Chapter 4. The effects of carrier solution pH have previously been investigated (Moon et al., 1998) and found to influence AF4 fractogram peak heights but not fractionation efficiency and are therefore not considered further here.

To investigate the effects of carrier solution ionic strength on particle size distribution within a leachate, 1:10 dilutions of the sample with varying NaCl ionic strength solutions (0.001 - 1 M) were prepared and analysed using DLS (Figure 3.3).

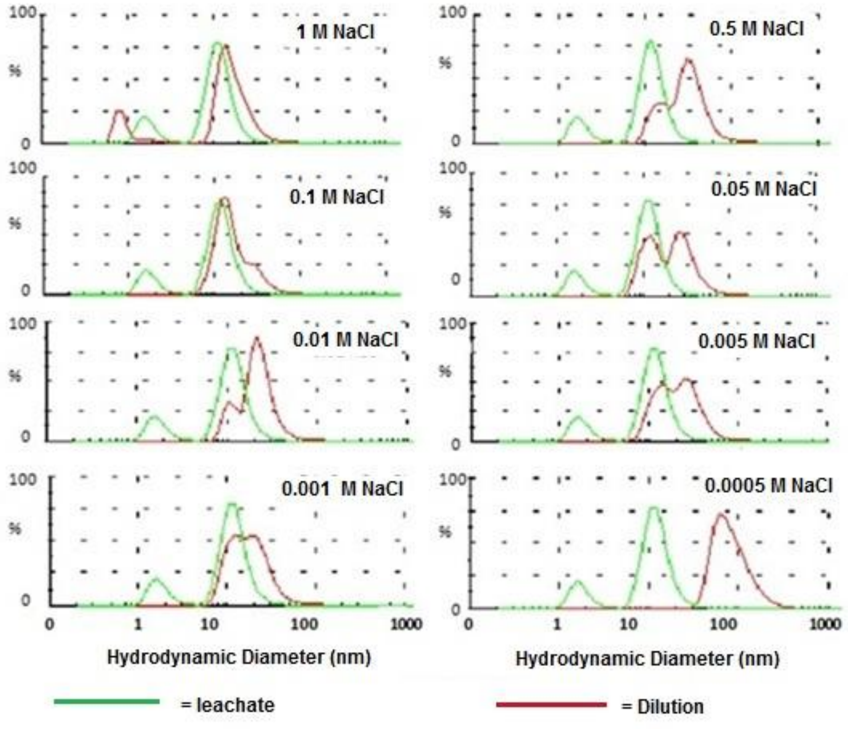


Figure 3.3 Dilution effects of varying ionic strengths on PSD

Figure 3.3 shows that dilution of the leachate with 1 M NaCl solution was most successful in preserving the PSD of the sample, because the distribution profiles of the sample and the sample dilution match most closely. Hence, even though dilution with 0.5 M NaCl was predicted to be most successful at preserving sample fidelity (because this solution matched the conductivity of the sample most closely), aggregation of particles in the leachate occurred (as shown by the increase in particle size and loss of smaller particles). Slightly lower sample fidelity was preserved by the 0.1 M NaCl solution than dilution with 1 M NaCl solution, as seen by the profiles in Figure 3.3. This observation is important, because it demonstrates that it is necessary to carefully test the effects of the nature of the carrier solution, rather than simply assume that matrix matching between the leachate and carrier solutions will necessarily yield the most reliable data. There is the possibility however, that the variation seen with the 0.5 M NaCl solution was due to a variation in PSD in the aliquot of sample used for dilution.

Decreasing the ionic strength of the dilutant solution below 0.1M resulted in the formation of larger particles (presumably due to particle aggregation) with the lowest ionic strength solution (0.0005 M) leading to the greatest apparent increase in the size of NP (maximum NP increased from 100 nm to 500 nm using DLS). It is important to note, however that all the dilutions resulted in some change in PSD.

Although the 1 M NaCl solution appeared from DLS to provide the closest PSD match with the leachate, the high total dissolved solids (TDS) content was not ideal for coupling with HR-ICP-MS. Although this problem could be overcome with dilution, that would result in lower concentrations of the elements of interest in the diluted sample and potentially compromise their detection limits. For this reason, 0.1 M NaCl was at this point chosen as the carrier solution concentration because it was the highest concentration of NaCl that was considered suitable for long term analysis with ICP-MS analysis whilst still preserving sample PSD fidelity.

The results from DLS analysis were surprising because it was expected that higher ionic strengths solutions would encourage aggregation however the opposite was observed. Further analysis; using AFM; to confirm the PSD results was therefore required. For AFM analysis, 1:10 dilutions of the MSW sample at 4 different NaCl ionic strength solutions (0.001 M, 0.01 M, 0.1 M and 0.5 M) were carried out and the solutions prepared as described in 3.6.2.3. Dilution with 1M NaCl was not investigated using AFM because the salt concentration would be too high for imaging and because a carrier solution at this strength had already been ruled out.

Table 3.5 shows the AFM analysis results obtained using the PicoImage software package. The mean diameters varied with all 4 dilutions confirming that the ionic strength of the solution had an effect on particle size. Dilutions with 0.001 and 0.01 M solutions resulted in particles almost double that of those solutions using 0.1 and 0.5 M NaCl suggesting that particles had aggregated when the sample was diluted with a solution of lower ionic strength. Dilution of the sample with 0.1 M NaCl resulted in the smallest particle size (suggesting no aggregation) and therefore this confirmed the use of 0.1 M NaCl as the carrier.

Table 3.5 MSW particle sizes from AFM analysis

Dilution	Min Diameter (nm)	Max Diameter (nm)	Mean Diameter (nm)
0.001 M NaCl	11.1 ±6.8	28.8 ±15	17.6 ±9
0.01 M NaCl	13.4 ±7.8	32 ±16	20.6 ±10
0.1 M NaCl	5.3 ±4	15.3 ±9	8.8 ±5
0.5 M NaCl	6.3 ±5	19.9 ±14	11 ±7

3.3.4.2 Membrane

Most studies using AF4 have used either regenerated cellulose (RC) or polyethersulfone (PES) membranes (Table 3.3). Little or no information is given on why particular membranes have been chosen, even though it has been recognised that the choice of the accumulation wall membrane can influence separation and sample perturbation e.g. aggregation (Baalousha et al., 2011b). Thang et al.,(2001) showed that use of a RC membrane with a humic acid solution resulted in higher sample recovery than PES due to less adsorption on the membrane. Conflicting views on membrane performance were found in Lyven et al.,(1997) where recoveries of creek water were higher using PES than RC membranes, but these studies investigated different samples with varied compositions making comparison of the membrane types difficult. Due to the high humic acid content that is present in landfill leachates, a RC membrane was selected on the basis of the results presented in Thang et al., 2001.

A 1 kDa cut off RC membrane was selected for use in this study because it was the smallest available in this material and permits for the smallest particles to be analysed. Particles < 1 kDa are transported through the membrane with the cross flow and are designated as the "truly dissolved fraction”. Fluctuations in the permeability of ultrafiltration membranes used in AF4 have been mentioned previously (Stolpe et al., 2005) and as such the 1 kDa cut-off is variable and may affect those elements with a higher dissolved fraction. This is because the cut off is actually dependent not only on the membrane pore size but the carrier solution and sample composition (Baalousha et al., 2011b).

A membrane with a small size cut-off e.g. 1 kDa can cause problems in maintaining stable pressure in the AF4 system. System pressure is monitored with an in built pressure detector and the software displays the pressure at all times giving an indication of the overall stability of the system. Stable pressure is required for good separation, but the high cross flow rate (see section 3.3.4.3.3) and small membrane pore size used in this study meant that both under and overpressure in the system were evident. A backpressure tube was added to increase the pressure when higher cross flows were used and this resulted in increased pressure throughout the fractionation run. Care had to be taken to ensure there was sufficient system pressure when higher cross flows were applied, but that the system did not overpressure when the cross flow was reduced (Figure 3.4). The detector flow rate was reduced to 0.3 ml/min in order to help maintain stable pressure during periods of high cross flow and reduce the chances of overpressure when no cross flow was applied.

3.3.4.3 Flow Rates

As noted in Chapter 2, there are 3 flow rates (tip flow, focus flow, cross flow) in the AF4, which are used to size fractionate the sample, and these are all controlled by the AF4 software. The resulting flow out of the channel (channel/detector flow) and to the detectors is controlled by varying these flow rates.

3.3.4.3.1 Tip Flow

The tip flow is a flow of carrier solution that constantly flows through the channel during a run, and flows through the channel prior to the injection of a sample. When a sample is added, the tip flow transports the sample remaining in the channel (particles) through the channel and towards elution at the channel outlet.

3.3.4.3.2 Focus Flow

The focus flow is a flow of carrier solution that opposes the tip flow and therefore does not allow particles in the sample to flow through the channel towards the outlet port and to elution. The focus flow is only applied at the beginning of a run prior to size fractionation to aid separation. Once the flow is removed, only the cross flow and tip flow remain. The duration of the focus flow is an important parameter for achieving efficient separation of particle sizes. It is important to note that larger injection volumes and samples with wide particle size distributions require longer focusing times because it takes longer for the particles to be sorted into size order in the channel.

Preliminary tests of the sample and injection volume used in this study showed that focusing times <10 minutes did not provide sufficient focusing of the sample and yielded poorly formed peaks shapes. Analyses of the size fractions require the particle elution to take place over a sufficiently long period (producing broad peaks) enabling distinct size fractions to be resolved. 10 minutes was found to be the optimum focusing time for the sample volume used here (1 ml). Extending focusing times beyond 10 minutes did not alter or improve the peak shape.

3.3.4.3.3 Cross Flow

The cross flow is one of the key parameters that determine AF4 resolution, quality and efficiency of size fractionation (Dubascoux et al., 2008d, Baalousha et al., 2011b). The cross flow forces particles in the sample towards the membrane and those which are smaller than the cut-off travel through the membrane. Optimisation of the cross flow to achieve the most efficient particle size separation must be carried out for each sample type, with the size of NP to be fractionated most strongly influencing the magnitude of the cross flows chosen. Lower cross flows enhance the separation of large NP and a higher cross flow enhances the fractionation of small NP (Baalousha et al., 2006). Bolea et al. (2010) used a gradient cross flow in order to separate heterogeneous samples with a wide size distribution in order to reduce run time and avoid steric elution in which the elution order is reversed and larger particles elute first (Hassellöv et al., 2006).

In this study the cross flow was optimised for the separation of low MW NP. This was because several studies (Gounaris et al., 1993, Geckeis et al., 2003, Li et al., 2009a) have shown that the majority of metals in landfill leachates are bound to OM which is expected to be present in the lower MW fraction (Figure 1.3). Therefore to examine the distribution pattern of metals in the nanocolloids, the fraction in which they are expected to reside must be examined.

Higher cross flows allow for a greater separation of smaller particles, which results in broader peaks, whereas as a very low cross flow may result in no separation of smaller particles at all because the flow is not strong enough to act against the diffusion towards the middle of the channel. In order to study differential partitioning of elements associated with different particle size fractions it is therefore necessary to achieve clear separation of the various particles sizes and thus as broad a peak as possible. Hence, this requires a high cross flow. High cross flows can, however, result in increased membrane interaction because the particles are more strongly drawn towards the membrane and this can potentially reduce sample recovery due to sorption of sample onto the membrane.

Cross flows higher than 3 ml/min were not possible with the equipment used in this study, because the AF4 lost pressure as the cross flow was increased, even with a low channel flow rate. This is presumably due to the small cut off size of the membrane. Flow rates between 2 to 3 ml/min resulted in unstable system pressure throughout the cross flow applied during the period of the fractionation run (Figure 3.4). The pressure in runs with a 2 ml/min cross flow did, however, remain stable and the cross flow was sufficiently high to separate smaller particles. 2 ml/min was therefore chosen as the optimum cross flow for low molecular weight NP and for stable pressure.

For this study, the low MW nanocolloids are of the greatest interest, but the cross flow was reduced to 0 ml/min for the second half of the run in order to elute the remaining larger NP (Figure 3.4) resulting in a residual peak.

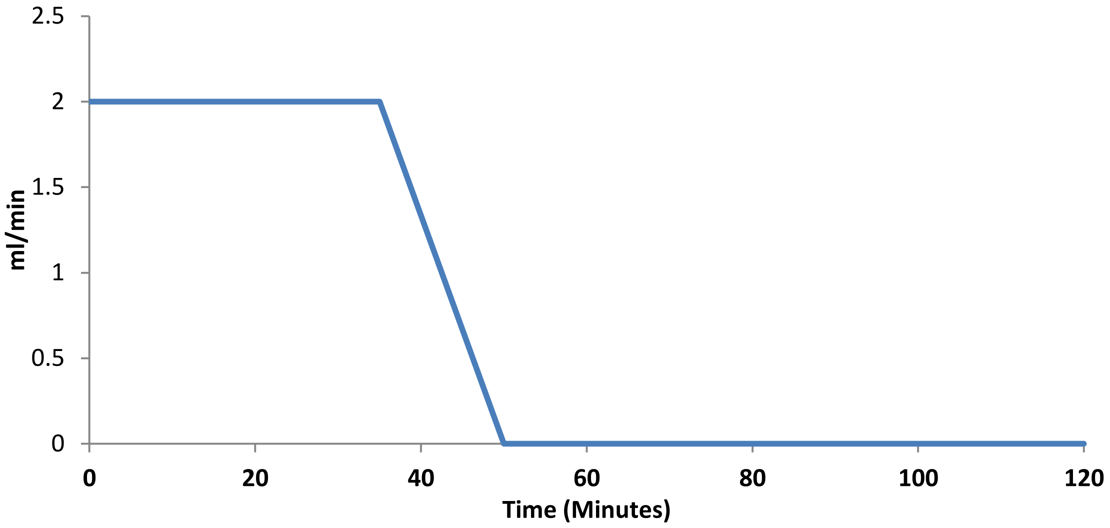


Figure 3.4 Cross flow programme for fractionation

3.3.4.4 Sample Volume

Preliminary experiments injecting variable volumes of MSW sample were carried out in order to determine the sample volume required to detect elements eluting from the AF4 after dilution using HR-ICP-MS. An injection volume of 1 ml of sample was sufficient to detect the majority of elements of interest after dilution. For some elements, however, sample volumes of > 1 ml were required, but injecting larger sample volumes would require longer fractionation times. For this reason 1 ml was chosen for this study as a compromise between the logistics of processing the samples and achieving high enough element concentrations to yield meaningful data.

3.3.4.5 Size Calibration- PSS Standards

Polystyrene sulfonate (PSS) particles with molecular weights of 1, 6, 15, 33 and 63 kDa were used for the size calibration process and to validate the size fractionation using the same separation conditions (e.g. carrier solution, flow rates) as used for the MSW leachate. 194 and 400 kDa PSS were also used, but elution was not observed during the measurement period when the cross flow was applied, hence these particles were eluted as part of the residual peak and their size fractionation cannot be calibrated. PSS were prepared at concentrations of 20 mg/L in the carrier solution. PSS standards have been used previously for calibration of humic substances (expected in leachates) due to the similarities in their random coiled structures (Prestel et al., 2005). Beckett et al.,(1987) noted that the accuracy of the use of PSS as calibration standards for natural humic substances calibration depends on how closely the standards represent the particle size distributions in the sample. As the shape of humic substances is unknown it has to be acknowledged that this remains an undetermined source of error in this approach.

Bolea et al.,(2006) recognised that although diffusion coefficients and hydrodynamic diameters can be calculated using FFF theory, deriving molecular weights using this approach is difficult because it does not account for the effects of the carrier solution composition. Instead they used a linear relationship between log t_r (Retention Time (Seconds)) and log M_w (Molecular Weight (Da)) for calibration (Equation 1). This has been used to calibrate MW for particles in the samples in this study.

$$\log(tr) = a + b\log(MW) \qquad (1)$$

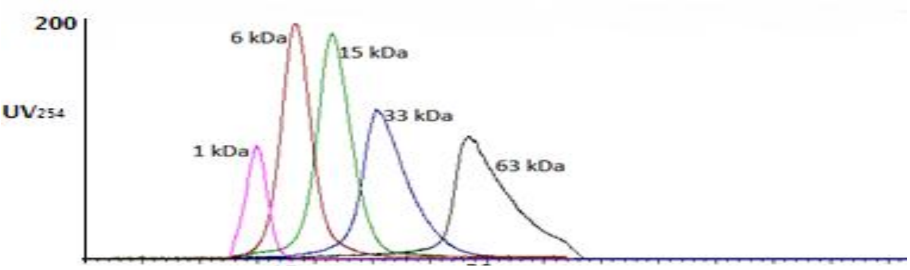


Figure 3.5 Calibration of molecular weight using PSS Standards 1- 63 kDa

Retention times of PSS (Appendix 1.4) used with the parameters in this study are illustrated in Figure 3.5 and the equation derived from this data is:

$$\log(tr) = 5.3984 + 0.177\log(MW) \qquad (2)$$

Although Equation 2 can be used to calibrate molecular weights, it is important to note that the equation has been derived using the peak maximum retention time for each PSS size. Figure 3.5 shows that the standards elute over a small time period rather than at a discrete point, hence the equation can be used to provide some limits on the range in particle size, rather than indicating a precise value due to the variation in size of the PSS.

The largest PSS that eluted within the cross flow period was 63 kDa , so only particles with lower molecular weights could be size calibrated. All fractions collected between 0 to 30 min (elution time of 63 kDa PSS) could then be designated < 63 kDa. In order to calibrate the size fractions in more detail, fractions were collected as frequently as possible in the time period before elution of the 63 kDa standard (see Section 4.2.3.1). Note that the 194 kDa and 400 kDa PSS standards were eluted with the residual peak and therefore cannot be used in this calibration.

3.3.5 Summary of Method and Parameters for AF4

The filtered sample was removed from the refrigerator and allowed to reach room temperature before an aliquot of the sample was injected into the AF4 using a 1 ml sample loop. At least 5 minutes prior to starting the run on the AF4 software, the tip and cross flows were slowly increased until the 2 ml/min cross flow was reached and pressure and flow rates were stable. The system was set up so that the AF4 software triggered the UV and Fluorescence detectors to start as soon as the sample was introduced in to the AF4 channel. The parameters used for fractionation and analysis are summarised in Table 3.6. Calibration for MW was conducted prior to the sample runs using the same method and parameters.

Table 3.6 Optimal parameters for separation

Parameter	Condition
Carrier Solution	0.1 M NaCl (Suprapur,VWR) with MQ
Injected amount	1000 µl
Cross flow	2 ml/min for 25 minutes, linear gradient decrease to 0ml/min from 50mins to 120mins
Detector Flow	0.3 ml/min
Focusing time	10 minutes
Run time	120 minutes
Membrane	Regenerated Cellulose 1 kDa
Spacer	350 µm
UV-VIS Wavelength	254 nm
Fluorescence	Ch 1- Ex:260nm, Em:450 nm, Ch 2- Ex 335nm, Em: 450 nm

3.4 Chapter Summary

This chapter has established methods suitable for characterising (AFM and DLS) and fractionating (AF4) nanocolloids in landfill leachates.

The AF4 method parameters (Table 3.6) have been optimised to consider compatibility with leachate samples (e.g. ionic strength and element concentrations) and compatibility with HR-ICP-MS analysis (e.g. composition and concentration of carrier solution).

Figure 3.6 illustrates the method steps involved in characterising and size fractionating a landfill leachate (detailed in this chapter) and determining the organic (UV and FLU) and elemental (HR-ICP-MS) composition of the nanocolloids in landfill leachates (detailed in Chapter 4).

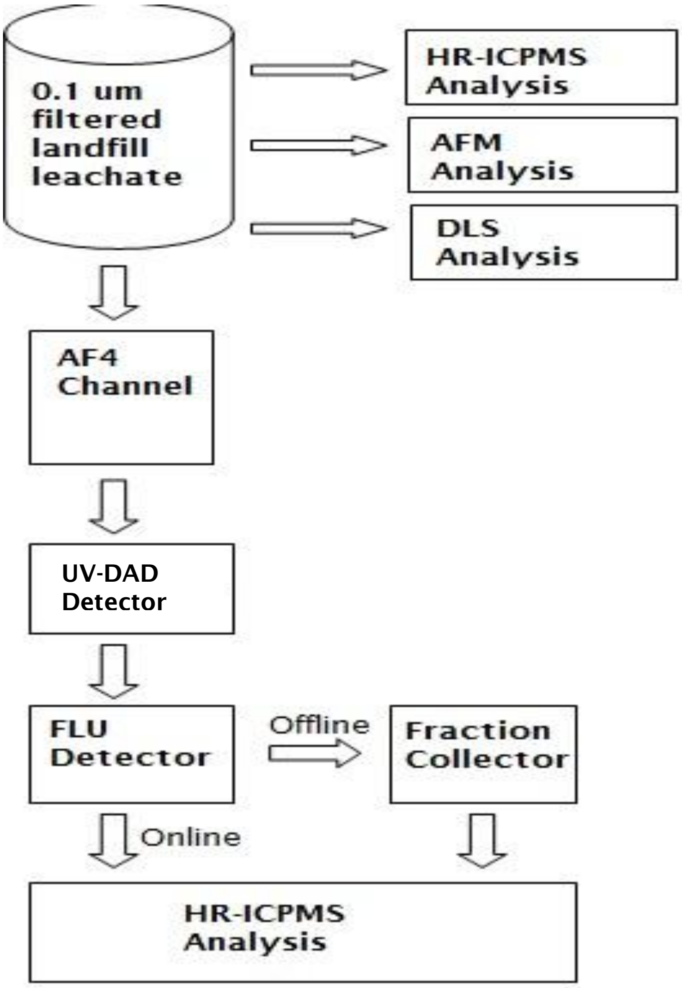


Figure 3.6 Method Steps for Landfill Leachate Analysis

Chapter 4: AF4-HR-ICP-MS Coupling for Quantitative Analysis: Challenges, Approaches and Validation

4.1 Introduction

Chapter 3 outlined the methods to be used to separate the particles in a landfill leachate into size fractions and to measure aspects of their organic composition using UV and Fluorescence detectors. To obtain quantitative trace element data associated with the various size fractions, the AF4 must be coupled with the HR-ICP-MS in a way which allows for all factors affecting quantification to be constrained. Although the AF4 separation parameters were optimised with coupling of HR-ICP-MS online in mind, extensive method development for coupling of the instruments was required, as well as development of analytical methods using the HR-ICP-MS.

Hassellov et al.,(1999) was the first to examine trace metals associated with environmental colloids by modifying an early non-commercial Sedimentation-FFF device to accept large sample volumes and removing metal parts to reduce contamination in the system. This early “AF4” did not feature an impermeable wall at the roof of the channel so although fractionation was carried out using the same principles as those outlined earlier, the flow profile and retention behaviour were different to those in the modern system used here.

Nevertheless, this study offered an approach to quantification that is more detailed than many outlined in more recent publications including information regarding the interface between the AF4 and the ICP-MS, monitoring of background concentrations in the system, of the method and the elemental detection limits. The importance of acidification and internal standards (see section 4.2.4.3 for a discussion of these issues in relation to this study) for optimising ICP-MS performance were discussed, as well as an examination of washout time of samples. Hassellov et al.,(1999) used quadrupole ICP-MS rather than HR-ICP-MS and, as a consequence, they were not able to detect as many elements or reach the same detection limits as has been possible in this study. In addition, although concentrations of elements were determined in their study, they were not able to determine the recovery efficiencies of individual elements or attempt an elemental mass balance of the system.

Lyven et al.,(2003) built on this work using the same method, and also discussed potential artefacts in the system resulting from losses of sample material during the analysis. They also noted the difficulty in performing a mass balance of the system due to the concentrations of several elements being below detection limits and the difficulty of separating losses of elements from the analysed fractions due to adsorption to parts of the system versus those due to losses through the membrane. Nevertheless, further research continued to improve their methods and Stolpe et al.,(2005) was able to couple the new, and now commercially available AF2000, with a HR-ICP-MS, thus lowering the detection limits and increasing the number of elements that were measured. Importantly, they concluded their study by noting that a proper mass balance of the method was still required.

Although several studies have used the AF4 coupled with either an ICP-MS or HR-ICP-MS for the measurement of elements in colloidal fractions, there is not yet an established technique for accurate and precise determination of elemental partitioning/quantification between different colloid size fractions. Indeed, several recent papers have applied the AF4-ICP-MS technique (Worms et al., 2010) to this problem without quantification, but instead simply monitor the elemental associations with particle size on the basis of signal intensities. In addition, these studies did not acidify the analysed fractions nor did they use internal standards (to monitor flow and ICP-MS drift). Instead, they chose to input the AF4 directly in to the ICP-MS nebuliser. Hence, while these studies are informative, they are essentially qualitative and highlight the difficulties in quantification of elemental partitioning in colloids.

In a review of AF4-ICP-MS by Dubascoux et al.,(2010), a detailed discussion of developments and applications was conducted. The interface between AF4 and ICP-MS was highlighted to be important in quantitative analyses. It was noted that while many publications reported apparently quantified data from coupling these devices, few of these studies documented the details of the AF4-ICP-MS interface and analytical procedures involved. Lesher et al., (2012) also recognised that serious challenges exist in accurately quantifying metal concentrations in FFF fractionated samples and that internal and external standards and sample recovery must all be carefully considered if recovery rates and potential background interferences are to be accurately measured. This issue was further highlighted in a recent review by Pornwilard and Siripinyanond (2014) who noted that quantitative information is not emphasised in most of the published articles using AF4-ICP-MS. Hence, while apparently quantified data may be being acquired, the lack of detail in publications is restricting the development of the method. Baalousha et al.,(2011b) suggested that the quality of particle size separation and elemental partitioning can be evaluated by sample recovery and fractogram repeatability, and that these parameters need to be further investigated.

Overall, in order to provide accurate and precise data concerning element partitioning between different NP size fractions, several factors need to be considered in more detail which have either been overlooked or poorly documented in the published literature:

- Interface between AF4-ICP-MS during online coupling
- The contribution of the carrier solution to the sample signal
- Calibration of signal intensities to elemental concentration and recovered masses
- Repeatability of fractionation and recorded signal intensities
- Sample recovery (mass balance)

This chapter aims to address the challenges facing quantification of elemental data using AF4-HR-ICP-MS, including instrument set up and to offer protocols to overcome these challenges in order to provide better constrained and documented quantitative data. The method and data treatment for both offline and online coupling will be discussed and compared.

An attempt to quantify the truly dissolved fraction and identify losses in the system due to method artefacts will also be carried out. This is important for environmental risk assessment because a truly dissolved element may have different mobility and environmental impacts to one in particulate form.

The results of this study will be discussed in terms of method implications in this chapter. Interpretation of these data will be addressed in Chapter 5.

4.2 Methods

The AF4 parameters used in method evaluation are detailed in Table 3.1. All experiments have been carried out using the MSW sample. All HR-ICP-MS preparation was carried out in a clean laboratory and vials were washed with 10% HNO_3 prior to use.

4.2.1 High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS)

The HR-ICP-MS used to measure element concentrations was an Element 2 XR (Thermo Scientific, Germany). The elements were measured in all 3 mass resolution settings, as mentioned in Chapter 2. Efforts were made to utilise as close to “normal” HR-ICP-MS operating conditions as possible, so as to avoid specialised or difficult to reproduce settings (see Table 4.1). The selected isotope of each element and the resolution required was generally determined by the ICP-MS software (also in Table 4.1). The AF4-specific HR-ICP-MS operating procedures will be detailed later.

Table 4.1 Operating conditions and resolutions for HR-ICP-MS

Parameter	**Condition**
RF Power (W)	1200W
Sample Uptake rate (ml/min)	0.2
Ar gas flow rates(l/min)	
Coolant	16
Nebuliser	0.7
Auxiliary	1.0
Add Gas	0.3-0.5
Nebuliser	PFA, peristaltic pumped
Spray Chamber	PFA, Peltier cooled
Sample Cone	Standard Ni
Skimmer Cone	Standard Ni “H”
Resolution - Low	Li(7), Sr(88), Mo(95), Cd(111), Sn(118), Cs(133), Ba(137), Au(197), Hg(202), Pb(208), U(238)
Resolution- Medium	Mg(24), Al(27), Si(28), Ca(44), Ti(47), V(51), Cr(53), Mn(55), Fe(56), Co(59), Ni(60), Cu(63), Zn(66)
Resolution- High	K(39), As(75), Br(79)

4.2.2 Bulk Concentration Analysis

Before analysis of element concentrations in size fractions of the sample could be obtained, it was important to first quantify the concentration of each element in the bulk sample (0.1 µm filtered (Section 3.3.1)). The variation of the element concentrations between aliquots also needed to be examined because this has implications for fractionation analysis results, hence 3 replicate aliquots were measured to establish the variation of sample concentrations measured in the most robust manner

Three 40 µL aliquots of the MSW sample were diluted in 20 ml of 3% HNO_3 (spiked with internal standard (In and Re at 10ppb and Be at 20ppb, see section 4.2.4.3)) to achieve a 1:500 dilution. All solutions were weighed to the nearest µg and concentrations calculated by weight. Solutions were analysed using the parameters in Table 4.2 alongside element calibration standards (see section 4.2.3.3.2)

4.2.3 Offline Coupling of AF4-HR-ICP-MS

Due to logistical issues associated with physically connecting the AF4 and HR-ICP-MS, initial studies were carried out in an “offline” mode. This meant that analyses of the particle size were carried out on the AF4 and samples taken using fraction collection for subsequent analysis by HR-ICP-MS to determine the elemental associations. To the knowledge of this author, the fraction collection technique coupled with ICP-MS analysis has previously been used only by Dubascoux (2010), in which the fractions were freeze-dried after collection before further analysis with ICP-MS, thus involving extensive sample preparation. Other studies have used collected AF4 fractions for further analyses such as TEM (Chen and Selegue, 2002) but not for use with ICP-MS. Hence, the lack of previously published work required substantial method development to establish optimal sampling conditions.

4.2.3.1 Offline Coupling: Fraction Collecting

In order to collect fractions at intervals that would be most useful for analysis fractionation was carried out using the optimised AF4 parameters (Table 3.6) apart from the run time, which was set to 50 minutes (because at this stage, this was deemed sufficient to elute the sample from the channel, see section 4.2.4). Initial fractionation profiles of the sample were reviewed to determine the fractions most useful for elemental analysis. Fractions were collected at time intervals set manually using the LC solutions software. Intervals were chosen by focusing on intervals where peaks in UV signals were visible and by increasing collection frequency during those peaks. On the basis of this evaluation, 17 fractions were selected and collected during each run (see Figure 4.1).

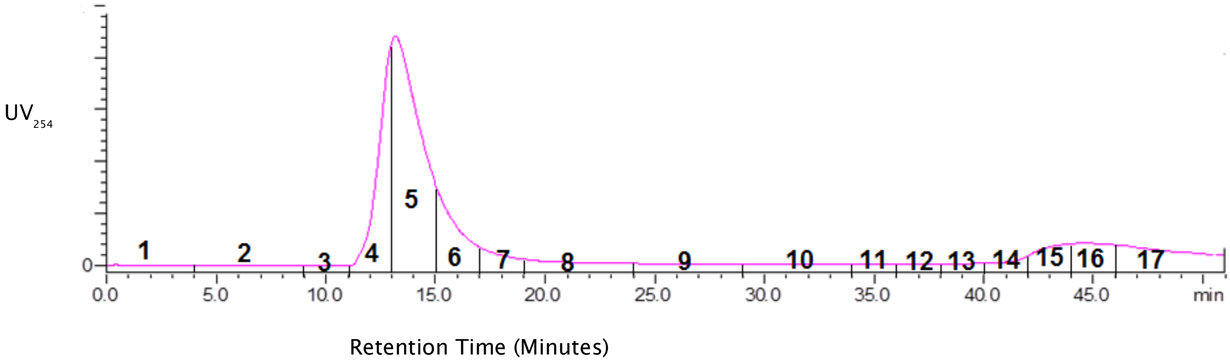


Figure 4.1 Fraction collection intervals for offline analysis

More frequent fractions were collected during the peaks, but due to the low system flow rate of 0.3 ml/min, the minimum time interval to collect sufficient volume for further analyses was determined to be 2 minutes.

The background concentrations of elements in the system were measured by injecting 1ml of carrier instead of a sample and collecting fractions at the same time intervals shown in Figure 4.1. Thus a corresponding baseline level of each element in the system at that time in the fractionation period was available for subtraction.

In order to assess recovery of the system the cross flow elutant (“truly dissolved fraction”) was also collected. This was collected in one pooled fraction rather than in time intervals.

4.2.3.2 Offline Coupling: Fraction Analysis

For analysis by HR-ICP-MS, all fractions (including the cross flow pool fraction) were diluted 1:10 with 3% HNO_3 to reduce their TDS. Samples were then spiked with internal standards (In and Re at 10ppb and Be at 20ppb, see section 4.2.4.3) to monitor and correct HR-ICP-MS instrument performance. All solutions were weighed to the nearest µg and concentrations calculated by weight. Solutions were analysed using the parameters in Table 4.2.

Each fraction collected was weighed (empty and after collection) so that the exact mass of each fraction was known, thus permitting the mass present in that fraction to be calculated. Weighing the fractions also served as an independent check on the system flow rate.

Table 4.2 Offline HR-ICP-MS Parameters

	Low Resolution	**Medium Resolution**	**High Resolution**
Acquisition mode	Mode 1	Mode 1	Mode 1
Mass Window (%)	50 for Li & Be, 20 for all others	150 for Be & Mg, 125 for all others	125
Search Window (%)	50 for Li & Be, 20 for all others	60	60
Integration window (%)	40 for Li & Be, 20 for all others	60	60
No. Sample per peak	20 for Li & Be, 50 for all others	20	25

All the diluted fractions (samples and baselines) were analysed in the same measurement sequence alongside a set of mixed element calibration standards. An aliquot of the original sample was also analysed to determine the total concentration of elements present before fractionation.

4.2.3.3 Offline Coupling: Data Treatment

In order to determine quantitative concentration data from the raw HR-ICP-MS CPS output, several data processing steps were required (Figure 4.2). Many of these steps are typically applied in HR-ICP-MS analyses, but additional steps were required to correct for the background concentration of the AF4 system. This will be hereafter referred to as the baseline concentration. Further steps were included to assess method recovery.

4.2.3.3.1 Offline Coupling: System Baseline Correction

Baseline fractions were collected at the same time intervals during a blank run as the sample (Figure 4.1) as described in 4.2.3.1. This meant that each sample fraction had a corresponding baseline fraction of the same volume so that the concentration of the baseline for that time interval could be directly subtracted. This gave the concentration present in each fraction for the sample only. The same methodology was applied to the cross flow. Results of baseline and sample data for offline coupling are shown in Figure 4.11 and Figure 4.12.

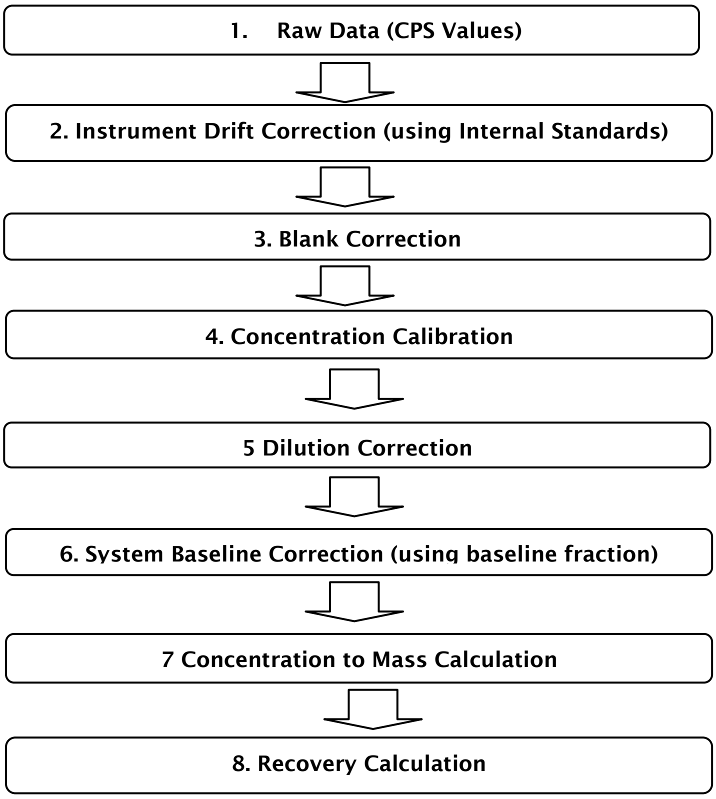


Figure 4.2 Data processing steps for offline AF4-HRICP-MS

4.2.3.3.2 Offline Coupling: Element Calibration

Pornwilard and Siripinyanond (2014) commented (without any clear evidence) that varying compositions in the fractionated peaks influences ionization efficiency within the ICP-MS plasma and can thus hinder exact external calibration. To address the issue of varying solution compositions reaching the plasma, matrix matched standards were created. Standards were initially created using 3% HNO_3 only, but this caused difficulty with calibration because the sensitivity of the HR-ICP-MS was higher and more stable when measuring the standards than the AF4 elutant samples. Standards of the same concentration in 3% HNO_3 and in 3% HNO_3 + NaCl gave different responses on the HR-ICP-MS, with the NaCl samples resulting in lower CPS because of the sample matrix. As a result of this finding, the standards were remade in the same matrix as the AF4 elutant so they could be used to calibrate the intensity signals from the HR-ICP-MS.

A concentrated stock solution containing elements at proportions similar to those in a landfill leachate sample was prepared by dilution of 1000 mg/L single element standards (Inorganic Ventures) in 3% $HN0_3$. This stock solution was then diluted to the required working concentration range with NaCl such that each standard was matrix matched to the AF4 Carrier solution (0.1 M NaCl). All solutions were measured to the nearest µg and concentrations calculated by weight.

A set of 5 standard solutions were prepared with concentrations designed to bracket the range expected for each element in a typical sample, post fractionation. The range was typically from 0.01 µg/L to 5 µg/L. This enabled a 6 point calibration to be constructed for the ICP-MS CPS versus the known standard concentrations. The equation of this relationship was then applied to all the CPS values obtained in order to transform them to concentration values once the previous data treatment steps (Figure 4.2) were accounted for.

4.2.3.3.3 Offline Coupling: Repeatability

Repeatability in this study refers to the variation that would be present if a fractionation run was repeated on the same day, by the same person under the same experimental conditions. Reproducibility would refer to the ability for results to be reproduced in another laboratory.

Due to the time-consuming nature of fraction collecting, repeat runs of offline coupling were not carried out. Repeatability of the instrument measurements was assessed from the Relative Standard Deviation (% RSD) of multiple measurements of the same samples.

4.2.3.3.4 Offline Coupling: Recovery

Recovery for fraction collecting was calculated by conducting a mass balance of the system using the elemental concentrations measured in each fraction (μg/L), and the volume (L) of each fraction (Eq.3). This enabled the total mass (μg) of each element recovered from the channel flow to be determined. The same process was applied to the cross flow. The mass (μg) of each element in the original 1 ml sample injected into the AF4 was also calculated and this enabled a mass balance of the system to be determined.

The total concentration and mass of each element in the original sample was determined from analysis of an aliquot of the initial leachate. Recovery results from this method are shown in Table 4.6.

$$R\% = \frac{\sum(\,(FC1-FCB1)+(FC2-FCB2)\ldots)+(FD-FDB)}{TM} \times 100 \qquad (3)$$

R% = Total Recovered Percentage of an element

FC1, FC2…= Mass of element in each Channel Fraction (μg)

FCB1, FCB2… = Mass of element in each Channel Baseline Fraction (μg)

FD = Mass of element in “ Truly Dissolved” Fraction (μg)

FDB= Mass of element in “ Truly Dissolved” Baseline fraction (μg)

TM = Mass of element in 1ml of sample.

4.2.3.4 Offline Coupling: **Method Summary**

- Optimisation of AF4 parameters
- Preliminary run to determine peak distribution
- Select fraction time intervals
- Collect fractions (baseline and sample)
- Dilute and spike fractions using 3% HNO_3 including an aliquot of initial sample
- Analyse using HR-ICP-MS
- Apply data treatment

4.2.4 Online Coupling of AF4-HR-ICP-MS

Online coupling of AF4-HR-ICP-MS for multi-element detection has been previously described in Stolpe et al. (2005) and Stolpe et al. (2010). Broadly, the method used in this study was approached in a similar way. There are, however, differences in set up due to the flow rates utilised in this study.

The initial intention was to conduct fractionation using the same parameters used for an offline analysis. However, during initial online experiments it was observed that although UV and FLU detector signals returned to baseline levels after 50 minutes (implying that the sample had passed through the system) not all element concentrations had returned to baseline values. In response to this observation the fractionation run time was extended to 120 minutes. This was to enable any elements (dissolved or particulate) remaining in the system to elute and therefore reduce possible sample cross contamination and provide reliable mass balances.

4.2.4.1 Online coupling: AF4-HR-ICP-MS Interface

Hassellov et al.,(1999) originally proposed dilution of the AF4 elutant with HNO_3 and use of an internal standard to optimise performance of the system. Acidification of the sample encourages better dissolution of many analytes and improves washout characteristics. The addition of internal standards allows instrument performance and sample interaction to be monitored (and where appropriate corrected). While many studies (Lyven et al., 2003, Alasonati et al., 2006, Bouby et al., 2008)) have used and developed this methodology, some studies have continued to interface instruments without sample acidification and dilution (Dubascoux et al., 2008b, Worms et al., 2010), thus restricting quantification of the data.

The low channel flow rate of 0.3 ml/min used in this study to overcome pressure issues (See Section 3.3.4.2) was particularly suited to direct interfacing because the flow rate was comparable to sample uptake rates typically used for solution analysis by the HR-ICP-MS (e.g. the 0.24 ml/min used in this study). Although the overall AF4 system flow rate was compatible with the typical uptake on the HR-ICP-MS, the channel elutant required further modification to address TDS and dilution.

Figure 4.3 shows a schematic diagram of the interface between the AF4 and HR-ICP-MS. After eluting from the channel the elutant passed through the UV-DAD and FLU detectors. At this point, the flow rate was 0.3 ml/min but the NaCl concentration (and therefore TDS) was considered to be too high for direct ICP-MS analysis. To address this problem the flow was passed through an adjustable T- piece where it was diluted 10:1 with 3% HNO_3. This step also enabled the flow to be spiked with the same internal standards used previously (Be, In, Re at 20, 10, 10 ppb, respectively).

As a consequence of this dilution stage, the overall flow rate was increased to a level that was too high (in the region of 3 ml/min) to pump directly into the HR-ICP-MS. To address this issue, an adjustable microsplitter valve was fitted to divide the output, so that the optimal flow (0.24 ml/min) could be pumped into the HR-ICP-MS. The excess flow went to waste, but it could easily have been diverted for other measurement or collection purposes. The output flow rate entering the HR-ICP-MS was monitored daily and the splitter valve adjusted as required to account for any fluctuations and to ensure that the flows remained constant.

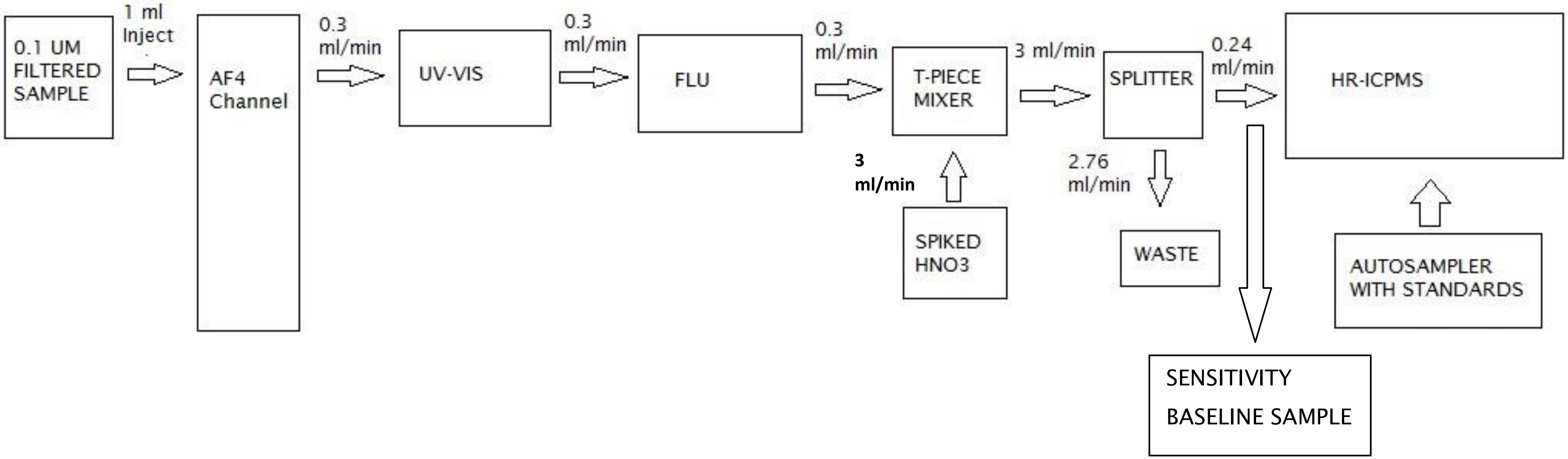


Figure 4.3 Schematic Diagram of AF4-HRICP-MS Online

4.2.4.2 Online Coupling: Software Set Up

In order to obtain more detailed information on elemental associations with particle size fractions than is possible with offline analysis, the measurement frequency for each element over the fractionation period was maximised. This also enabled direct comparison of the elemental signatures with the UV and FLU signals. Strictly speaking, however, it is not possible to get a continuous measurement of each element using HR-ICP-MS because the elements are analysed sequentially. However, to get as close as possible to a continuous measurement of each element throughout the fractionation run, the operating parameters of the HR-ICP-MS were optimised to measure a complete 'sweep' of all the analytes of interest as often as possible (Table 4.3). To achieve the fastest scanning frequency, elements in all resolutions were assigned the smallest mass windows and fastest sampling rates that could achieve acceptable data quality. This was done by trial and error, until a best compromise was identified.

Table 4.3 Online HR-ICP-MS Analysis Parameters

	Low Resolution	**Medium Resolution**	**High Resolution**
Acquisition mode	Mode 2	Mode 2	Mode 2
Mass Window (%)	20 for Li & Be, 10 for all others	100 for Be & Mg, 50 for all others	125
Search Window (%)	25 for Li & Be, 10 for all others	100 for Be & Mg, 50 for all others	60
Integration window (%)	25 for Li & Be, 10 for all others	20 for Be & Mg, 50 for all others	60
No. Sample per peak	40	40	20

For online AF4 analysis, the Element 2 XR software was operated using the "Mode 2" scanning cycle (as first used for AF4-HR-ICP-MS analysis by Stolpe et al.,(2005)) instead of the "Mode 1" used in offline analysis. In this mode, the resolution was alternately switched after each sweep of analytes to achieve a time-resolved measurement. In this way, a complete sweep of all LR and MR analytes could be achieved and reported in the smallest time interval. In practise this was about one complete elemental sweep every 8 seconds. During initial experiments a HR sweep was also included to analyse for K and As.

However, the additional time required to accommodate a third resolution switch was excessive and disproportionate given the limited amount of extra data acquired. It was therefore decided to analyse the HR elements in a separate, dedicated run. Data were acquired using the Element 2 XR's inbuilt CHROM package. This provides a chromatogram-style display of analyte signal versus time and allows the data to be exported as a spreadsheet containing a matrix of time slice versus. analyte intensity.
The overall HR-ICP-MS analysis time was extended to be slightly longer than the AF4 fractionation time to ensure that a signal was present before injecting a sample into the AF4.

4.2.4.2.1 Online Coupling: HR-ICP-MS Tuning and Conditioning

All instrument operating parameters were optimised for maximum sensitivity, signal stability and low oxide levels using a 0.1 ppb mixed element tuning solution.

During method development and early observations it was noted that the sensitivity of the HR-ICP-MS was generally unstable for the first hour after interfacing with the AF4. As is the case with most saline analyses, this was presumed to be caused by changes and a gradual conditioning of the ICP-MS interface region as it adjusted to the high NaCl content. It is thought that as NaCl deposits build up on the cones the gas flow dynamics and pressure between the sample and skimmer cones changes, resulting in changes in sensitivity and stability (Montaser, 1998).

To encourage consistent interface a 'conditioning' solution with a similar matrix to that being analysed during the AF4 runs was injected into the HR-ICP-MS for 1 hour prior to any sample analysis (Sturrock et al., 2013). The solution used had the same matrix as the carrier solution (0.1 M NaCl) diluted ten-fold with internal standard spiked 3% $HNO_{3.}$ Following this conditioning period the signal stabilised. At this point the mass offsets (required for MR and HR measurements) were checked and adjusted if necessary.

It was important that the NaCl content of the solution reaching the HR- ICP-MS remained constant during a run, otherwise undesirable changes may occur.

4.2.4.3 Online Coupling: Internal Standards

Ranville et al.,(1999) explained that internal standards can be used to correct AF4-ICP-MS signal for baseline drift, artefacts and unusual signals. Internal standards were used in this study to monitor instrument drift and also the flow rate from the AF4.

Be, In and Re were selected for use as internal standards because they are widely applied in ICP-MS, had previously been used with high TDS samples (Sturrock et al., 2013), are well spaced across the mass range and were elements that were not expected to be present in high concentrations in landfill leachates (Öman and Junestedt, 2008).

During the method development phase, measurement of the internal standards by the HR-ICP-MS showed that the signals varied systematically and repeatably through the run. Initial investigation suggested that the flow rate must have dropped in the second half of the run. However, this event coincided precisely with the point where the cross flow of the AF4 was turned off (50 minutes). To examine the implications of this observation in more detail, the actual flow rate was measured by collecting fractions and weighing them to calculate the total mass collected (Figure 4.4).This experiment demonstrated that the AF4 flow rate was not constant during the run. This implied underperformance of the cross flow pumps resulting in an increase in the AF4 flow rate once the cross flow was removed (and hence greater dilution of the internal standards). This problem was corrected by applying a pump correction to the tip and focus pumps (after consultation with the manufacturer) so that a constant flow was achieved. This observation highlights the importance of using internal standards – otherwise this flow variation might not have been observed or corrected for.

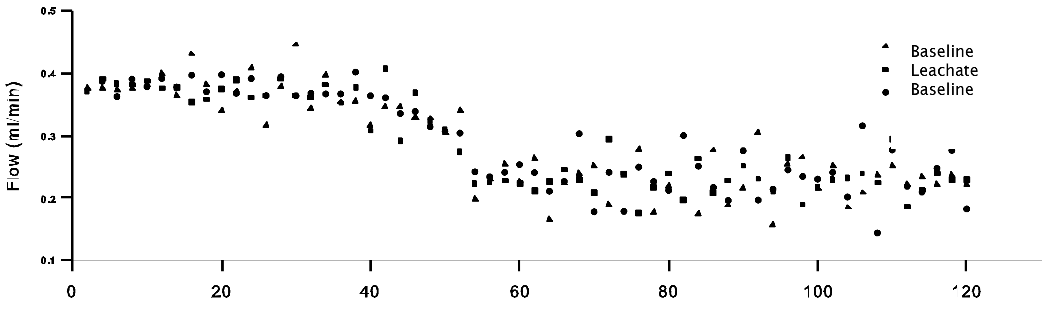


Figure 4.4 Flow Rate Check for Sample and Baseline Runs

Initial trials in this study found that neither Be or In were suitable for use as internal standards when added to the carrier solution because they interacted in some way with the sample. Figure 4.5 shows an increase in the intensity of the Be and In signals coinciding with an increase in the concentration of organic matter (UV intensity), suggesting that both Be and In interact with organic particles in the sample. Re did not show a similar interaction with the sample. It must be noted that a dip in signal is visible in Re at the point at which the cross flow is turned off because these experiments were carried out prior to the tip pump correction mentioned above. In order to eliminate any possible sample interaction artefacts, it was therefore decided to introduce the internal standards into the diluting acid after the sample had passed through the AF4.

An additional benefit of spiking the diluting acid (3% HNO_3) was the ability to monitor the flow rate out of the AF4. Any variation in the intensity of the internal standards implied a change in the output flow rate of the AF4. The diluting acid was spiked with 20 ppb Be, 10 ppb In and 10 ppb Re. The mixing ratio of spiked acid to the outflow of the AF4 was 1:10, with 0.3 ml/min of AF4 flow being met with a 3 ml/min flow of diluting acid (see Figure 4.3).

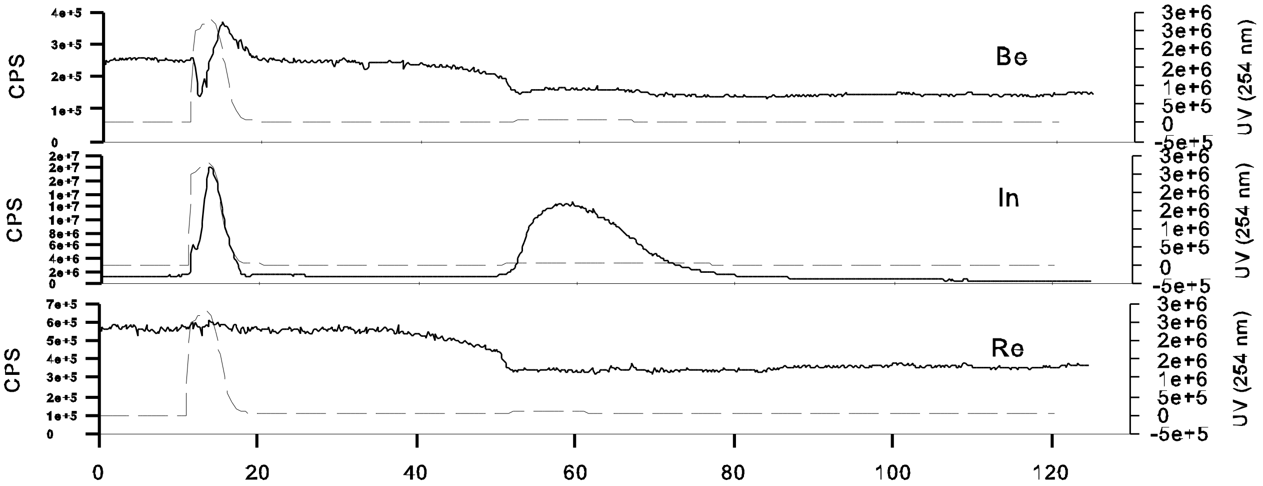


Figure 4.5 Be/In/Re signals for online fractionation with spiked carrier solution

All 3 elements were originally intended as internal standards to correct for instrument drift but due to the low intensity of the signal of Be in MR and HR, and for calculation simplicity, In alone was used as internal standard. In was chosen over Re because it is more central in the distribution of the masses analysed. Together, the internal standards were used to simultaneously check the AF4 flow stability and HR-ICP-MS performance, but only In was used to mathematically correct for changes in instrument sensitivity.

The flow rate from the splitter valve to the HR-ICP-MS remained constant at 0.24 ml/min, as did the flow rate of the diluting acid at 3 ml/min. The variation in the intensity of Be, In and Re was therefore indicative of any system-related variation and could thus be used for correcting instrument and/or matrix-related drift.

4.2.4.4 Online Coupling: Sample Analysis

For several reasons (principally large sample volumes and incomplete flushing of all parts of the AF4 system), there was a possibility of sample carryover from one separation run to the next. Wahlund (2013) suggested that a residual peak (observed after the cross flow is turned off), was ascribed to remobilization of material that was previously absorbed by the membrane unless a high cross flow and low channel flow were used. With those conditions, the residual peak was suggested to be caused by hydrodynamic immobilisation of particles due to high cross flow. Therefore in this study, immobilisation of larger particles was expected until the flow was removed and thus the particles are mobilised and able to elute from the channel.

In order to determine if particle carryover after sample injections was present with the parameters in this study (Table 3.6), baseline concentrations in the system before and after sample injection were examined. Separation run analyses were already extended to 120 min to minimise the effects of sample carryover as noted in Section 4.2.4 and to include elution of all sample particles within the analysis time.

To establish a baseline concentration of the elements of interest in the system, 1 ml of carrier solution was injected and analysed in the same way as a sample normally would be. 1 ml of sample was then injected and analysed, followed by another carrier solution injection. Although baseline measurements of elements might be expected to return to levels prior to the injection of the sample, some baseline variation was seen to occur, indicating that some particle carryover occurred and thus a new system baseline was established for the next sample injection. Variations in sample baseline may also be due to sensitivity (See Section 4.2.4.5.4)

To fully account for possible carryover effects, a baseline run was conducted before each sample injection to monitor cross-contamination and also to establish the background levels in the complete AF4 and HR-ICP-MS system corresponding with that sample. This procedure also provided an indication as to whether various components of the AF4 system (e.g. membrane and tubes) became contaminated as more samples were processed through the instrument and acted as a rinse step (allowing extra time for potential residual particles to elute) in between samples. Baseline data is discussed further in 4.2.4.5.4 and illustrated in Figures 4.13 and 4.14.

4.2.4.5 Online Coupling: Data Treatment

Several data processing steps were explored to obtain quantitative results from the raw data and to evaluate the method (Figure 4.6 Data Processing Steps for online AF4-HR-ICP-MS DataFigure 4.6).

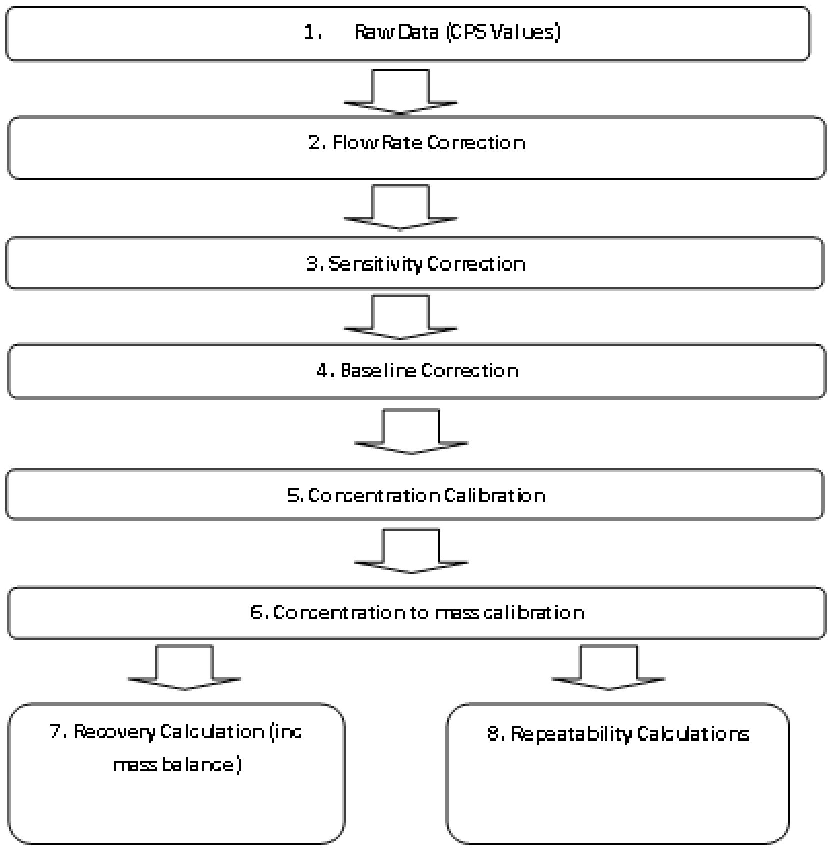


Figure 4.6 Data Processing Steps for online AF4-HR-ICP-MS Data

4.2.4.5.1 Raw Data

The raw data was exported from the HR-ICP-MS software in the form of CPS versus time for each element throughout the run, similar to the example shown in Figure 4.7. Before performing any further corrections, the raw data was examined by plotting time versus CPS to check that the run had completed correctly (i.e., no flow blockages, sensitivity was stable, etc.). These were identified by unusual fluctuations in internal standard CPS data. A drawback to the online data collection software was that it was not possible to track the element data as it was being measured because the data capability required was beyond that of the PC used. Therefore any problems with the AF4-HR-ICP-MS system could not be detected until this stage was completed, except those in the AF4 only, which were visible with the UV-Vis or FLU detectors. It was, however, still possible to make sure data were being collected using the HR-ICP-MS software “SHOW” setting which displayed individual element peaks or the entire mass range in real time (one resolution at a time).

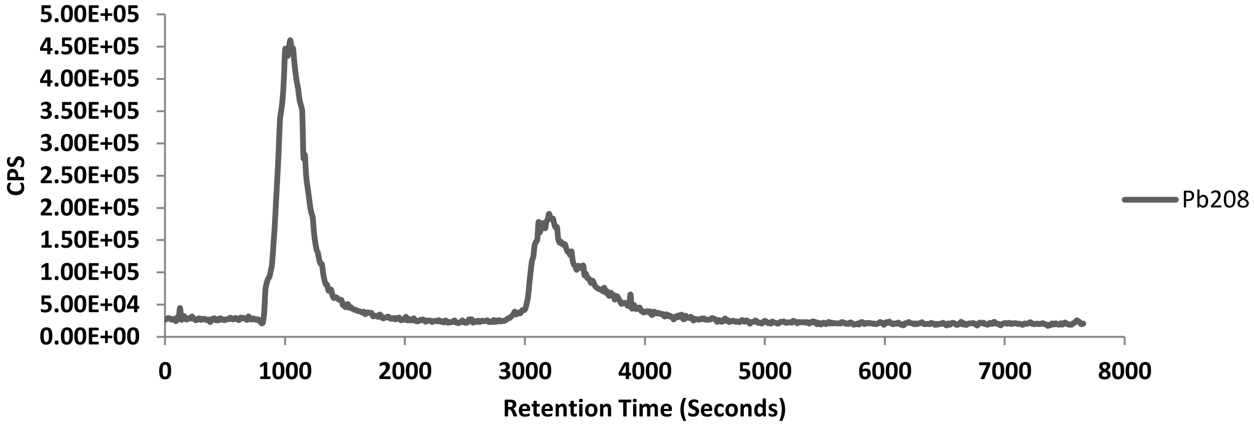


Figure 4.7 Example of Raw Time v CPS (Pb) data plot

4.2.4.5.2 Instrument Sensitivity Corrections

The first correction applied to the raw data was an internal correction. Given that the flow rate from the AF4 and the online spiking/dilution was constant (see section 4.2.4.3), it would be reasonable to expect the intensity of the internal standards to be consistent throughout the run. Any variation from this implies a measurement artefact in either the AF4 or the HR-ICP-MS measurement. Using an internal correction intensity (and the assumption that the intensities should be constant), it was possible to correct these variations and apply them across all CPS values. In practise, for example, the In signal was corrected to a constant value throughout the run by normalising subsequent In time slice signals to the first time slice value. The factor determined for each time slice is then applied to all other analytes. This is standard practise for most elemental analysis by ICP-MS (Montaser, 1998).

4.2.4.5.3 Instrument Drift Correction

Throughout (and between) each measurement period (sometimes over 24 hours), the HR-ICP-MS sensitivity varied. Figure 4.8 shows an example of sensitivity variation over a typical measurement period (Table 4.4) as monitored using the In internal standard signal during each 120 minute analysis.

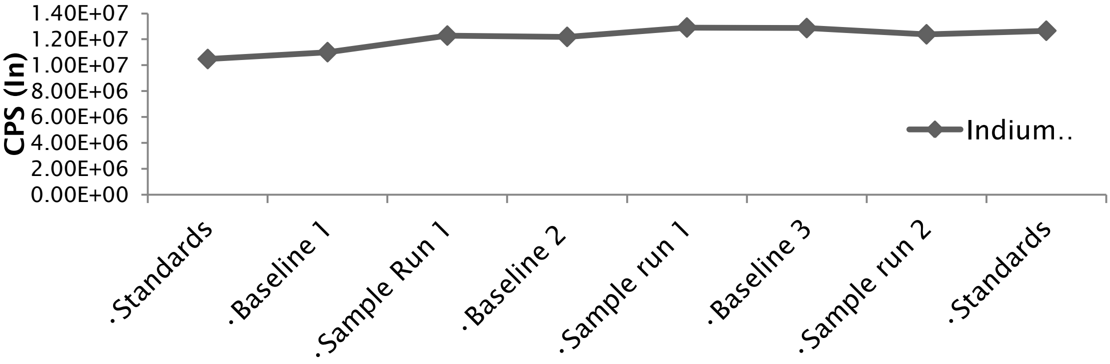


Figure 4.8 Sensitivity Variation over a Measurement Period

So that data collected at different times within each measurement period (or between measurement periods) (Table 4.4) could be directly compared, a sensitivity baseline sample (SBS) was introduced.

The SBS was a sample of diluted elutant collected at the point where the flow would normally enter the HR-ICP-MS when connected (Figure 4.3) but was instead diverted to a 20 ml vial. This was collected at the beginning of a measurement period prior to the connection of the AF4 to the HR-ICP-MS, immediately after the ‘conditioning’ phase.

As the concentration of internal standard in the diluting acid did not change during the measurement period (the diluting acid was made up in 20 L bottles), the CPS values of the internal standards as measured at the start of the session (in the collected SBS) should not vary throughout the session unless the system sensitivity varies. A correction factor derived by comparison of the internal standard CPS value in the SBS to the CPS value during subsequent runs allowed for all runs during a measurement period to be compared and corrected for sensitivity variation. The same correction factor was applied to all elements and a factor was determined for all resolutions using the In signal of the corresponding resolution.

4.2.4.5.4 Baseline correction

To ensure that the elemental concentrations (and subsequently the recovered masses of elements within a sample) could be fully quantified, the contribution of the system baseline to the sample signal was investigated to establish the most appropriate method of correction.

Figure 4.9 shows three baseline profiles analysed prior to consecutive samples within the same measurement period. It is clear that the baseline signal (for Sn, in this example) varied during this measurement period with Baseline 1 showing over 60,000 CPS and Baseline 3 recording under 40,000 CPS. Given this variation, it was therefore necessary to establish a baseline correction for each sample run rather than use a single baseline correction for an entire measurement period. To exemplify the importance of between-sample baseline runs, the peak around 32 minutes in baseline 2 shows contamination in the system which could potentially be from particles attached to the tubing or the membrane that were subsequently remobilised during later runs. The reduction in CPS after the peak suggests that the contamination quickly left the system.

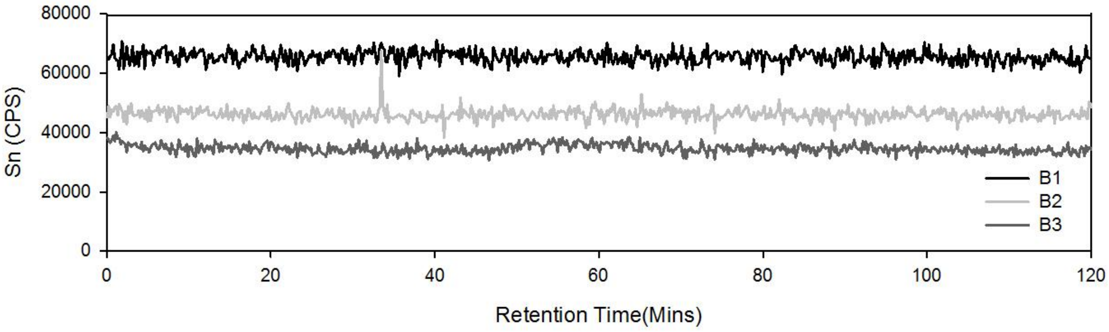


Figure 4.9 Baseline signals for Sn during a measurement period

The last 70 minutes of each fractionation run were without cross flow, so the majority of particles and contamination in the system should be eluted by this point. Some small peaks were seen at 50 minutes (if any were present at all) for some elements, corresponding to the time when the cross flow was turned off and larger carryover particles (which were not sufficiently eluted in the 120 minute elution time) were able to freely elute from the channel. Background signal intensities typically reduced towards the end of the run, indicating that any carryover particles had eluted from the system in the baseline run.

To establish a representative baseline value for subtraction from the subsequent sample runs, the mean CPS value for each element during the last 20 minutes of a baseline run was used. This was because less fluctuation and fewer spikes were observed during this time. A mean of the whole baseline period would be unrepresentative because it would include carryover particles which had been eluted and were no longer in the system.

The baseline CPS value was subtracted from the sample CPS values so that only the contribution of the sample was used for the concentration calibration. Due to the varying sensitivity, detection limits and concentrations of the elements, the contributions of the baseline to the sample signal varies for different elements and therefore impact the detection limits, precision and accuracy of individual element concentration measurements.

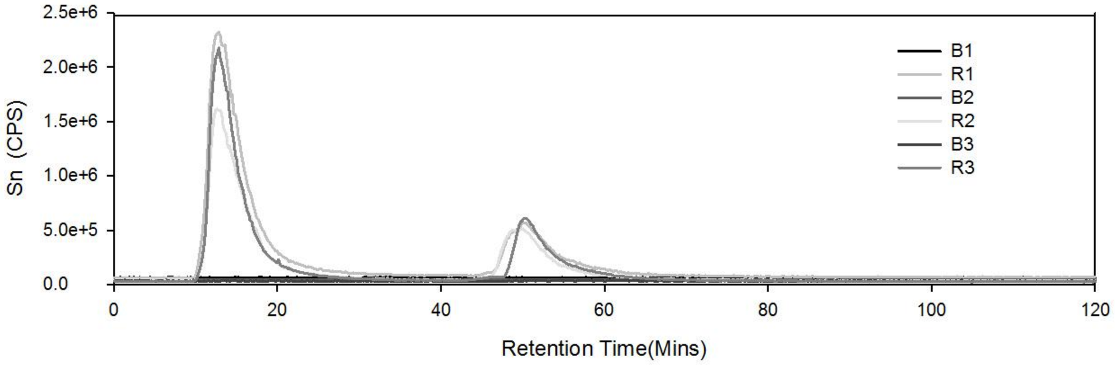


Figure 4.10 Baseline and Fractionation run signals for Sn

Figure 4.10 shows the variation in CPS for Sn during the course of a run. In this case, the maximum CPS values were approximately 2000x higher than the baseline values, so the effects of the baseline on the overall concentration are minimal. Nevertheless, the contribution of the baseline to the sample signal must be investigated and accounted for in all runs and throughout each run for every element. Figures 4.13 and 4.14 illustrate the baseline and sample signals for all elements under consideration.

4.2.4.5.5 Concentration Calibration

Dubascoux et al.,(2010) and Lesher et al.,(2012) reviewed AF4-ICP-MS calibration techniques and demonstrated that 2 types of approaches to this problem were common; (1) Injecting a small, quantified element concentration after the AF4 channel, just before the ICP-MS and then integrating the corresponding peak area with the peak area of the sample to establish the relative proportions, and (2) injecting larger volumes with known concentrations after the channel, just before the ICP-MS to create flat topped peaks with continuous signal and then transforming that signal to concentrations.

To apply these strategies to our experimental setup, both of these methods involve the reference standards being diluted (if acidified) at the point where the eluted flow from the AF4 would be diluted (mixer valve, Figure 4.3) with the standards then flowing through the splitter valve. Although an advantage of this approach is that the standards are treated in the same way as the sample, the major disadvantages are that high concentration standards may contaminate the interface tubing. For the second method a large volume of each standard is required. Even with peek tubing, in initial set up experiments it was found that after high concentration injections, an elemental signature remained in the system tubing after the solution had left the system. Therefore sufficient wash time is needed in between standards for signals to reach previous baselines. This washout time would thus increase the analysis times and require large amounts of additional reagents.

The type of online analysis of standards previously reported also means that exact dilution of the sample is unknown. Hence, there are implications for accurate calibration of the sample data. If the samples have not been acidified and flow directly into the nebuliser then the standards will not be matrix matched with the samples, which also means accurate and precise calibrations cannot be performed.

An alternative approach to overcoming the contamination, dilution and matrix matching issues is to use standards run offline using an autosampler during the same measurement period. The tube connecting the splitter valve to the ICP-MS nebuliser was disconnected and connected to the autosampler probe. A range of synthetic standards were analysed in the normal way using the same operating parameters as discussed previously. Standards were freshly made for each measurement session and diluted with the same spiked diluting acid used for the online samples. In this way the equivalent internal standard intensities could be used to cross-calibrate the on-line and off-line measurements.

Multi-element standards were prepared (as described in 4.2.3.3.2) at concentrations 10 fold higher than those used for the off-line analyses. The standards were then diluted to the required level using the same spiked HNO_3 used to dilute the AF4 flow after elution, thus mimicking the online dilution of the sample. Standards were run at the beginning and end of each measurement session (Table 4.4).

Table 4.4 Typical Order of HR-ICP-MS Analysis in a measurement period

Analysis Order	**Sample**
1	Conditioning solution
2	Multi-element standards- offline
3	Baseline run 1
4	MSW Sample run 1
5	Baseline run 2
6	MSW Sample run 2
7	Baseline run 3
8	MSW Sample run 3
9	Multi-element Standards- offline

The multi-element standards run included the sensitivity baseline sample (see section 4.2.4.5.3). The initial run of standards were used for calibration. The repeat standards measured at the end of a session were used to ensure that the sensitivity correction was working [i.e. they were treated as unknowns].

4.2.4.5.6 Repeatability

Only a handful of previous studies (Whitley, 2012, Bolea et al., 2010, Stolpe et al., 2005) have discussed the use of multiple runs to check that fractograms are repeatable and that the method yields data with defined precision. This lack of data quality evaluation may be due partly to the lengthy analysis time of an AF4 analysis, such that a single fractionation run is deemed to be acceptable. As a consequence, many studies do not attempt any assessment of the repeatability of their data. Nevertheless, for a method to be considered robust and the results to be reliable, the repeatability of the data generated by any method must be assessed.

Most studies coupling AF4 and ICP-MS have used peak areas (UV or ICP-MS signals) to analyse repeatability by calculating the variation in peak areas from repeat analyses. Assessing peak area alone, however, does not take in to account variations in the retention time and therefore the size of the particles. For example, two profiles could have identical peak areas but different profile shapes. Assessing peak areas is therefore a measurement of the repeatability of the composition of particles and the retention time is a measurement of the fractionation process. In order to evaluate the repeatability of the method, variations in peak areas and retention times should be evaluated. Thus, a true measure of the repeatability of elemental concentrations and associations with specific particle sizes can only be accomplished by considering retention times and peak areas together.

The parameters in this study were optimised for small, low molecular weight particles in landfill leachates and therefore the method precision was assessed by comparing the peaks during cross flow. The total peak areas of all the peaks were assessed, but the repeatability of peaks outside of the calibration range (> 63 kDa) were not assessed individually. Peak integrations and peak retention times were calculated and identified using Origin 9.1 software (OriginLab, Northampton, MA) with the baseline for integration set at zero.

Replicate dilutions of the bulk filtered sample were also analysed in order to assess the repeatability of these analyses. Results from replicate sample injections can be found in Figures 4.13 and 4.14 and Table 4.5.

4.2.4.5.7 Recovery

Lesher et al.,(2012) reported that even when using an optimised system recovery is often <100% and that the recovery rate can also be particle size dependent. It is therefore important to report quantified recovery rates when analysing the results. Due to the high fractionation field strength in this study, it was expected that sample losses would be high because of the compression of the sample against the membrane (Giddings and Caldwell, 1989).

Previous studies (Dubascoux, 2008c and Neubauer, 2011) assessed recovery rates using only the UV signal and therefore only the organic particles detectable at the chosen wavelengths were analysed. Stolpe et al.,(2005) recognised that this method of recovery calculation was incomplete and discussed the need for a more robust method to determine recovery rates and that the mass balance of elements should be addressed. Recent studies have integrated peak areas of each element fractogram rather than the UV signals, in order to calculate a mass balance and found that a proportion of sample was not recovered from the channel.

All previous studies have calculated recovery rates by comparing the signal eluted from the channel when no cross flow is applied (and therefore no sample is passing through the membrane) with the signal obtained when cross flow is applied (i.e. when the dissolved fraction passes through membrane). This method does not account for losses inside the system or for the presence of any of the “truly dissolved fraction” that passes through the membrane when no cross flow is applied.

Bolea et al.,(2006) found that using this method indicated that >50% of most metal ion species were lost during field flow fractionation. These low recoveries were attributed to permeation through the membrane of the truly dissolved species, but losses within the system were not considered. Wahlund (2013) recognised that the issue of sample loss by adsorption to the membrane needed to be addressed and that it was not possible to assess such losses without separating them from those in the truly dissolved fraction (passing through the membrane) but no investigation in to these losses were made.

In this study, it is important to distinguish between the truly dissolved fraction and any sample sorbed to the membrane or to the system tubing, so that any lack of recovery is fully quantified.

In order to quantify the colloidal (channel) fraction, the truly dissolved (cross flow) fraction and losses within the system, both the channel elutant and cross flow were collected throughout a sample fractionation. These fractions were collected in 125 ml bottles and treated in the same way as offline collected fractions (4.2.3.2) using the HR-ICP-MS parameters listed in Table 4.2. A recovery percentage (within a 120 min period) for each element was obtained as well as a distribution between dissolved and particle fractions using equation 4, below. The total concentration, and therefore mass, of each element in the original sample was calculated from analysis of an aliquot of the leachate measured in the same sequence as the fractions. Baselines were quantified in the same way and intensities of the baseline channel and cross flow fractions were subtracted from the sample intensities prior to calibration.

$$R\% = \frac{(TC-TCB)+(TD-TDB)}{TM} \times 100 \tag{4}$$

R% = Total Recovered Percentage of an element

TC= Mass of element in Total Channel fraction (μg)

TCB= Mass of element in Total Channel Baseline fraction (μg)

TD = Mass of element in Total “Truly Dissolved” fraction (μg)

TDB = Mass of element in Total “Truly Dissolved” Baseline fraction (μg)

TM = Mass of element in 1ml of sample.

Recovery masses of the particulate fraction were also calculated using the origins 9.1 software (Origin Lab, Northampton, MA) and values compared with those obtained using the method outlined above. Recovery results are shown in Table 4.6.

4.2.4.6 Summary of Online Coupling Method

- Optimisation of AF4 parameters
- Optimisation of HR-ICP-MS parameters e.g. mass offsets
- Preparation of conditioning solutions and standards
- AF4-HR-ICP-MS connection
- Flow rate checks and HR-ICP-MS conditioning
- Online analysis
- Data treatment for quantification

4.3 Results and Discussion

The results for both offline and online AF4-HR-ICP-MS fractionation of the MSW sample are presented and discussed here in terms of the method development. Interpretation of the results in the context of landfill leachate study will be discussed in Chapter 5.

4.3.1 Offline Fractionation

Figure 4.11 and Figure 4.12 show the results for offline fractionation of a baseline (grey shaded area) and MSW sample using conditions in Table 3.6 (except for run time which was 50 minutes). The figures indicate the concentration present in each time resolved fraction of the sample and the corresponding baseline. As discussed in section 4.2.3.3.1, the baseline for each fraction was subtracted from the corresponding sample fraction so that the sample mass could be quantified (mass calculated using sample concentration and volume of collected fraction).

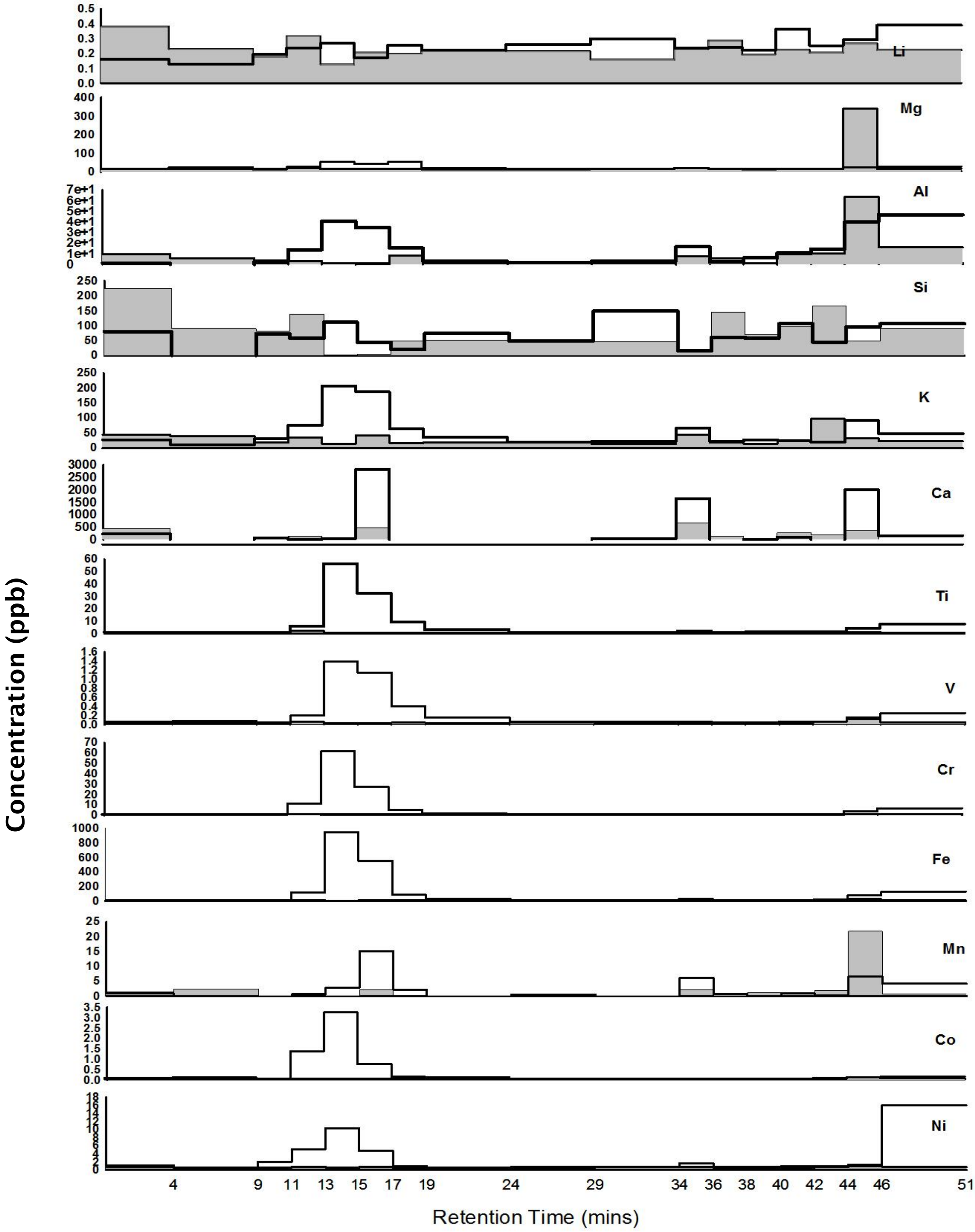


Figure 4.11 Baseline and Sample fraction concentrations of elements Li- Ni (Mass order) collected using offline fractionation

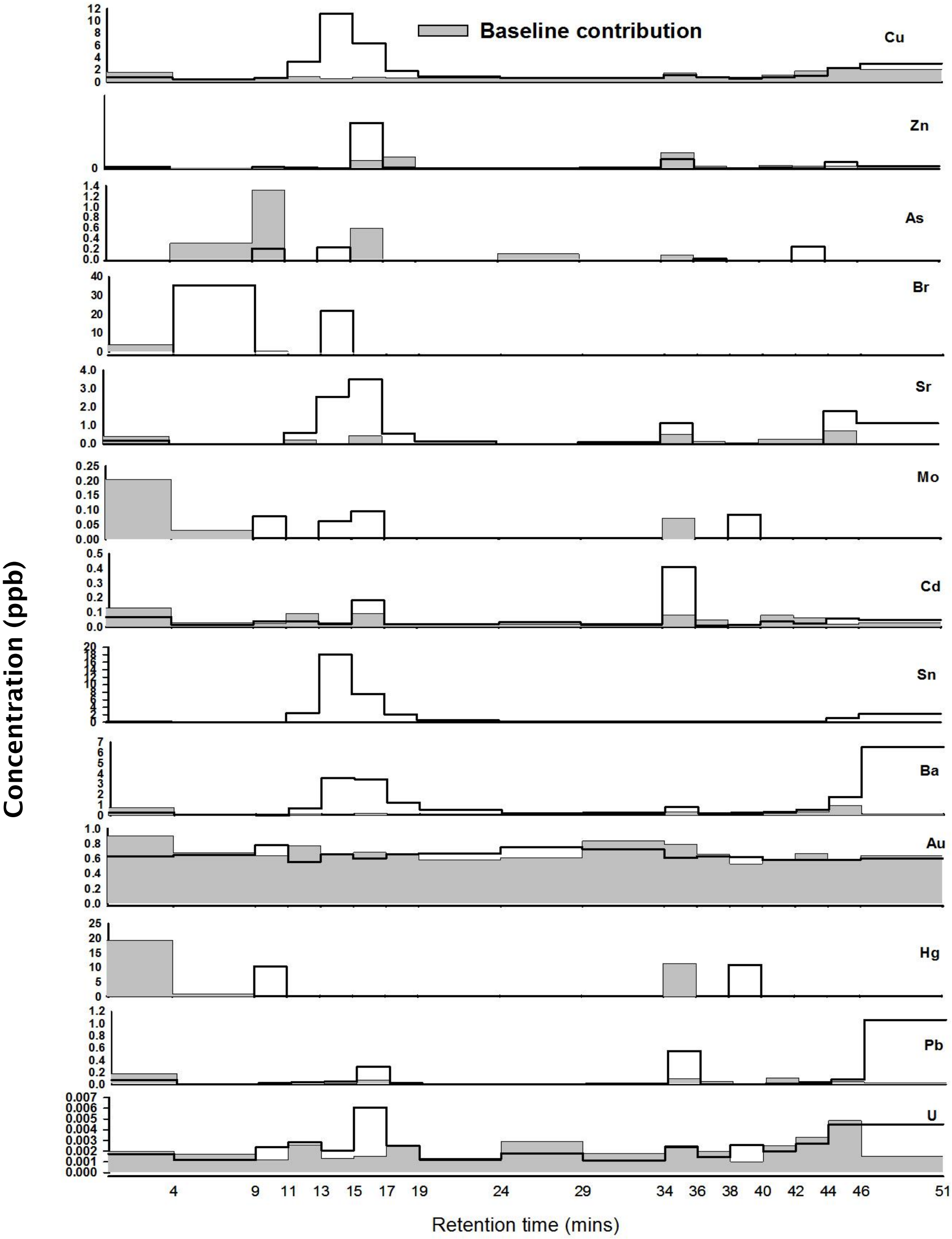


Figure 4.12 Baseline and Sample fraction concentrations of elements Cu- U (Mass order) collected using offline fractionation

Observations from these data were:

- The baseline fraction concentrations for offline collection varied across the time period of a fractionation run (50 minutes) and the percentage contribution of the baseline to the concentration in the sample varied between elements.
- Li, Mg, Al, Si, K, Mn, Zn, As, Mo, Cd, Au, Hg and U all had at least one fraction in which the baseline concentration was higher than the sample concentration. This resulted in no mass determined for those fractions after subtraction of the baseline fraction.
- Ti, V, Cr, Fe, Co, Ni, Sn and Barium fractions were all above baseline fractions meaning that sample masses can be determined for all fractions.
- During the focusing step (0-10 minutes), there were higher baseline than sample concentrations for Li, Si, Ca, Cu, As, Br, Mo, Au, Hg and Pb
- High concentrations of Li, Al, Si, Fe, Ni, Cu, Sr, Sn, Ba, Pb and U can be seen in the last collected fraction. This suggests that not all of the colloids have been eluted from the system within the 50 minute run time because the concentration would be expected to be back to baseline levels at the end of the run.

4.3.2 Online Fractionation

Figure 4.13 and Figure 4.14 show fractograms for 3 replicates of baseline and MSW sample injections (120 minutes of analysis each, conditions in Table 3.6) collected in one measurement period (Table 4.4). These results are prior to the subtraction of the baseline signal.

Observations from the data were:

- For the majority of elements, the baseline signals were negligible compared with the sample signals.
- Only Si, Cd, Au, and Hg sample signals were difficult to resolve from the baseline.
- There were narrow spikes in the baseline for Ti, Ba and U but these did not occur in each baseline for these elements
- The baseline signals showed little variation across the 120 minute fractionation period other than these spikes.

- During the focusing step, the baseline and sample signals were the same apart from for Hg.
- With the exception of Si, the sample signal returned to baseline levels after 120 minutes.
- Retention times and peak shapes for replicate fractionations for all elements appeared to be in reasonable agreement.
- There was variation however in the intensity of the repeat runs and Run 1 appeared to have the highest intensity of the 3 repeat runs.

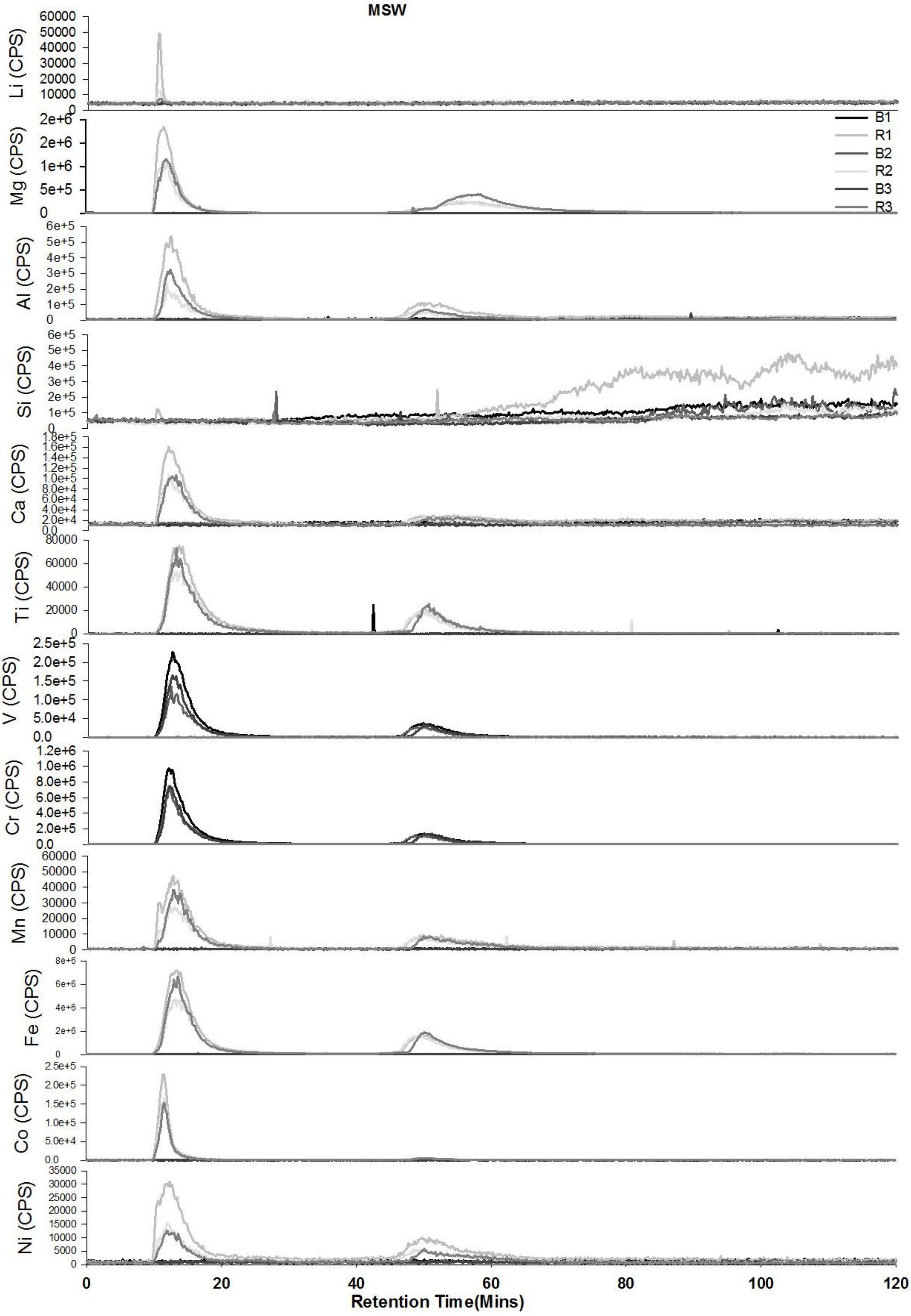


Figure 4.13 Baseline and sample fractograms for elements Li-Ni (mass order) obtained with online AF4-HR-ICP-MS (3 replicates)

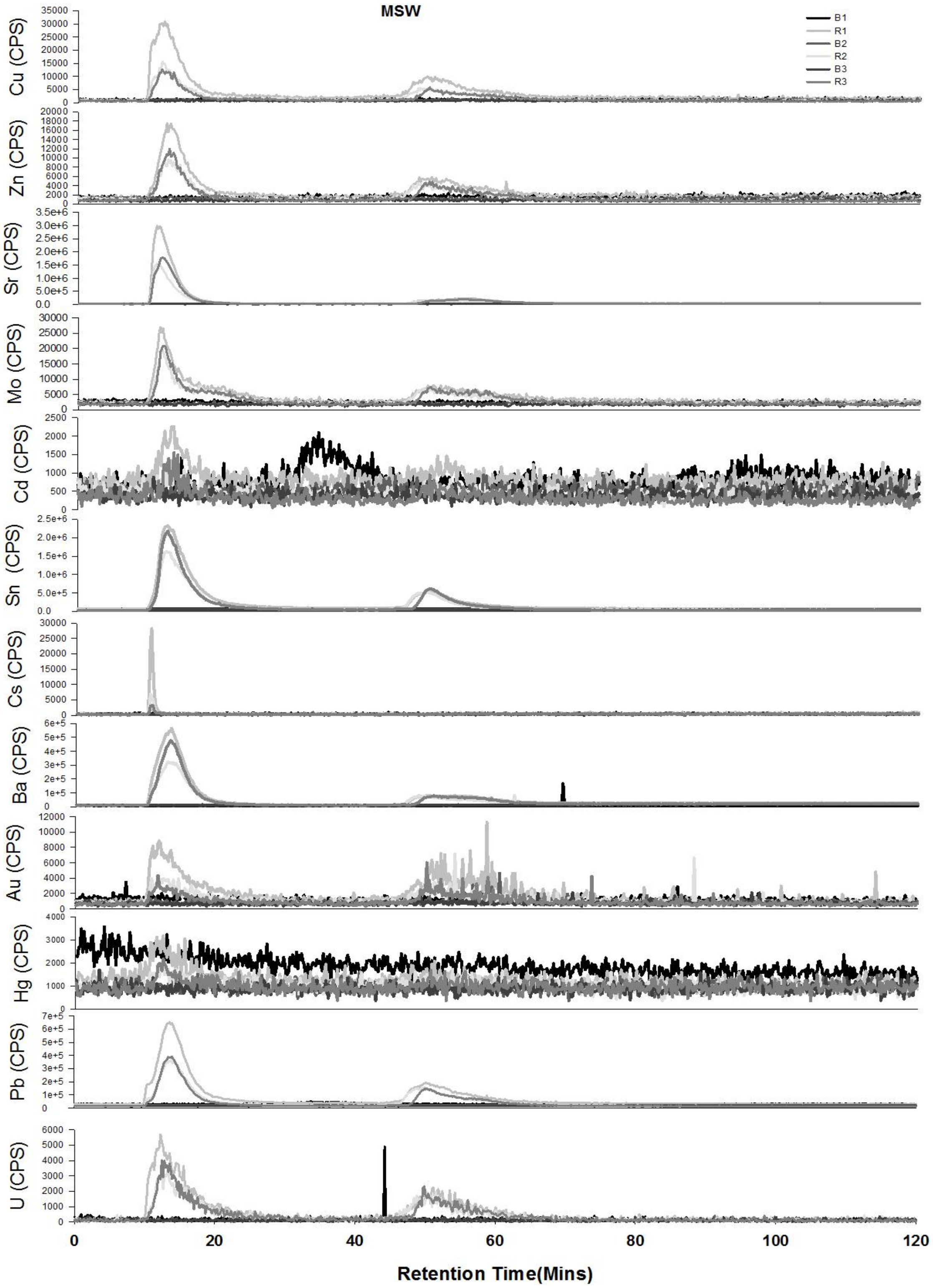


Figure 4.14 Baseline and sample fractograms for elements Cu-U (mass order) obtained with online AF4-HR-ICP-MS (3 replicates)

4.3.3 Implications of Baseline Measurements

The variations in baseline signals were more noticeable in the offline method than online because with the online method, spikes in signal were detected instead of an elevated concentration for a whole fraction. These spikes and elevated fraction concentrations suggested contamination in the system caused by carryover from previous runs.

It was difficult to detect particle carryover with the offline system. The high concentrations for many elements in the last collected fraction observed with offline analysis were not enough alone to indicate that there was particle carryover, due to the UV and Fluorescence detectors reaching baseline levels. It was only when the AF4 was coupled online that the extent of the carryover became clear as elements were seen eluting after the 50 minute period. This demonstrates how powerful the online coupling of AF4-HR-ICP-MS is and also the importance of the use of multiple detectors so that both organic and inorganic particles can be detected. Extending the fractionation time to 120 minutes for the online method showed that with the exception of Si, this was sufficient time for the baseline to return to a stable level for all elements indicating that the particles have eluted from the system.

Geiss et al.,(2013) recognised that system contamination was a problem and in their study the AF4-ICP-MS interface (T-piece mixing AF4 with diluting acid) was disconnected between runs to allow for cleaning of the system. This study was one of the few that recognised the problems that system contamination may cause for quantification however they only investigated Ag NPs so only ^{107}Ag levels were monitored using ICP-MS. Levels were monitored until an acceptable and stable level was reached and only then was a new sample injected. Baseline signals were mentioned in Stolpe et al.,(2005) however only 12 baseline measurements were taken over a 6 month period therefore fluctuations over a smaller time period were not accounted for.

Due to the 28 elements of interest and the range of particle sizes and samples in this study, it was difficult to administer the approach of Geiss et al.,(2013) because there were more element levels to monitor and the contamination was less predictable than that from studies using manmade NP. Monitoring of the baseline after each sample injection to establish the new baseline was therefore a compromise between their method and that of most AF4-ICP-MS studies in which there was no rinse or baseline monitoring.

It is thought that the carryover in the system can be caused by insufficient elution time for larger particles as well as particles attaching to the membrane and tubing and subsequently remobilising. When changing the membrane used with this study, what is presumed to be sample material was observed at the focusing point and appeared as a lightly stained brown area on the membrane (leachate samples used were brown in colour) indicating that attachment to the membrane had occurred and thus, there would inevitably be an impact on recovery rates (See 4.3.5). Wahlund (2013) also observed that sample was seen on the membrane at the point of sample injection/focusing point after several fractionation runs. They noted however, that the presence of sample did not impair the quality or separation or the peak height and retention time and that it appeared that the membrane became conditioned with sample, therefore only affecting the initial runs after a new membrane.

It was clear from initial hook-up studies that after a new membrane and several sample injections, the system baseline concentration increased for most elements although this appeared to stabilise after 5-6 runs. The study by Geiss et al.,(2013) noted that a membrane was useful for at least 50 runs before the efficiency of the separation and the quantification was compromised and that a membrane conditioning step was necessary until the signals observed were stable (presumably when the membrane became saturated with Ag on its sorption sites and the baseline Ag level in the system remained constant).

These recent studies along with the data shown here reinforce the importance of monitoring the baseline in the system as well as conditioning the membrane prior to analysing samples to ensure meaningful analysis and also to determine recovery.

Although the carrier solution elemental concentrations were compared with that of the bulk sample during initial selection tests, it has been shown with the offline fractionation method that concentrations in some sample fractions were lower than that in the carrier solution resulting in no mass being determined for that fraction. This indicates that the concentration of elements in size fractions differs from that of the bulk sample. Without baseline measurements, concentrations of the carrier solution could be wrongly attributed to that of the sample.

The high concentration of Br during the focusing step of the offline method when particles should not be eluting from the system may be due to contamination during the dilution or fraction collecting process rather than from contamination in the system because there should not be any carryover in the system after a baseline. This highlights the risk for contamination from the extra sample handling steps required when using the offline method. There is also the possibility however, that the Br was attached to the membrane or tubes from previous injections and became mobilised during the focusing step.

In summary, the introduction of a baseline run between sample fractionations has shown to be a useful tool not only because of its ability to monitor system contamination but because it also acts as a rinse in between runs so that the system does not need to be disconnected each time. This allows for multiple runs to be completed in one measurement period giving the user confidence that the results are faithful and providing accurate sample concentrations.

4.3.4 Repeatability of Online Fractograms

Table 4.5 shows the repeatability results for the online replicates in Figure 4.13 and Figure 4.14 after baseline correction. Repeatability is reported as % RSD values for the retention time of peak 1 and for the total peak area (representing the colloidal fraction) (Section 4.2.4.5.6). The raw CPS values for the peaks are also shown alongside the % RSD values of replicate aliquot analysis of the bulk concentration.

Table 4.5 Repeatability of Online Coupling and Repeat Bulk measurements

Element	**Peak 1 Max Retention Time (% RSD)**[a]	**Total Peak Area (% RSD)**[b]	**Peak CPS** [c]	**Bulk Conc. (%RSD)**[d]
Li	1	113	50,000	11
Mg	2	18	2,000,000	9
Al	3	58	550,000	24
Si	No Peak	169	550,000	4
K	0	11	1,200,000	9
Ca	5	37	160,000	7
Ti	2	16	80,000	9
V	1	30	250,000	9
Cr	1	23	1,200,000	10
Mn	1	23	60,000	7
Fe	56	18	8,000,000	7
Co	1	23	250,000	10
Ni	1	28	35,000	6
Cu	2	64	35,000	5
Zn	1	92	20,000	24
As	3	13	1600	10
Br	0	54	2500	10
Sr	4	15	3,500,000	6
Mo	2	27	30,000	5
Sn	1	18	2,500,000	2
Cs	173	173	30,000	13
Ba	2	34	500,000	10
Pb	1	39	700,000	13

a. Peak 1 retention time maximum identified using OriginsPro software
b. Total peak area calculated using OriginsPro. Represents total colloidal fraction µg recovered
c. HR-ICP-MS counts for peak 1
d. % RSD of 3 aliquots of the initial 0.1 um filtered MSW sample (Measurement precision all < 5% RSD)

Observations from the data:

- % RSD values for the retention time of Peak 1 maximum were highly repeatable with all elements below 5% apart from Cs which was 173% and Fe which was 55% (see below).
- % RSD values for the total colloidal mass (peak areas) were higher and more variable than the retention time values for the same runs, ranging from 15 to over 100%.
- Considering total peak areas Li, Cs and Si % RSD values were over 100%.
- Al, Cu and Zn all had values over 50%. All other elements had RSD values less than 50% with Sr, Sn, Mg, Ti, and Fe below 20%.

- % RSD values of aliquot analyses of bulk concentrations (section 4.2.2) of the sample showed variations between aliquots but these were lower than those of the peak areas.
- Al and Zn showed the highest variation between aliquots of bulk samples but all other elements showed variation in total concentrations less than 10%.
- High peak area % RSD values did not correspond with high bulk % RSD values e.g. Li had 113% variation for peak areas but only 11% for bulk replicate variation.
- Peak CPS values varied from 1600 for As to 1,200,000 for K

The low % RSD values for retention time indicated that the fractionation process was highly repeatable and that replicate injections of sample showed the same particles size distributions even if the element concentrations altered, thus validating the fractionation parameters used. The higher values observed for Cs and Fe retention time variability can be explained by 2 different reasons.

The high value for Cs is explained by the presence of a peak in the first run only. The narrow peak of Cs as indicated in Figure 4.13 suggests that Cs is present in the smallest fraction only (from low retention time) and therefore it may be possible that in the subsequent runs, all of the Cs present was eluted with the cross flow rather than in the colloidal fraction. Due to fluctuations in membrane cut off as noted in Stolpe et al.,(2005) some Cs may have been retained in the channel in the first run but eluted through the membrane in subsequent runs. This will be discussed further in Chapter 5.

The retention time for Fe was also found to be less repeatable than other elements but after inspecting the peaks shown in Figure 4.13, the reason for the variation was that the peak was multi-modal. When analysing peaks using the OriginsPro software, peak maximums were detected but the peak shape was not considered. This reinforces that inspection of the data by eye alongside analysis with software packages is recommended when determining repeatability and analysing results.

% RSD values for the total colloidal concentration calculated from the total peak area were much higher than the retention times indicating that the fractionation process was more repeatable than the detection of the colloidal fraction of elements. The low retention time values show that the peak area variation was likely due to varying element concentrations between sample aliquots (as indicated by the bulk concentration % RSD) or due to HR-ICP-MS response (due to nebulisation and transport of sample to the plasma, atomization of elements in the plasma and transport of ions into mass spectrometer) rather than variations in the PSD. It is important to note that the % RSD values for bulk concentrations indicate the variability of the leachate concentrations measured in the most robust manner therefore the % RSD values for total peak area cannot be expected to archive lower values than those for bulk concentration.

Of those elements with RSD values over 100% for peak areas, Si can be explained by it's presence only in the residual peak of the fractionation. All Si injected within the sample did not appear to elute within the 120 min run (Figure 4.14) and therefore the system was likely to contain carryover particles which would affect subsequent runs and therefore introduce variation. This may also have implications for the recovery of Si.

Li showed high variation for peak areas (173%) but the bulk concentration did not show high enough variation (11%) to explain the variations in the colloidal fraction. Due to the narrow peak occurring early on in the fractionation run (Figure 4.13), Li was shown to be present in the smallest fraction and therefore as shown with the variations in Cs retention times, fluctuations in the cut off size of the membrane may explain the variation in colloidal fraction.

High variations of Al (58%) and Zn (92%) colloidal fractions can be partly explained by their high variations in bulk concentrations of the MSW sample (24% for both) however this does not fully account for the high values. If the variation is not wholly due to the concentrations then other factors must be considered.

Counting statistics are known to influence precision of data and analytical precision improves with an increase in the number of ions counted (Thomas, 2013) therefore this must also be investigated to identify the source of variation. The Al peak CPS value was more than 20 times that of Zn but both showed the same variation therefore counting stats could not be the only factor affecting the variation.

Cu variations of 64% could not be explained by the bulk variation (5%) however the relatively low response of the HR-ICP-MS compared with elements with lower % RSD values suggested that for some elements including Cu, counting statistics were important.

There were other factors which may affect the repeatability of the samples and although these were constrained, there was still the possibility of variations in sample volume injected, sensitivity of the signal, as well as the possibility of the particular aliquot in question containing higher concentrations than expected from the bulk replicate analysis.

The online measurement parameters used (Table 4.3) were optimised to measure multiple elements continuously and therefore search, mass and integrations windows were minimised. This reduced the repeatability of each individual measurements and thus impacted on the repeatability of the element signal. If fewer elements were to be measured, these windows could be widened and thus lower % RSD values would be expected.

As mentioned previously, there are limited repeatability values of similar analyses available for comparison. Stolpe et al.,(2005) achieved better repeatability (<10%) for peak areas of many elements in this study but this was with a 45 ml sample injection. When injection volume was reduced to 10 ml, the RSD values increased to more than double for many elements therefore the repeatability values in this study with a far smaller sample injection of 1 ml are better than might have been expected when considering this data. This is likely due to the internal standard sensitivity corrections, and the inclusion of the baseline applied in this study. It must be reiterated however, that the repeatability of the replicate analyses is limited by the variation of the sample and thus it is important to also quantify the sample variation.

The assessment of the repeatability of fractograms has shown to be an essential tool to validate AF4-HR-ICP-MS fractionation and to accompany element distribution data particularly when quantitative information is required. Even if colloidal concentrations are shown to vary, it is important to report this so it can be considered with the interpretation of the data. It is also important to report the variation of the bulk sample concentrations because this is the limitation on the repeatability that can be achieved with fractionation.

4.3.5 Mass Balance Recovery

Table 4.6 shows the total recovered mass for each element collected using the two recovery approaches (section 4.2.3.3.4 and 4.2.4.5.6). These are compared with the injected mass (determined from an aliquot of the same sample) to establish system recovery. It must be noted that the data in Table 4,6 represent the results from only one baseline and one sample injection for each approach and therefore there are limitations which may have implications for the uncertainty budget of the technique.

Approach 1 uses the offline fraction collection method data to calculate total recovered mass of each element. These calculations therefore consisted of 36 values (17 colloidal fractions and 1 cross flow fraction for the baseline and 17 colloidal fractions and 1 cross flow fraction for the sample).

Approach 2 uses data from the channel and cross flow elutant, each collected in 1 fraction. These calculations therefore consisted of 4 values (1 colloidal fraction and 1 cross flow fraction for the baseline and the same for the sample).

The measurement precision values shown in Table 4.6 are the sum of the analytical variation values for each fraction therefore more values contribute to this value for approach 1.

The LOD values shown in Table 4.6 are based on repeat measurements of fractions collected within a single baseline run and thus reflect the variability of the HR-ICP-MS analysis rather than the variability of the AF4 baseline.

Table 4.6 Detection Limits, Precision and Recovery Values for Fractionation using Offline and Online Parameters (Equation 3 and 4) (one sample injection)

	Approach 1 (Offline Parameters-50 Minute Fractionation Equation 3)				**Approach 2 (Online Parameters-120 Minute Fractionation Equation 4)**			
Element	**LOD[a] (ppb)**	**R^2[b] Value**	**Recovery[c] %**	**Measurement Precision[d] (% RSD)**	**LOD (ppb)**	**R^2 Value**	**Recovery[e] %**	**Measurement Precision (% RSD)**
Li	0.0039	0.9994	**68**	3	0.0101	1	**68**	0
Mg	0.387	0.9983	**51**	1	0.3484	1	**66**	1
Al	0.129	0.9995	**290**	3	0.4596	0.9999	**1766**	1
Si	1.58	0.9986	**16**	27	0.0816	0.9998	**9**	1
K	0.757	0.9997	**113**	1	1.0141	0.9998	**89**	2
Ca	81.3	0.9999	**14**	4	3.8957	1	**193**	1
Ti	0.013	0.9989	**48**	2	0.006	1	**77**	8
V	1.12E-03	0.9999	**14**	18	0.0014	1	**82**	10
Cr	0.021	0.9999	**39**	1	0.0163	1	**64**	1
Mn	0.047	0.9999	**140**	1	0.001	0.9995	**128**	5
Fe	0.381	1	**19**	3	0.0392	0.9999	**128**	2
Co	9.21E-04	0.9999	**73**	2	0.0013	1	**73**	13
Ni	0.016	0.9998	**119**	13	0.1173	0.9997	**164**	46
Cu	0.02	0.9998	**1016**	21	0.0137	1	**223**	12
Zn	0.923	0.9998	**BD**	**BD**	0.5693	0.9983	**98**	70
As	0.0039	0.9982	**46**	74	0.001	0.9999	**77**	18
Br	1.142	0.9993	**105**	3	4.4836	0.9999	**69**	1
Sr	0.0117	0.9997	**1**	2	0.009	0.9977	**62**	3
Mo	0.0001	0.9999	**51**	3	0.0119	1	**268**	6
Cd	4.32E-04	0.9881	**355**	29	0.0013	1	**1550**	2
Sn	2.70E-03	0.9995	**30**	2	0.0145	1	**81**	4
Cs	2.00E-04	0.9999	**102**	3	0.0012	0.9995	**102**	36
Ba	0.006	1	**4**	15	0.0095	0.9997	**3**	18
Pb	2.73E-04	0.9958	**391**	3	0.0044	0.9997	**212**	8

a. LOD= limits of detection = 3 standard deviations of the blank
b. R^2 value of the standard calibration
c. Total mass (µg) recovered in 18 sample fractions and 18 baseline fractions n= 36
d. Sum of analytical error for each measured fraction inc. baseline fractions
e. Total mass (µg) recovered in 2 sample fractions and 2 baseline fractions n=4

4.3.5.1 Approach 1

- Recovery ranged from 1 to 1016% therefore for some elements a higher mass was measured in the recovered fractions than was injected
- The analytical precisions values on these values ranged from 1 to 74% therefore the results achieved using this method were highly variable.

- 9 out of 28 elements showed feasible (<100%) mass balance results using this method.
- Ag was not quantified due to the low R^2 values using both methods.
- Hg and U were not detected in the initial sample so recovery values could not be determined.

Using approach 1 (36 fractions) to evaluate recovery did not provide an accurate mass balance (100%) of the system for the majority of elements and this can be partly attributed to the error introduced from measuring a large number of fractions with low concentrations. The high variation values for As (74%) and Cd (29%) can be attributed to the low CPS values (Table 4.5) for these elements which reduce the quality of their measurement.

4.3.5.2 Approach 2

- Recovery values ranged from 3 to 1776% therefore this approach still showed that for some elements, more was recovered from the channel than was injected.
- 14 out of 28 elements provided feasible (<100%) mass balance results so this was an improvement on approach 1.
- Only Al and Cd, showed recovery values that were more than a factor of 10 out of the expected range
- Mo, Pb, Ca, Mn, Fe, Ni and Cu all showed that more was recovered from the system than was injected even when considering the analytical precision errors and the bulk concentration variability.
- The precision errors for this method ranged from 1 to 70%.
- 14 out of 28 elements showed mass balance recovery within the expected range (0 to 100%).
- Ba and Si had the lowest recoveries (3 and 9%) with all other elements showing at least a 62% recovery.
- Similar recoveries were detected for Li and Cs values using both approaches.

4.3.5.3 Mass Balance Recovery Findings

The lower precision values obtained using the second approach were due to the lower number of collected fractions for the same sample volume (therefore higher concentrations and higher CPS values were measured in the fractions) and this approach is therefore more useful for assessing recovery than approach 1.

Those elements which were difficult to measure above baseline concentrations resulted in particularly abnormal values e.g. Cd 355 and 1550% using both of the methods and so it is clear that carrying out an accurate mass balance with lower signal to baseline ratio and low CPS values was difficult. This also reinforces the importance of measuring the system baseline and is another reason why repeatability values may vary between elements.

Elements which showed that more was recovered from the system than injected can be attributed either to system contamination, variations in injected sample concentrations, analysis and data transformation steps or the contribution of the carrier solution.

Al was found to have a very high (290% and 1550%) recovery in both methods and this suggests that there was Al contamination in the system or during the sample analysis preparation. There was no way however, to know if this was contamination post collection, from within the system or in the sample. Online analysis would show a spike in Al if this was caused by contamination in the system or the sample and therefore online analysis alongside recovery experiments would provide more details on the contamination source.

It is possible that due to the large sample dilution by the carrier solution throughout the AF4 run, some of the metals originating from the injected sample were below detection. Even though the carrier solution was very pure, the contribution to the total mass of elements from the carrier solution is magnified with such a large dilution and this could perhaps explain the >100 % recoveries for some metals.

The LOD values reflect the limits of the HR-ICP-MS rather than the LOD above the baseline therefore to be more relevant to the method, they should ideally be based on multiple AF4 runs. Multiple AF4 runs would likely result in considerably higher LOD values due to variations in the level of contamination in the AF4 system but would reflect the true LOD of the approach and remove the effects of the carrier solution contribution.

It was expected that recoveries using approach 1 would be lower than approach 2 because the fractionation run was only carried out for 50 minutes however there were elements in which recovery did not differ using either 50 minutes or 120 minutes e.g. Li which suggest that the element was only present in the smallest colloidal fraction and is in agreement with previous findings for Li in this study.

Recovery methods previously used have not found recoveries of 100% and this has mostly been presumed to be due to losses through the membrane of the dissolved fraction. Geiss et al.,(2013) did note that the current method for determining recovery rates does not considers losses of material on the membrane or adsorption on tubing walls (see section 4.3.3) and that the current recovery method in which comparing the eluted volume of samples with and without crossflow also presumes that samples losses are equal with and without cross flow.

The mass balance approach presented here has shown that real losses to the system occur. Ba and Si in particular showed that there were high losses of these elements to the system and whilst it can be explained that Si recovery was low because not all Si particles have eluted from the system in the 120 minute period, it was not clear why the recovery of Ba is so low and perhaps may be due to the adsorption to the membrane or tubing.

Giddings and Caldwell (1989) reported that sample loss was highest with high cross flow due to the compression of the sample against the accumulation wall which can cause inter-particle association and adhesion to the membrane. Variations in recovery could also be caused by fluctuations in membrane cut off which occur as the membrane becomes more conditioned(by particle adhesion to the membrane) (Wahlund, 2013). It is possible that the particles absorbed to the membrane not only physically alter the cut off size of the membrane but that inter-particle electrostatic repulsion between the particles on the membrane and those in the channel occur, thus potentially restricting the movement of < 1 kDa nanocolloids through the membrane. These artefacts are likely to increase the variability of fractionation results and the truly dissolved/nanocolloidal distribution.

The recovery method used previously in studies (See Section 4.2.4.5.7) does not account for sample losses due to cross flow whereas the mass balance approach presented here, accounts for the cross flow. As such, this is a more appropriate approach to quantify recovery in the system particularly when high cross flows are used.

Thang et al., (2001) found that AF4 recovery decreased when carrier solution ionic strength and cross flow was increased therefore it may be possible that higher recovery rates may be achieved using different fractionation parameters. It is therefore important to measure recoveries for each set of AF4 parameters e.g. carrier solutions, membranes, cross flow and for each sample.

Stolpe et al.,(2005) highlighted the need to determine the truly dissolved fraction to establish real system losses and to date this is the first study to analyse the cross flow from AF4 to determine this. Although all elements present in the cross flow were presumed to be that which are truly dissolved and in this case, below the 1 kDa cut off, it was possible that during the focusing step, only those elements which were strongly associated with the particles remained in the channel and that because of the high cross flow, a rinsing process occurred.

The high cross flow means that there was a large volume of carrier solution in contact with the sample which not only has implications for the detection of elements but also for the distribution between nanocolloidal and truly dissolved fractions. It is possible that elements which were loosely bound to particles may have been rinsed off when exposed to the carrier solution and because the flow was consistently replenished with new carrier solution this rinsing step could continue throughout the fractionation process. This process is an artefact of the AF4 technique that may result in variability in the recovery and dissolved/nanocolloidal distribution results. This could be confirmed by conducting an ultrafiltration experiment alongside a cross flow collection and comparing the results.

The recovery approach implemented here presumed that the sample aliquot injected, and the aliquot of sample analysed for bulk concentration contained the same concentrations. The repeat measurements of the bulk sample (Table 4.3) however, showed that variations do occur. The approach also presumes that losses in the system were independent of size and that an equal amount of dissolved and colloidal elements are lost in the system.

In order to obtain a more precise mass balance of recovery and dissolved and colloidal fractions, an experiment in which the exact inputs in to the system were known would be required. It is not possible however, to know prior to a run what is expected in the colloidal and dissolved fractions and this makes a mass balance of natural samples difficult. Whilst recovery of a solution of manmade NPs in which the elution fraction was known could be calculated, the results of these recoveries cannot be used to suggest recovery of a leachate sample due to the differences in the matrix and the characteristics of the particles.

So that an accurate and precise mass balance recovery could be calculated, a completely clean system would be needed e.g. new tubing, membrane and in this way an exact recovery of the system could be determined. The influence of membrane conditioning and system contamination however would mean that these results would not be transferable because the fluxing baseline in the system could alter samples whilst conditioning is taking place.

To summarise, determining a rigorous mass balance recovery in the system remains a difficult and ambiguous factor and a challenge to substantive quantification of elements in natural samples. Nevertheless, this method provides an improvement on previous recovery methods and as long as repeatability and details of the methods used are reported and discussed then this information can be useful when interpreting AF4-HR-ICP-MSICP-MS analyses. The finding that a proportion (0 to <99%) of elements are not recovered from the system impacts on previous studies in which any sample not eluted from the channel was attributed to the “dissolved” fraction thus having implications for their research.

4.3.6 Offline v Online Analysis

Offline and online coupling of AF4-HR-ICP-MS have both shown to be successful in providing element distribution information and there are benefits to both methods.

The detection capabilities between the two methods differ and Li, Si, Cd, Au and Hg cannot be quantified using the offline method but Li and Si can using the online method, therefore the online method provides better resolution/detection limits than offline (in this study).

The nature of offline analysis means that fraction collection can be less resource intensive. To complete offline analysis the AF4 and ICP-MS do not need to be in the same laboratory and indeed not in the same institution as fractions can be collected and transported elsewhere for analysis. Offline analysis does not need to be carried out simultaneously and potentially allows for better time management. It is vital however, that all analysis is carried out in trace element clean conditions; fraction collecting increases the risk of contamination because of the increased steps involved in analysis.

Offline analysis was only carried out for 50 minutes because UV and Flu detectors showed that the signal returned to baseline after this period and therefore it was expected that all particles had been eluted in this period. Once AF4 was coupled online with ICP-MS it was clear that not all particles were eluted within this period and this is a drawback with the offline method which could affect reliability of the results.

Offline analysis also enables the user to measure elements in all resolutions simultaneously and provides an indication of the precision of the data that is not possible with online analysis.

On the other hand, online analysis reduces the risk of sample contamination and allows for contamination in the AF4 system and interface to be easily monitored. The online fractograms are much easier to resolve from the baseline and provide clearer visual results as well as more detailed time resolved data.

Although online analysis requires large periods of analysis in one measurement period and lots of equipment set up, once it is set up, it has the ability to generate large data sets in relatively short time periods (compared with offline) and also provides almost instant results making it a much more desirable method than offline analysis.

4.4 Conclusions

This study shows the first approach to quantifying multiple elements associated with colloidal size fractions in a landfill leachate considering the baseline, mass balance of the system and repeatability of the method.

It has shown that fraction collecting and offline analysis is a useful tool for preliminary and complimentary experiments to online coupling however online coupling of AF4-HRICP-MS provides better resolution than the offline fraction collecting method.

The interface between AF4-ICP-MS and the data transformation steps presented in this study have shown to be successful in quantifying element concentration and masses and it is important to report the exact methods so that the data can be assessed by the reader with these in consideration.

Even if element associations with size fractions and other characteristics are not to be quantified, acidification and the use of internal standards are recommended to optimise the sensitivity and stability of the analysis.

The internal standards must be chosen wisely for each sample to reduce interaction and interference with sample concentrations, the carrier solution and the AF4 system.

Retention times determined online are repeatable to within 10% for all elements and the colloidal fraction is repeatable to within 50% for 14 of the 28 elements measured.

It was difficult to quantify Cd, Au, and Hg above baseline concentrations due to low concentrations in the sample but for all other elements baseline contributions using online coupling are neglible.

The baseline concentrations in the system do not remain constant and are in flux due to system inputs therefore the baseline must be monitored to ensure that representative results are obtained and a baseline is recommended between each sample injection.

Fraction collecting the cross flow and channel elutant provided an indication of the mass balance in the system and was a useful method for determining sample losses in the system. It can infer the split between colloidal and dissolved fractions and is recommended to be conducted for each sample and set of AF4 parameters.

Chapter 5: Multi-element Distribution in the Nanocolloidal Fraction of Landfill Leachates using AF4-HR-ICP-MS

5.1 Introduction

Current regulations regarding landfill leachates (EU Groundwater Directive 80/68/EEC and the Groundwater Daughter Directive 2006/118/EC) are based on research in which all elements present under < 0.45 μm are classed as truly dissolved and thus do not consider that the elements in this fraction may be present in colloidal form (See Section 1.4). This chapter aims to investigate the distribution of elements within the conventionally termed “dissolved fraction” of landfill leachates and identify mechanisms of element partitioning by applying the techniques previously optimised (Chapter 4).

In brief (Chapter 1), the nanocolloidal fraction (< 100 nm) is important in terms of the fate of elements in the environment because the large surface area to volume ratio of nanocolloids compared with the bulk minerals, can result in increased reactivity (Plathe, 2010). The high surface area to volume ratio of smaller particles can result in higher apparent adsorption coefficients of dissolved metals to colloids, larger diffusion coefficients of colloids relative to larger particles, and thus greater travel distances of metals associated with colloids compared to those present in larger particles or when truly dissolved (Baalousha et al., 2011a).

The more that we understand about how natural nanocolloids effect their surroundings, the more we are also able to potentially predict the distribution and interactions of man-made NP in the environment (Wiesner et al., 2011).This is particularly important with the ever increasing release of man-made NP into the environment (naturally or through waste disposal sites) and the comparative absence of knowledge about their fate. For example, Mueller and Nowack (2008) have estimated that 50% of manmade NP will eventually reside in landfills, so if we are to understand the fate of this additional loading of NP it is important that we first characterise those presently available, their interactions (particularly with inorganic and organic hazardous materials) and potential transport/co-transport properties and impact (Wagner et al., 2014) .

This study investigates landfill leachates from both Class 1 and 2 (Section 3.2.3), and therefore the different composition and characteristics of these leachates will allow for potential differences between element associations and size fractions which may arise from the implementation of recent legislation (e.g. introduction of MBT as a requirement to reduce the mass of biodegradable waste (Section 1.1.1)) to be investigated.

Thus far (Chapter 1) studies identifying element associations with different size fractions (including < 0.1µm) in landfill leachates have relied on conventional filtration methods, such as ultrafiltration (Matura et al., 2010, Gounaris et al., 1993, Jensen and Christensen, 1999, Jensen et al., 1999, Wu et al., 2012, Li et al., 2009a) yet no clear distribution patterns have been determined.

Gounaris et al.,(1993) were the first to examine colloids in landfill leachates and observed that the major component of colloids was OM and that these colloids associated strongly with Zn, Pb and Cr. The study hypothesised that OM existed on the surface of inorganic colloids, keeping them stable and in suspension. However, because of the filtration methods used they were not able to investigate this further.

Jensen et al., (1999) spiked groundwater samples with Cd, Ni, Zn, Cu and Pb at concentration ranges found in landfill leachates. It was found that more than 87% of TOC was present in the fractions < 10 nm. The heavy metals were strongly associated with the OM however Zn did not complex as strongly as the other heavy metals and was instead associated mainly with inorganic colloids. The major elements (Na, Ca, Mg, Mn, and K) were found primarily in the truly dissolved fraction however Fe was found only in the colloidal fraction.

Jensen and Christensen (1999) found that a substantial but highly variable proportion of heavy metals was present in the colloidal fraction of four different leachates (all from MSW sites). No distinct distribution behaviour of metals between dissolved and colloidal species was observed. However, the colloidal fraction ranged from 1-400 nm and was thus a much larger size range than to be investigated in this study. The varying cut off sizes for fractions and the membrane types used during filtration techniques also makes it difficult for direct comparisons between studies.

Li et al.,(2009a) examined organic nanocolloids and heavy metals (As, Cr, Cu, Ni, Pb, Zn) in MSW leachates at different stages of leachate treatment using ultrafiltration. They found that OM in raw and treated leachates primarily existed < 1 kDa and that heavy metals were found to predominate in colloidal fractions (> 1 kDa) but there were large variations in dissolved/colloidal species between the leachates. The size distribution of heavy metals was significantly affected by the treatment nature of leachates, apart from Zn. This study, as with previous did not distinguish between humic and fulvic substances and inorganic particles were not considered.

Matura et al.,(2010) used ultrafiltration alongside TEM and ICP-MS to examine binding of trace elements to organics and inorganics in leachates from a closed and an active landfill site. TEM showed that colloids present in the leachates were inorganic, composed of carbonates (Mg and Ca) and clays and that these were larger than 100 nm. Organic and Fe-oxide particles were shown to be present but to have a minor role in binding compared with inorganic clays and carbonates. Colloids in the active landfill site were shown to bind larger amounts of trace elements than those in the closed landfill site.

Wu et al.,(2012) used ultrafiltration combined with fluorescence to identify types of OM in leachates and found that OM interacts with metals in leachates and that Cd preferentially bound to fulvic-like components, wheras Cu complexed with humic like components. The MW of nanocolloids was found to exert less influence on metal binding than that of specific metals or components (e.g. humic or protein like) and is thus an indirect factor. This is consistent with the variations in element distributions observed between different studies due to their different metal compositions and components.

Hennebert et al., (2013) is the most recent ultrafiltration study to investigate leachate colloids and to the author's knowledge, this was the first to examine the distribution of a wide range of elements between colloidal and dissolved fractions in landfill leachates although they did not consider organic nanocolloids. They examined all of the elements as in this study apart from Li, Br, Sr Ag, Au, Hg, and U but additionally, were not able to quantify Al and Si due to background contamination. The majority of elements were found to be present in the colloidal fraction but Fe, Mn, Ba, Cr and Ni were not found in the < 3 kDa fraction. This study did not however suggest reasons for distributions of the elements between size fractions.

These previous landfill leachate studies have all shown that there are strong associations between OM and metals in landfill leachates however further investigation has been limited by the amount of data that can be produced with filtration techniques. The use of AF4-HR-ICP-MS will allow for better resolution of the nanocolloidal fraction to be obtained.

One previous study (Dubascoux et al., 2008a) used AF4-ICP-MS (not HR) to evaluate landfill leachate nanocolloids however this study focused on the speciation of organo-tins in landfill leachates rather than conducting a multi-element investigation. This study found that two distinct nanocolloid populations were present, an organic and an inorganic rich fraction, although Sn did not show complexation with either preferentially. Although both organic and inorganic colloids have been detected in previous studies, the filtration techniques used have not been able to distinguish between them by size. Since organic and inorganic colloids typically show different behaviour and play different roles in environmental processes, it is of importance to differentiate them (Hassellöv et al., 2006).

Although studies investigating nanocolloids in landfill leachates are limited, more recent studies (Bolea et al., 2006, Bolea et al., 2010, Laborda et al., 2011) have used AF4-ICP-MS to examine colloids in compost leachates which had similar characteristics to landfill leachates (e.g. TOC, pH and range of elements). These studies focused on organic nanocolloids, and all have shown that NOM was associated strongly with metals as seen with the findings of previous landfill leachate studies (Gounaris et al., 1993, Jensen et al., 1999, Dubascoux et al., 2008c).

AF4-ICP-MS has also been used more extensively for studies investigating river samples (Lyven et al., 2003, Alasonati et al., 2010, Benedetti et al., 2003, Stolpe et al., 2012) and this technique has enabled size distribution patterns of both inorganic and organic nanocolloids in rivers to be established (e.g. fulvic-rich nanocolloids between 0.5 to 3 nm and iron-rich nanocolloids between 4 to 40 nm in Alaskan rivers (Stolpe et al., 2012)).

As well as the source and nature of particles and the physical, chemical and biological processes, the physicochemical parameters of the environmental system also affect the size distribution of nanocolloids in the environment (Baalousha, 2009). Physicochemical parameters include pH and ionic strength, both of which are likely to change when a leachate escapes from a landfill site into a groundwater system (freshwater), an estuary (saline) or when rainwater infiltrates a landfill.

It is difficult to constrain the pH and ionic strength effects on environmental samples because they are not independent characteristics and therefore most investigations are based on experimental work using engineered NP and simplified environmental conditions (Wang et al., 2014, Tan et al., 2007) and well characterised humic substances such as Suwannee River Humic Acid (SRHA) (Mohd Omar et al., 2014, Yang et al., 2011). Ionic strength experiments have been carried out using laboratory simulated mixing with synthetic sea/groundwaters (Krachler et al., 2010, Stolpe and Hassellov, 2007).

A previous pH experiment (Mohd Omar et al., 2014) investigated zinc oxide NP and found that pH influences the stability of particles and that at higher pH values, particles were more likely to aggregate in the absence of humic acid. However, aggregation was reduced with the addition of SRHA, which appeared to coat the NP and fragment aggregates thus reducing the effects of pH.

Yang et al., 2011 highlighted the need to consider the influence of pH and ionic strength when investigating the role of NOM in colloidal mobility. Using a simplified laboratory experiment, pH and ionic strength were shown to influence the deposition of colloids (manmade microspheres) in a sand packed porous column in the presence of SRHA. Deposition of colloids in the column was influenced more by pH than ionic strength but higher pH and lower ionic strength solution values together resulted in the highest deposition of colloids and thus both influenced colloidal mobility and are likely to be important factors in the distribution of nanocolloids and associated metals in landfill leachates.

5.1.1 Aims and Objective

To the author's knowledge, this is the first study to couple AF4 online with HR-ICP-MS to investigate the distribution of multiple elements within the "dissolved" fraction of landfill leachates. This chapter will apply the method developed in Chapters 3 and 4 to three leachates in order to validate it and to determine the composition and distribution of nanocolloids and the elements associated with them, so as to identify colloidal carriers of potentially toxic metal(loids). The differences between leachates created from different waste classes and the effects of altering environmental characteristics will also be explored. By investigating the distribution of elements within this fraction, data which may be used to enhance landfill risk assessment models will be discussed.

5.2 Materials and Method

Materials and methods used in this chapter have been detailed in Chapters 3 and 4 however brief supplementary information will be given. The methods applied in this chapter will be used to validate and demonstrate their ability for use with leachate samples other than MSW (used for method development in Chapter 3 and 4).

5.2.1 Leachates

Three different UK leachates were used in this comparison study as detailed in Chapter 3-: (1) MSW leachate (used in Chapters 3 & 4), (2) MBT leachate (created synthetically from MBT waste) and (3) AMSW leachate (created synthetically using aged waste (buried > 10 years)). These have been previously characterised (Dalton, 2014) (Table 3.1) and briefly discussed in Section 3.2.3.

In order to explain elemental distributions within the leachates, the differences in their characteristics must be further discussed.

All the leachates studied had pH values that were in the mid to high range for typical MSW landfill leachates (7.21 to 8.32). MSW had a particularly high conductivity (41 µS) compared with both MBT (7.7 µS) and AMSW (2.9 µS) and with typical landfill leachates (2.5 to 35 µS). This was as expected because MBT has many particles/constituents removed in the treatment process (Robinson et al., 2005) and AMSW will have experienced degradation, thereby also reducing it’s conductivity (Kjeldsen et al., 2002). MSW TOC (2380 mg/L) was double that of MBT (1080 mg/L) and more than 10 times that of AMSW (139 mg/L) however it was within the typical range for landfill leachates as was both MBT and AMSW.

5.2.2 Method

Methods used for characterisation and analysis of the three leachates in this study are described in detail in Chapters 3 and 4. Briefly, leachates were characterised using AFM and DLS and fractionated using AF4 with parameters optimised for the fractionation of low MW nanocolloids. AF4 has been coupled online with UV-DAD, FLU and HR-ICP-MS to obtain a distribution of particle compositions and associations with size. Repeatability of multiple sample injections has been assessed using peak retention times and total peak area integration (Section 4.2.4.5.6). Recovery was also assessed offline using fraction collection of cross flow and channel elutants (Section 4.2.4.5.7).

5.2.2.1 pH and Ionic Strength Effects

pH and ionic strength alteration of leachate may occur in the environment as leachates escape from the landfill or water infiltrates. The potential effects of these changes on elemental distributions were examined by fractionating online using AF4-HR-ICP-MS. MSW was chosen out of the three leachates available to evaluate these influences because it contained the highest concentrations of most elements (Table 5.3) and so was most likely to retain detectable concentrations after dilution. The concentrations of elements were also more homogeneous compared with MBT and AMSW (Table 5.3), so the variability of different aliquots was less likely to affect the results of ionic and pH alteration.

5.2.2.2 Ionic strength

To alter the ionic strength of the MSW leachate, aliquots of filtered (0.1 µm) MSW leachate (as used in Chapters 3 and 4) were diluted with 0.1 µm filtered Milli-Q in order to lower the ionic strength of the solution (also resulting in lower TOC). There were 4 different dilution values, 1:10, 1:20, 1:50 and 1:100.

Dilution beyond 1:100 was not feasible for this study because many of the element signals would not be detectable.

5.2.2.3 pH

The pH of MSW (0.1 µm filtered) was adjusted to lower values with the addition of 3% HNO_3 solution (high purity sub-boiled diluted with 0.1 µm filtered Milli-Q) using the amounts of HNO_3 in Table 5.1. The pH was lowered from 8.32 to pH 7, 6 and 5 because these lowered values are representative of the range of rain (Environment Agency, 2010a) and seawater (Environmental Protection Agency, 2006) which may mix with leachate and alter pH.

HNO_3 was selected to adjust the pH of MSW because it had been previously used in leachate adjustment studies (Kim et al., 2002, Christensen and Christensen, 2000, Fan et al., 2007) and because it was readily available in high purity grade (also used in this thesis for acidifying samples for HR-ICP-MS analysis).

Drops of 10 µL were continuously added to 10 ml of MSW and the pH change was monitored with each drop. Once the required pH was reached, each solution was left to equilibrate and measured again after 12 hours. The MSW was well buffered and therefore the pH of the solutions rose after the initial addition of HNO_3. More drops were subsequently added, whilst measuring the pH, and the solution pH measured every 12 hours until the desired pH levels were reached and were stable. The EC (electrical conductivity-ionic strength indicator) was also monitored to establish the effect of altering pH on the EC and only minor changes were observed.

Table 5.1 3% HNO_3 addition for pH alteration of MSW samples

Required pH	**3% HNO_3 added (µL)**
5	1830
6	1710
7	1340

5.3 Results and Discussion

This section presents the results from the fractionation of three different UK landfill leachates in order to identify their nanocolloidal element distribution and to validate the method developed in Chapters 3 and 4. Results of the fractionation of a modified leachate (pH and ionic strength) are also presented.

Results will be presented and discussed in sections dependent on the method used. Those relating to the validity of the AF4-HR-ICP-MS method will be presented before a comparison of MSW v MBT v AMSW results. Initial results of pH and ionic strength effects on elemental distributions of MSW will also be shown.

5.3.1 DLS Results

DLS was used to analyse the PSD of MSW, MBT and AMSW. The results shown are the number (%) of particle sizes detected in particle size fractions in the 3 leachates. These are presented in Figure 5.1 to Figure 5.3.

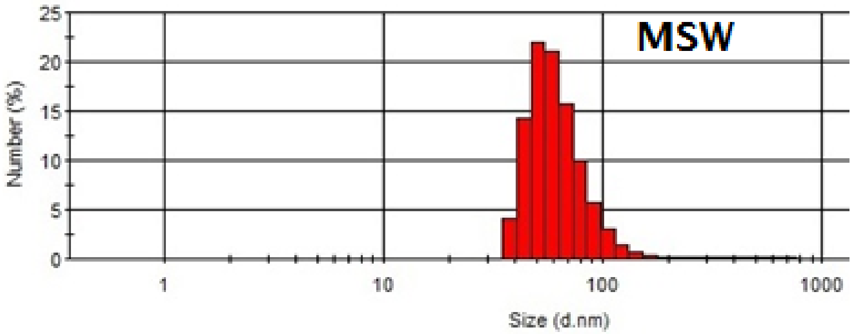


Figure 5.1 MSW Particle Size Distribution from DLS Analysis

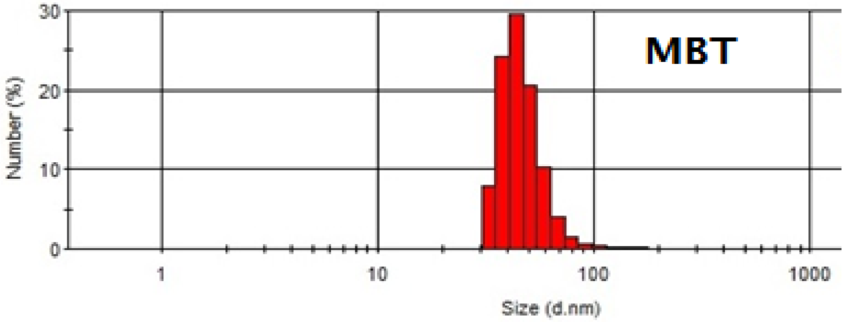


Figure 5.2 MBT Particle Size Distribution from DLS Analysis

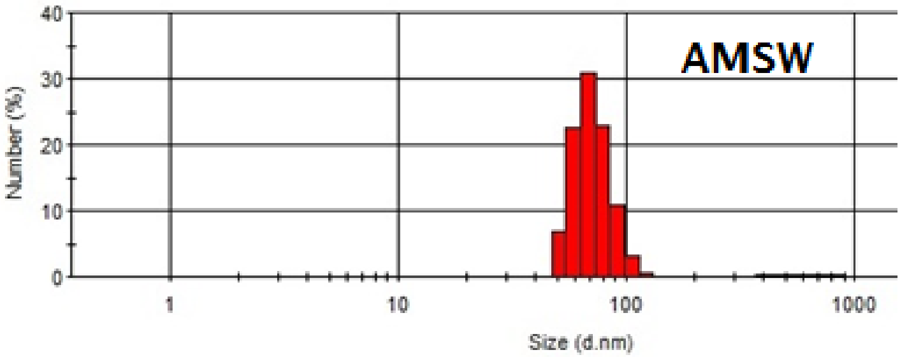


Figure 5.3 AMSW Particle Size Distribution from DLS Analysis

The results from DLS analysis show that:

- No particles <30 nm were detected in any of the leachates.
- All leachates contained particles larger than the 100 nm filter size used in sample preparation indicating that particle aggregation occurred after initial filtration (See Section 3.3.1).
- MSW PSD ranged from 35 to <200 nm with a mode of 50 nm
- MBT PSD ranged from 30 to < 200 nm with a mode of 40 nm
- AMSW had the largest particles with PSD ranging from 48 to <200 nm with a mode of 70 nm.

The results from the PSD comparison showed that MSW and MBT leachates had a similar particle size range; however AMSW demonstrated a higher range, indicating larger particles. This could be explained by the age of AMSW which results in a lower leachate TOC value and therefore less organic particles present in the leachate and proportionally greater numbers of inorganic. Organic particles such as humic and fulvic acids are known to be generally of a smaller size than inorganic particles (Lead and Wilkinson, 2006)(Figure 1.3) therefore AMSW can be expected to have larger particles.

No other studies have used DLS to investigate PSD in landfill leachates therefore no direct comparisons can be made. However Baalousha et al.,(2006b) used DLS to investigate the size of humic substances by using SRHA as a synthetic standard. This study found that humic substances were < 10 nm which suggests that the leachate samples in this study did not contain any humic substances because no particles of this size were detected, although this maybe a gross oversimplification, as humics have been shown to be very variable in nature.

It is possible that the leachates contained aggregated humic substances or humic substances complexed with larger inorganic which is why they were not detected at smaller sizes but it is also important to note that, as discussed in Section 2.2.1.3, DLS is less sensitive to the detection of smaller particles. This is particularly known to occur in polydisperse samples because the large particles mask the presence of smaller particles because they scatter light more effectively (Filella et al., 1997) and therefore this may be occurring in these measurements. These results must be therefore be used in conjunction with AFM and AF4 results before drawing any final conclusions.

5.3.2 AFM Results

AFM analysis was carried out for each of the three leachates (Table 5.2).

Table 5.2 Leachate Particle Sizes from AFM Analysis

Dilution	Min Diameter (nm)	Max Diameter (nm)	Mean Diameter (nm)
MSW	5.29	15.3	8.88
MBT	1.96	54.8	24.1
AMSW	9.78	88.5	41.9

The AFM analysis confirmed the presence of nanocolloids in each of the three leachates but there were different size ranges in the leachates.

In summary:

- MSW particles had the smallest size range from 5.29 nm to 15.33 nm.
- MBT particles were detected from 1.96 nm to 54.8 nm.
- AMSW particles were larger than MSW and MBT particles and ranged from 9.78 nm to 88.5 nm.
- The PSD detected with AFM was smaller than that with DLS.

It was surprising that the largest particles detected in MSW were 15.33 nm because this contrasts with the results from DLS for MSW in which particles were between 35-200 nm. It appears that the particles detected in MSW using AFM may be the organic particles that were masked by larger particles in DLS however it is not clear why no larger particles were detected in this sample. Smaller particles in the size range of humic acid (Baalousha et al., 2006) were also detected in MBT and AMSW using AFM that were not detected using DLS. AMSW PSD was larger than MBT and MSW and this is in agreement with the findings by DLS.

When comparing the PSD obtained from AFM with DLS, it was clear that the DLS detected a larger particle population in all leachates than AFM and this was because each method uses a different principle to determine particle sizes (See Section 2.2.1). Variation between AFM and DLS was also recognised by Baalousha and Lead (2012) in which it was found that comparison between the sizes obtained by the two methods can only be carried out when using highly monodisperse hard spheres. Therefore the results of samples with polydisperse populations, such as those in this study, must be reported alongside details of the actual methods used. For the benefit of this study however, both AFM and DLS were useful to confirm the presence of nanocolloids within the leachates prior to analysis using AF4-HR-ICP-MS.

5.3.3 Bulk Elemental Concentrations

Total element concentrations of the bulk samples analysed by HR-ICP-MS are presented in Table 5.3 (with variation of sample concentrations from analysis of replicate aliquot dilutions of the bulk sample).These were prepared as described in Section 4.2.2.

Table 5.3 Total Element Concentrations for MSW, MBT and AMSW

	MSW			**MBT**			**AMSW**		
	LOD (ppb)	**Conc. (ppb)**	% RSD	LOD **(ppb)**	**Conc. (ppb)**	% RSD	LOD (ppb)	Conc. **(ppb)**	% RSD
Li	0.004	**568**	11	0.010	**57**	5	0.002	**50**	18
Mg	0.122	**56160**	9	0.204	**58822**	2	0.363	**49355**	16
Al	1.540	**1443**	24	1.008	**1211**	12	3.318	**155**	13
Si	1.068	**55283**	4	4.244	**4870**	3	0.800	**6768**	14
K	1.014	**1028**	9	1.47	**494**	10	0.060	**361**	16
Ca	8.350	**37437**	7	2.918	**31156**	97	9.402	**36052**	10
Ti	0.007	**490**	9	0.112	**18**	20	0.067	**0.54**	14
V	0.002	**105**	9	0.013	**5.2**	1	0.006	**0.22**	18
Cr	0.066	**640**	10	0.021	**83**	3	0.019	**6.43**	20
Mn	0.003	**29**	7	0.003	**0.71**	70	0.006	**0.41**	13
Fe	0.263	**3128**	7	0.139	**2885**	37	0.342	**70**	18
Co	0.002	**77**	10	0.011	**25**	11	0.007	**3.0**	16
Ni	0.005	**288**	6	0.072	**230**	12	0.035	**57**	14
Cu	0.050	**18**	5	0.066	**248**	9	0.075	**54**	16
Zn	0.377	**1897**	24	0.625	**1037**	15	0.488	**656**	6
As	0.001	**2.5**	10	0.001	**0.01**	2	0.063	**BD**	5
Br	4.484	**219**	10	2.20	**14**	11	1.349	**20.2**	18
Sr	0.015	**1099**	6	0.115	**210**	74	0.040	**271**	14
Mo	0.041	**6**	5	0.022	**BD**	NA	0.031	**21**	16
Cd	0.019	**BD**	NA	0.010	**BD**	NA	0.015	**BD**	NA
Sn	0.023	**106**	2	0.003	**6.9**	19	0.044	**2.3**	15
Cs	0.0001	**21**	13	0.0002	**1.4**	11	0.000	**0.14**	17
Ba	0.084	**907**	10	0.006	**4.8**	54	0.073	**8.0**	14
Au	0.034	**BD**	NA	0.006	**BD**	NA	0.033	**BD**	NA
Hg	0.045	**BD**	NA	0.016	**BD**	NA	0.056	**BD**	NA
Pb	0.019	**31**	13	0.002	**9.3**	71	0.015	**2.5**	14
U	**0.0002**	**0.15**	18	0.00003	**0.13**	23	0.001	**0.07**	37

% RSD = Relative standard deviation (n=3) LOD= Limits of Detection (3 standard deviations of the blank)
BD= Below Detection

The most prominent observations from these data are:

- MSW leachate had the highest concentration of all elements other than Mg and Cu which were highest in MBT leachate and Mo which was highest in AMSW leachate.

- MSW leachate concentrations ranged from 0.15 ppb of U to 56,160 ppb of Mg, MBT ranged from 0.1 ppb of U to 58,822 ppb of Mg and AMSW ranged from 0.07 of U to 49,335 ppb of Mg.
- Mg, Si and Ca, were the major elements in all leachates ranging from 37,437 ppb (Ca) to 56,160 ppb (Mg) in MSW, 4870 (Si) to 58,822 ppb (Mg) in MBT and 6,768 ppb (Si) to 49,355 ppb (Mg) in AMSW.
- Fe was a major element in MSW (3129 ppb) and MBT (2885 ppb) but not AMSW (70 ppb).
- U was present at the lowest concentrations in all leachates.
- For Li, Si, Ti, V, Cr, Mn, Co, Cs, Ba and Pb concentrations in MSW were at least an order of magnitude higher than AMSW.
- Cd, Hg and Au were not detected in bulk samples of all the leachates.
- % RSD values (calculated from 3 replicate analyses of diluted aliquots) for MSW were all under 18 % except for Al (24%) and Zn (24%) which both had high concentrations (>1 ppm).
- MBT had five elements with % RSD values over 20% (Ca, Mn, Fe, Sr, and Ba) indicating that the concentration of these elements was variable.
- All elements in AMSW had % RSD values < 20%.

The concentrations of elements in MSW and AMSW leachates were within the ranges expected when compared with an MSW leachate components review (Christensen et al., 2001). However, MBT values for Mg, K, Ca, Mn, Fe, Ni, Cd, Pb, As and Hg, were lower than typical MBT concentrations reported by (Robinson et al., 2005), except Cr (60% higher), Cu (500% higher) and Zn (300% higher). Due to limited literature, element concentrations for all the elements in thus study were not available for comparison. The MBT leachate was however synthetically leached, therefore this may explain the lower concentrations observed.

It was interesting that MBT had higher levels of Cu than MSW because it was expected that MSW would have higher concentrations because there had been no metal removal process. The lower values of Cu may therefore be due to less metals being present in the original MSW landfilled waste than the MBT waste prior to treatment or due to greater leaching in the MSW. It is important to note here that although there are typical values of leachate concentrations derived from studies of many landfill leachates, it is not possible to characterise or predict leachate compositions without knowing the initial waste composition and therefore typical ranges must be taken as an indicator and individual site characteristics must be considered.

The AMSW waste concentrations were lower than MSW and this is expected because in an older landfill there will have been more water intrusion, thus diluting element concentrations over time.

The variability of the concentrations was mostly lowest in MSW and this may be because the leachate contained higher concentrations and was therefore less affected by counting statistics (Section 4.3.4). This explains the increase in variability from MSW to AMSW (as concentrations decrease) however the variability seen in replicate MBT aliquots cannot be attributed to this and must therefore be a real characteristic of the MBT waste and resulting leachates or an artefact of the synthetic leaching process used.

5.3.4 Fractionation Recovery (mass balance) Results

In order to assess the recovery of each element during the AF4 fractionation process, a mass balance experiment was conducted for each of the three leachates (Section 4.2.4.5.7). These were analysed using offline AF4-HR-ICP-MS (collecting channel and cross-flow elutants) and the results are shown in Table 5.4.

The mass balance results of the leachates show that there were elements for which a higher mass was recovered from the system than was injected (as previously observed and discussed for MSW in Chapter 4). The measurement precision was calculated using the sum of the % RSD value for each fraction collected.

Table 5.4 Recovery (mass balance-offline) and measurement precision for MSW, MBT & AMSW Leachates

	MSW		MBT		AMSW	
Element	Recovery %	Measurement Precision (%RSD)	Recovery %	Measurement Precision (%RSD)	Recovery %	Measurement Precision (%RSD)
Li	**68**	0	**130**	7	**130**	3
Mg	**66**	1	**81**	1	**42**	1
Al	**1766**	1	**108**	4	**6**	0
Si	**9**	1	**NA**	NA	**128**	5
K	**89**	2	**80**	1	**40**	1
Ca	**193**	1	**26**	0	**5**	1
Ti	**77**	8	**211**	16	**55**	27
V	**82**	10	**NA**	NA	**79**	68
Cr	**64**	1	**38**	1	**63**	5
Mn	**128**	5	**1**	9	**40**	1
Fe	**128**	2	**63**	0	**81**	0
Co	**73**	13	**147**	16	**93**	13
Ni	**164**	46	**87**	8	**59**	2
Cu	**223**	12	**82**	4	**43**	0
Zn	**98**	70	**109**	1	**9**	7
As	**77**	18	**159**	25	**NA**	**NA**
Br	**69**	1	**54**	1	**65**	1
Sr	**62**	3	**64**	1	**25**	6
Mo	**268**	6	**NA**	NA	**71**	5
Ag	**NA**	**NA**	**NA**	NA	**NA**	**NA**
Cd	**1550**	2	**NA**	19	**41**	15
Sn	**81**	4	**37**	1	**43**	0
Cs	**102**	36	**NA**	**NA**	**139**	49
Ba	**3**	18	**93**	19	**339**	140
Au	**NA**	**NA**	**NA**	NA	**NA**	**NA**
Hg	**NA**	**NA**	**NA**	NA	**NA**	**NA**
Pb	**212**	8	**60**	7	**42**	7
U	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**

RSD% represents analytical variation using HR-ICP-MS NA= Data not available (below detection limits)

In summary:

- MSW recovery values were previously discussed in chapter 4 and showed 9 elements with >100% recovery and that all, except Ba (3%), had a recovery of at least 62%.
- For MBT, 6 elements had a recovery value higher than 100%. Recoveries ranged from 1% to > 100%.

- Mn in MBT had a low recovery of 1% however the sample variation (Table 5.3) was 9% suggesting that a high proportion of Mn is lost in the system.
- AMSW recovery values showed that only Li (130%), Si (128%) and Ba (339%) had recovery values above 100 when the sample and measurement precision were considered (Cs was 139 ±49%.).
- There was no consistency between the leachates as to which elements were subject to loss in the system. For example Ba shows low recovery (3%) in MSW but higher (93%) and (339%) in MBT and AMSW, respectively.
- Mn also showed different recoveries in each leachate (128%, 1% and 40%) in MSW, MBT and AMSW, respectively. This cannot be explained by the amount being measured because MSW had higher concentrations than MBT and AMSW and would therefore be expected to show higher recoveries.
- Si, V, Mo, Cd, Cs and U were not detectable in the MBT leachate recovery experiment above baseline fraction concentrations.

There is no mass balance data in the literature to compare the results of this study with. However, as discussed in section 4.3.5, the results of these recovery experiments confirm that real sample losses occur within the system, perhaps through adsorption to the membrane or system tubing. This confirms the need to detect the baseline for each sample fractionation in order to rule out system contamination altering the results of the study, as will be discussed further in Section 5.3.6.1.

Thang et al., (2001) investigated the recovery of groundwater humic colloids and purified humic and fulvic acids using AF4 and found that colloidal recovery decreased when ionic strength and cross flow was increased. This suggests that higher recovery rates may be achieved using different fractionation parameters (e.g. carrier solution), but these may alter the fractionation results obtained by influencing the PSD of the sample in question and the efficiency of the separation (e.g. width of the peaks). It is likely that lower cross flows may also reduce the variability of the recovery results because there will be less sample exposure to carrier solution and less adhesion to the membrane. It is therefore important to determine the recovery and state the possible variations on results for each set of fractionation parameters for each sample.

5.3.5 Dissolved/nanocolloidal distribution

The mass balance recovery data can also be used to show the distribution between the truly dissolved (cross flow elutant <1 kDa) and colloidal phases (channel elutant >1 kDa). Figure 5.4 shows the recovered mass for each element as a percentage distribution between the truly dissolved and colloidal phases.

In summary:

- For MSW leachate Al, Ti, V, Fe, Ni, Sn, Ba and Pb were mostly in the colloidal fraction, and apart from Fe and Ti, this observation was the same in the MBT and AMSW leachates.
- Fe was more than 90% colloidal in MSW leachate.
- For MBT, only Ti, Mn and As were at least 50% present in the colloidal fractions with the remaining elements predominantly dissolved.
- For AMSW, Fe was the only element that had more than 50% in the colloidal fraction however, Fe in the colloidal fraction (55%) was only slightly higher than that in the dissolved fraction.
- Li was observed to be 100% in the dissolved fraction of the MSW and MBT leachates and 97% in the AMSW leachate.
- Br showed a similar pattern to Li, in which 100% was in the truly dissolved phase in MSW and MBT and 91% in AMSW.
- Sr was at least 90% present in the dissolved fraction for all the leachates and Mg, K, Ca, Mo, and U were more than 70% present in the dissolved fraction.
- The first row transitional metals and Pb have higher colloidal fractions than the other elements particularly in MSW.

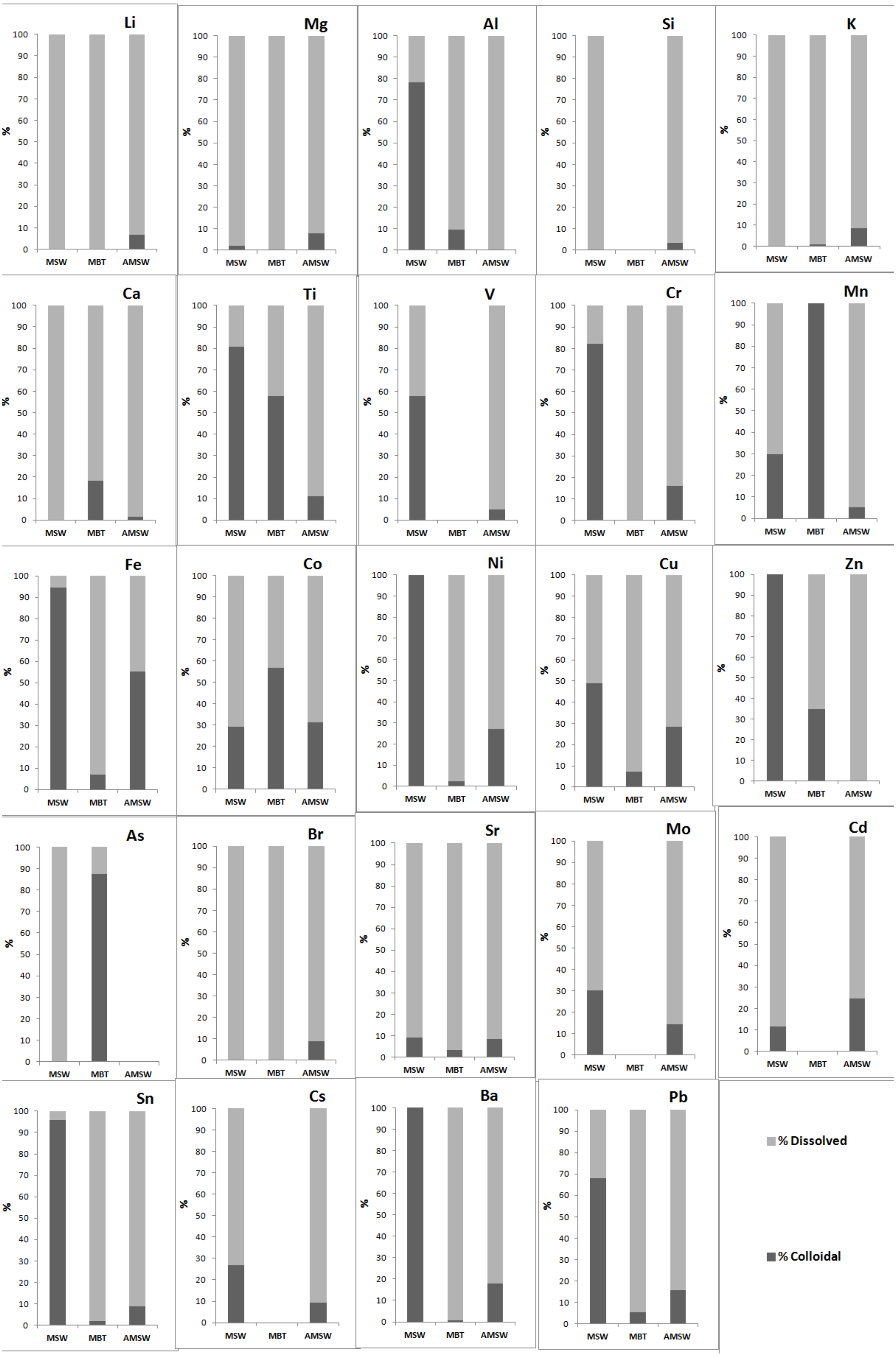


Figure 5.4 Percentage distribution between dissolved and colloidal fractions determined by collection of cross-flow and channel flow elutants

The collection and analysis of the cross flow fraction revealed that Sr, Mg, K, Ca, Mo, and Cd were predominantly present in the truly dissolved fraction in all leachates. This was in agreement with Oygard et al.,(2007) in which Mg, K, and Ca were shown to be truly dissolved, but their study found Cd to be present predominantly in the colloidal fraction. Observations by Jensen and Christensen (1999) however agreed with the findings reported here in which Cd was predominantly found in the dissolved fraction. It must be noted however, that neither of these studies used the AF4 elutant collection approach for their research and instead used ultrafiltration which make comparisons difficult.

Li and Br distributions both showed that nearly 100% was present in the truly dissolved fraction in all leachates suggesting that these elements do not readily complex with particles and remain as free ions confirming their suitability as passive tracers in transport studies (Woodman et al., 2011, Öman and Rosqvist, 1999).

As was expected to be present solely in the truly dissolved fraction because it was previously found in this fraction by both Li et al., (2009a) and Matura et al., (2010). Whilst this was the case for the MSW leachate, in MBT As was found to be mostly colloidal (87%).

Fe showed a higher affinity to the colloidal fraction in MSW and AMSW and this was expected because many studies have found Fe to be predominantly colloidal (Oygard et al., 2007, Jensen and Christensen, 1999). Although MBT showed a portion to be colloidal, Fe was found to be predominantly dissolved although recovery for Fe in this leachate was lower than MSW and AMSW and the initial concentration was more variable (37%) which could explain this unexpected observation.

5.3.6 Online AF4-HR-ICP-MS Analysis Results

The following results are those obtained using the AF4-HR-ICP-MS online configuration (Figure 4.3)

5.3.6.1 Baseline and Sample Fractograms

Baseline and sample data for repeat AF4-HR-ICP-MS fractionations that have been corrected for signal and sensitivity drift are presented in Figures 5.5-5.10. These figures are shown as Retention time v CPS because only the signal above the baseline was corrected to concentration values (ppb) as this represents the concentration of the sample only. Interpretation of the fractograms will be discussed in Section 5.3.7. Only results related to analytical methods validation are presented in this section.

5.3.6.1.1 MSW

Figures 5.5 and 5.6 show MSW results which have previously been discussed in Chapter 4. Briefly however:

- Only the MSW colloidal fraction signals of Cd, Au and Hg were difficult to discriminate from the baseline.
- All elements in MSW showed a peak in intensity before 20 minutes and a second smaller peak at around 50 minutes apart from Li, K, Ca, As, Br and Cs which only showed the first peak and Si.
- Si showed a unique distribution and did not show a peak, however counts increased after 50 minutes (elution of residual peak) and did not decrease.
- All element signals above the baseline showed the same signal shape for all of the replicates however, the intensity of the signals varied between runs.

5.3.6.1.2 MBT

- Figures 5.7 and 5.8 showed that As, Br and Hg signals were not detectable above the baseline.
- There was a very narrow peak in Au in one of the replicates which indicates contamination in the system from previous injections of Au

nanoparticles. No Au was detected in any of the samples used in this study (Table 5.3) therefore it cannot be contamination from sample carryover and may be attributed to contamination from the use of Au NPs from another study using the AF4. No Au signal was detected above the baseline apart from this peak.

- All element signals in MBT showed a peak in intensity before 20 minutes and a second smaller peak at around 50 minutes apart from Li, Mg, K, Ca, Mo, and Cs which showed only the first peak.
- Si showed a unique distribution and did not show a peak, however counts were observed to increase after 50 minutes, the same as seen in MSW.
- All element signals in MBT above the baseline showed the same distribution in each replicate however the intensity of the signals varied between them and the peak widths differed for some elements.

5.3.6.1.3 AMSW

- Due to the lower concentrations of many elements in AMSW (Table 5.3) compared with MSW and MBT which resulted in lower signal to noise ratios, only Mg, K, Fe, Co, Ni, Cu, Br and Pb were detectable above the baseline.
- The U signal was visible above the baseline; however the CPS values (300) were below detection limits (Table 5.3) and thus cannot be quantified.
- The Si signal was similar to that in MSW and MBT in that no distinct peaks were visible but there was an increase in CPS after 50 minutes.
- All detectable element signals showed a single peak before 20 minutes apart from Fe, Pb and Br.
- Fe and Pb showed the peak before 20 minutes as well as a small increase in sample signal at around 50 minutes but no clear peaks were observed apart from the initial peak.
- Sn showed a peak in all replicate runs around 20 minutes however the signals were lower than the baseline runs so could not be quantified.

- A narrow Ti peak was present in a baseline run indicating contamination in the system.
- As well as a Br peak before 20 minutes, a Br peak was also present at 70 minutes, but only in run 2 which suggests that this may be due to system contamination. The 1% RSD of Br concentration in replicate aliquot analysis also supports this.
- Other than Br, the peak intensities and retention time of the replicates appeared to be similar.

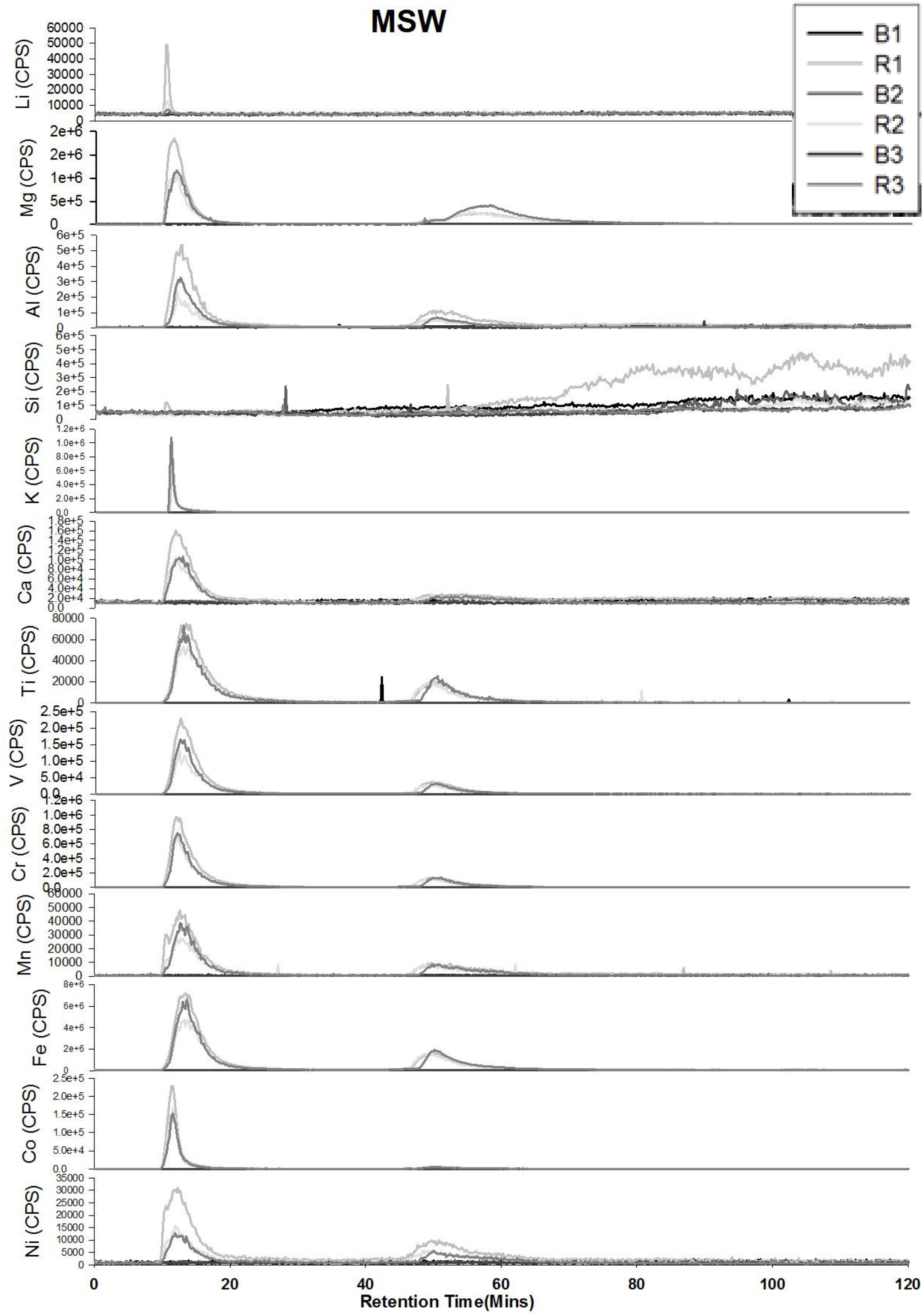


Figure 5.5 MSW Sample and Baseline runs for Li – Ni (Mass order) from AF4-HR-ICP-MS

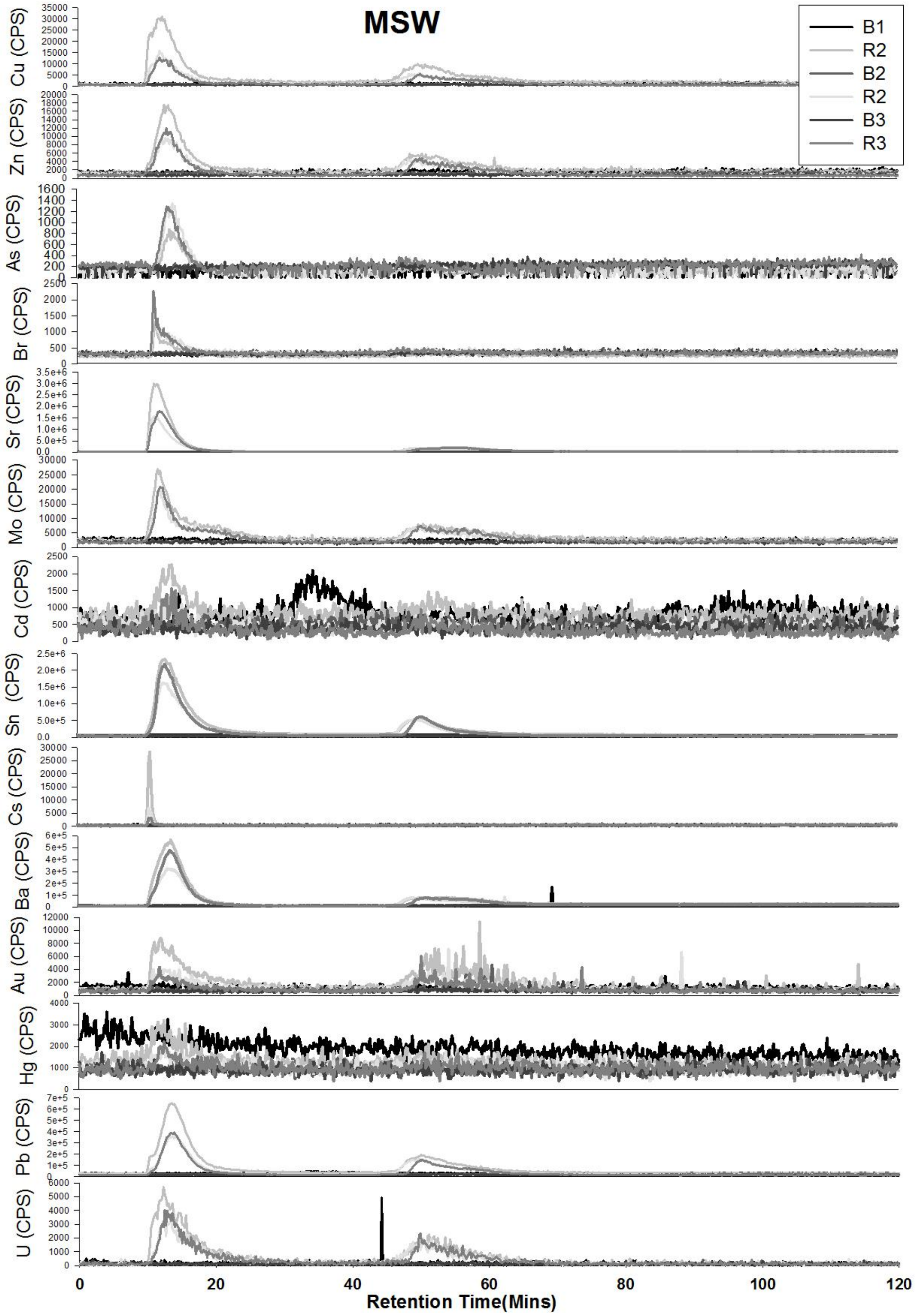


Figure 5.6 MSW Sample and Baseline runs for Cu – U (Mass order) from AF4-HR-ICP-MS Analysis

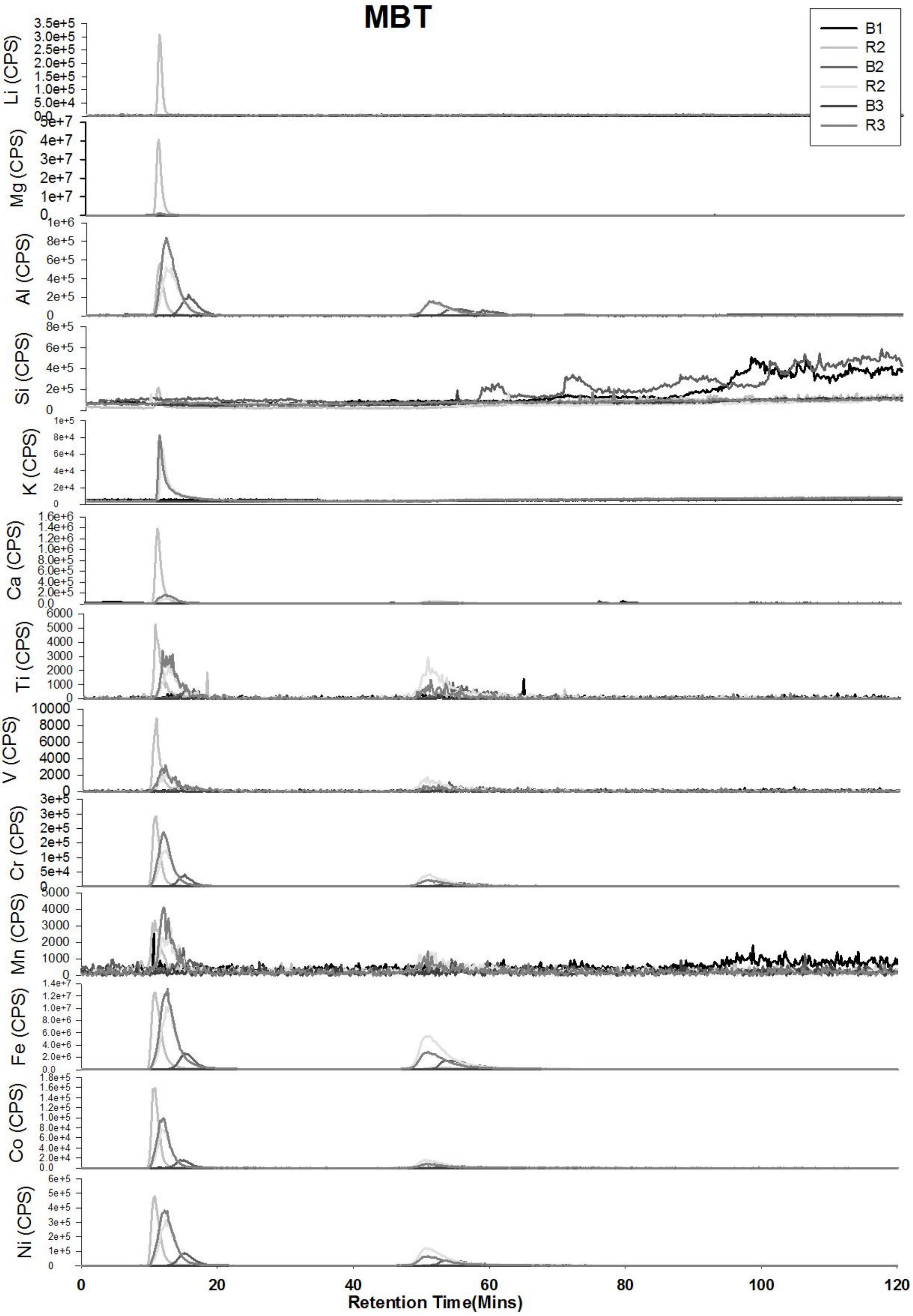


Figure 5.7 MBT Sample and Baseline runs for Li-Ni (Mass order) from AF4-HR-ICP-MS Analysis

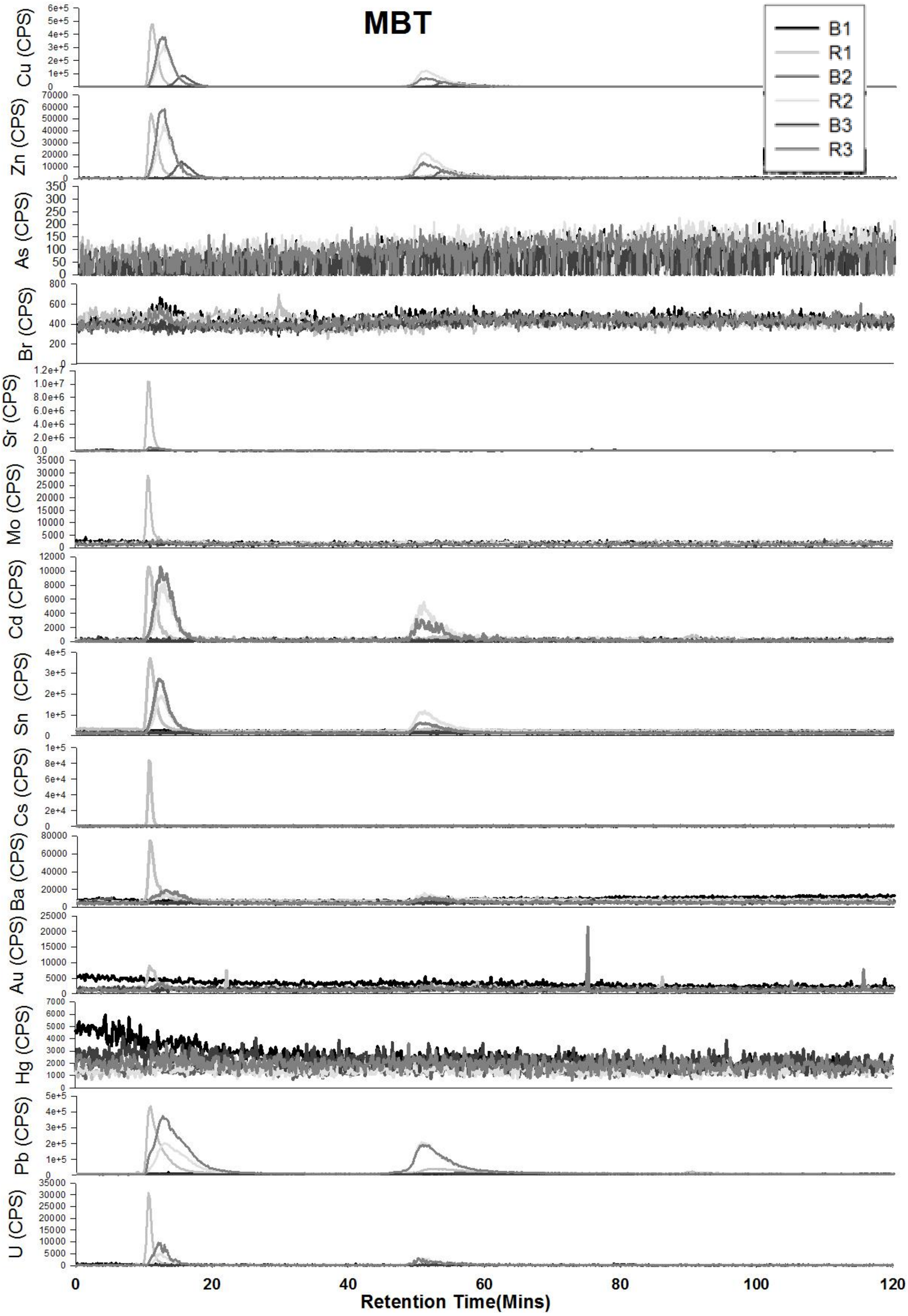


Figure 5.8 MBT Sample and Baseline runs for Cu-U (Mass order) from AF4-HR-ICP-MS Analysis

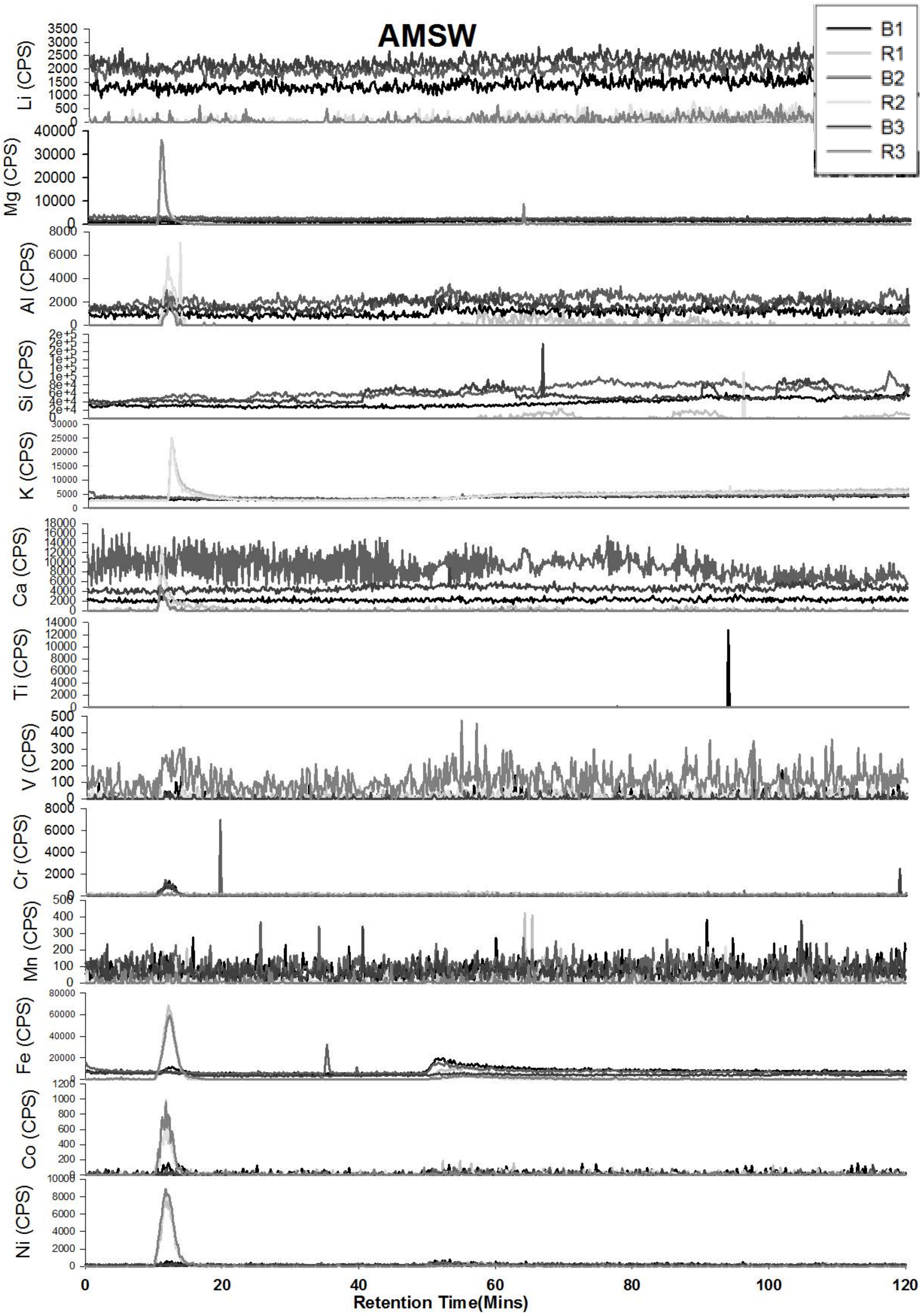


Figure 5.9 AMSW Sample and Baseline Runs for Li- Ni (Mass order) from AF4-HR-ICP-MS Analysis

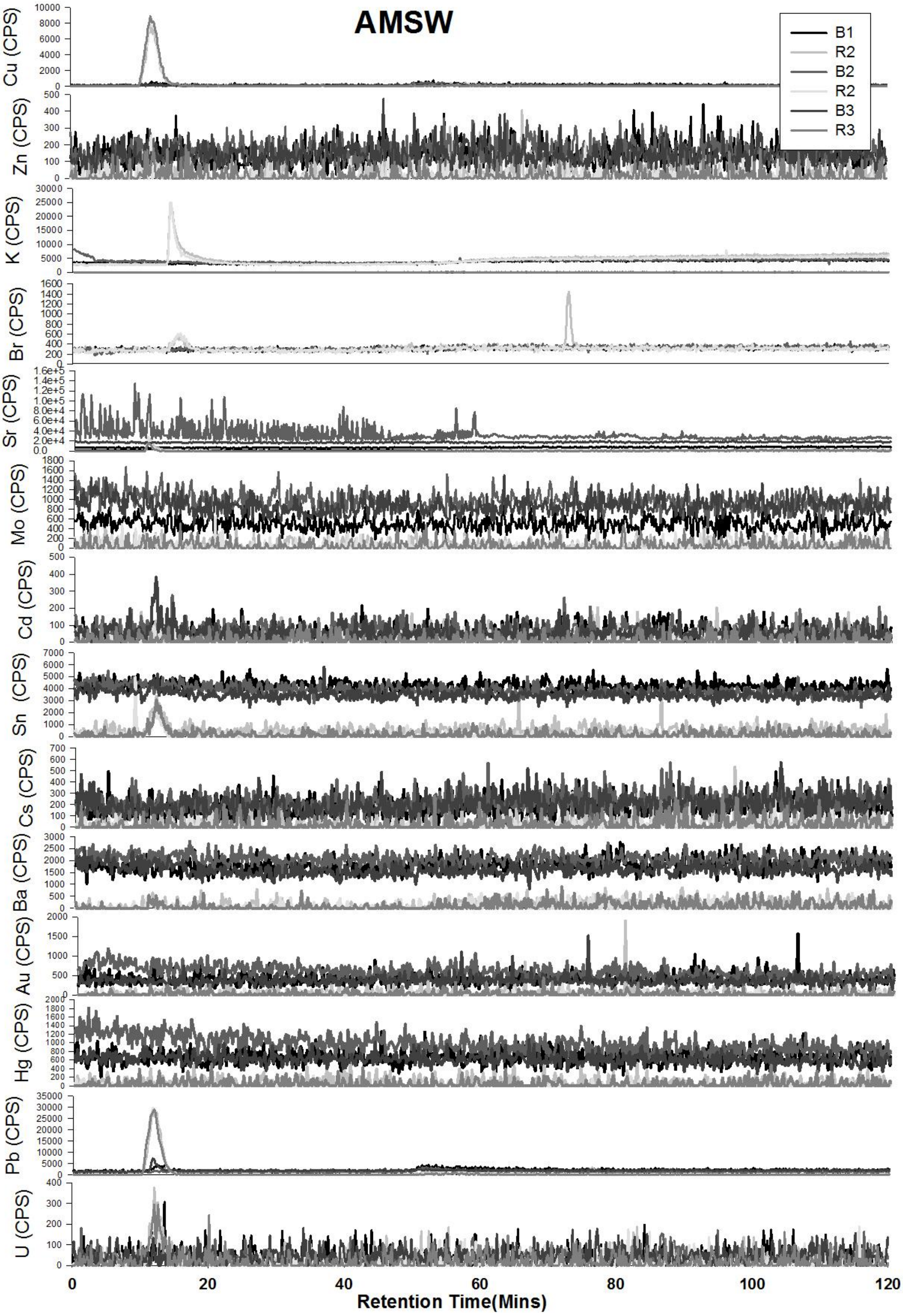


Figure 5.10 AMSW Sample and Baseline Runs for Cu- U (Mass order) from AF4-HR-ICP-MS Analysis

The replicate injections of sample and baseline for MSW have already been discussed in Chapter 4 but briefly they showed that that the baseline between replicates was a useful tool for quantifying sample-only concentrations and for acting as a rinse run between sample injections and this is true for all of the three leachates. Carryover particles represented by sharp narrow spikes were present in baseline runs for some elements in all the leachates and thus reinforced the need for a baseline/rinse run between sample injections.

Fewer elements were quantifiable above the baseline in MBT and AMSW than with MSW however this was due to the low nanocolloidal concentrations, which were expected due to the lower bulk element concentrations in MBT and AMSW, rather than the high baseline concentrations.

Replicate runs for MSW and AMSW appeared (by visible observation) to be repeatable in terms of retention times, however the signal intensities varied. MBT replicates appeared much more variable than the other leachates with respect to both peak width and intensity. This may be attributed to variations in the injected aliquot of sample and will be discussed further in the next Section (5.3.6.2)

5.3.6.2 Fractionation Repeatability

So that the repeatability of the replicate fractionations could be assessed statistically, three subsequent injections of baseline and sample were carried out. The results for the assessment of repeatability of the nanocolloid elemental distribution of each of the leachates are reported in Table 5.5.

In order to determine the repeatability of the fractionation process, the first peak maximum was used as an indicator of the repeatability of the fractionation process and of the nanocolloid distribution in the sample because replicate sample injections would expect to show similar distributions and therefore peak retention times. The total peak area (calculated using OriginsPro software) was used as an indicator of the repeatability of the sample nanocolloidal concentration for each element.

In summary and for further discussion:

- For MSW, element distributions were highly repeatable (<5 %) apart from Cs (173%) and Fe (55%) (See Section 4.3.4).

- The high % RSD value for Cs, observed in MSW, was also seen in MBT and this can be explained by the presence of Peak 1 in only 1 of the 3 replicates.
- The same result (high % RSD value) was also recorded for Li and Mo in MBT and Al in AMSW, again due to the peak presence in only 1 replicate.
- MBT retention times were less repeatable than MSW (all <12% except where mentioned) and for all elements detected in AMSW RSD were < 2%, so were highly repeatable (discussed below).
- The total peak area (above the baseline) represents the mass of the element in the colloidal fraction and was used to indicate the repeatability of the sample signal response and thus the recovered colloidal mass.
- % RSD values for the total colloidal mass (peak areas) were far higher and more variable than the retention time values for the same runs, ranging from 15 to over 100% in MSW, 1 to over 100 % in MBT and 1 to 28% for AMSW excluding those where only 1 peak was observed.

Repeatability for MSW has already been discussed in Chapter 4 and the factors affecting fractogram repeatability are applicable to all of the leachates used in this chapter.

The results of the replicate injections have shown that it is important to assess how variable the initial sample is prior to performing replicates because this may explain variability in the results. The MBT fractogram repeatability was found to be higher than that of AMSW and MSW for most elements, for both peak retention time and for the total colloidal concentrations. This can be explained by the expected variability in the concentration of injected aliquots of sample which was higher than AMSW and MSW as discussed in Section 5.3.3. Although it may be expected that repeatability of replicate runs would be reduced with lower element concentrations (because of lower signal to noise ratios and counting statistics), this does not appear to be the case when comparing repeatability of MSW and AMSW. Fewer elements however, were detectable in AMSW.

The variability of total colloidal concentrations may also be attributed to the HR-ICP-MS analysis parameters used. The analysis time was optimised to measure multiple elements by reducing the mass windows (see Section 4.2.3.2) and therefore this could result in variations in signal intensity. Increasing the mass windows may result in more repeatable results, however this would reduce the number of elements able to be analysed in a single run and therefore a compromise between number of elements and measurement repeatability may need to be assessed to meet the needs of individual studies.

The aim of this study was to investigate the nanocolloidal element distribution within landfill leachates and therefore although the results may be variable, this method has enabled multiple elements to be assessed in a single fractionation run. In order to obtain less variable results, it would be possible to focus on single elements and optimise the HR-ICP-MS parameters for their analysis thus providing more precise data for modelling purposes. It is therefore important to report all analytical parameters used in order to obtain substantiated quantitative data and subsequent formative interpretation, which are lacking in studies reported in the literature, of all sample types, so far.

Table 5.5 Repeatability of replicate sample injections determined using retention time of Peak 1 and integration of total peak area

	MSW		MBT		AMSW	
Element	Retention Time(% RSD)	Total Peak Area (%RSD)	Retention Time (%RSD)	Total Peak Area (%RSD)	Retention Time (%RSD)	Total Peak Area (%RSD)
Li	1	113	173*	17*3	BD	BD
Mg	2	18	1	153	0	85
Al	3	58	5	51	173*	173*
Si	NA	169	NA	173*	BD	BD
K	0	11	0	47	2	17
Ca	5	37	5	37	BD	BD
Ti	2	16	9	31	BD	BD
V	1	30	7	2	BD	BD
Cr	1	23	7	26	3	3
Mn	1	23	7	26	3	3
Fe	56	18	8	12	1	15
Co	1	23	6	11	0	28
Ni	1	28	6	14	2	3
Cu	2	64	8	32	BD	BD
Zn	1	92	9	38	BD	BD
As	3	13	BD	BD	BD	BD
Br	0	54	1	76	BD	BD
Sr	4	15	2	132	BD	BD
Mo	2	137	173*	173*	BD	BD
Cd	BD	BD	BD	BD	BD	BD
Sn	1	18	10	1	BD	BD
Cs	173*	173*	173*	173*	BD	BD
Ba	2	34	12	34	BD	BD
Au	3	97	3	97	BD	BD
Hg	BD	BD	BD	BD	BD	BD
Pb	1	39	8	49	2	1
U	BD	BD	BD	BD	BD	BD

BD= Below Detection Limits % RSD= % Relative standard deviation n= 3 *= Only 1 peak detected in 3 replicates

5.3.7 Comparison of Nanocolloidal Fraction of UK Landfill Leachates

The nanocolloidal distribution of the three leachates are shown in the fractograms (Figure 5.11 and Figure 5.12) of MW v UV$_{254.,}$ MW v FLU and MW v ppb of all detectable elements.

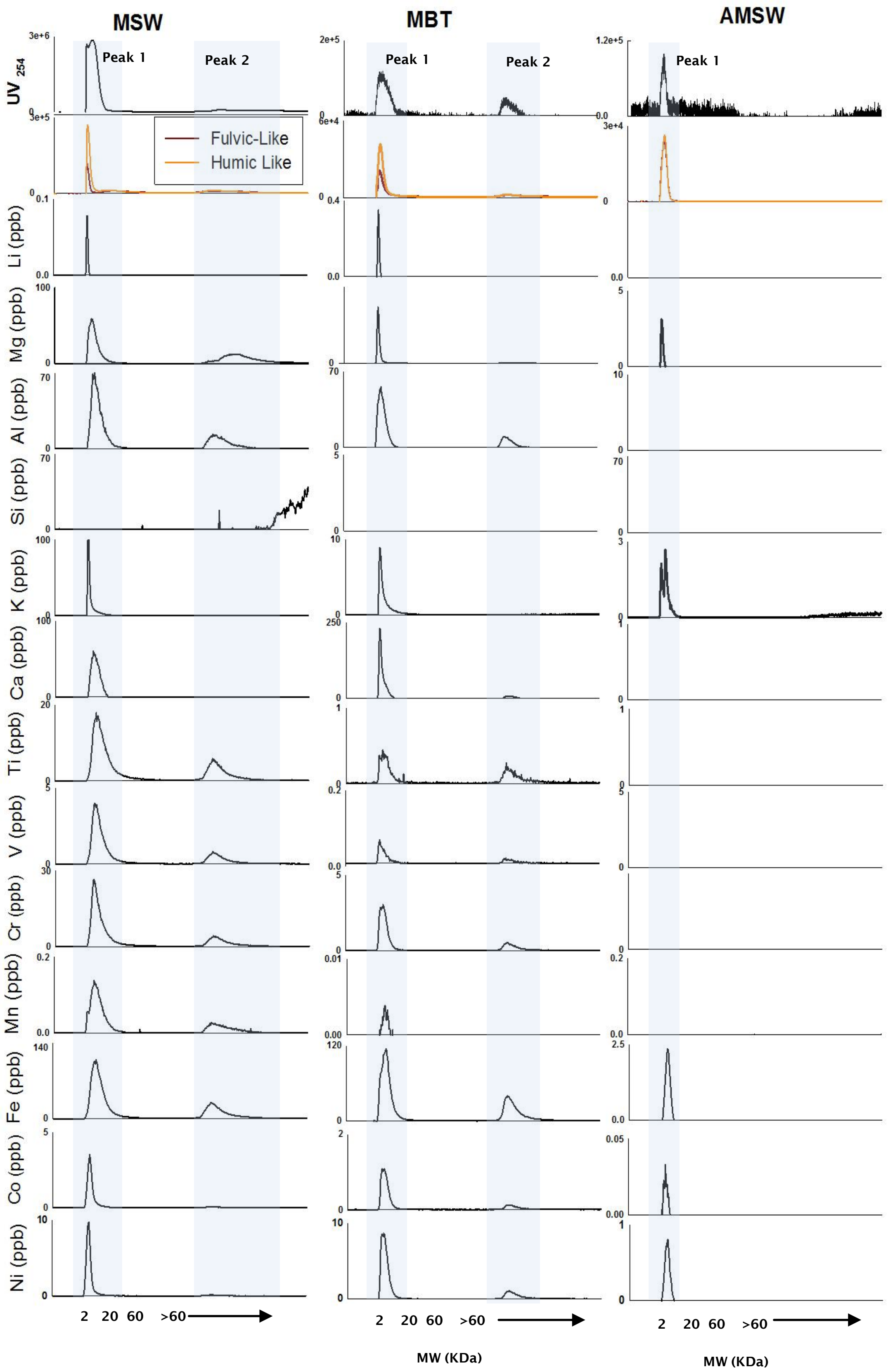


Figure 5.11Nanocolloidal size distribution of UV_{254}, humic-like, fulvic-like and elements Li- Ni in mass order for MSW, MBT and AMSW leachates

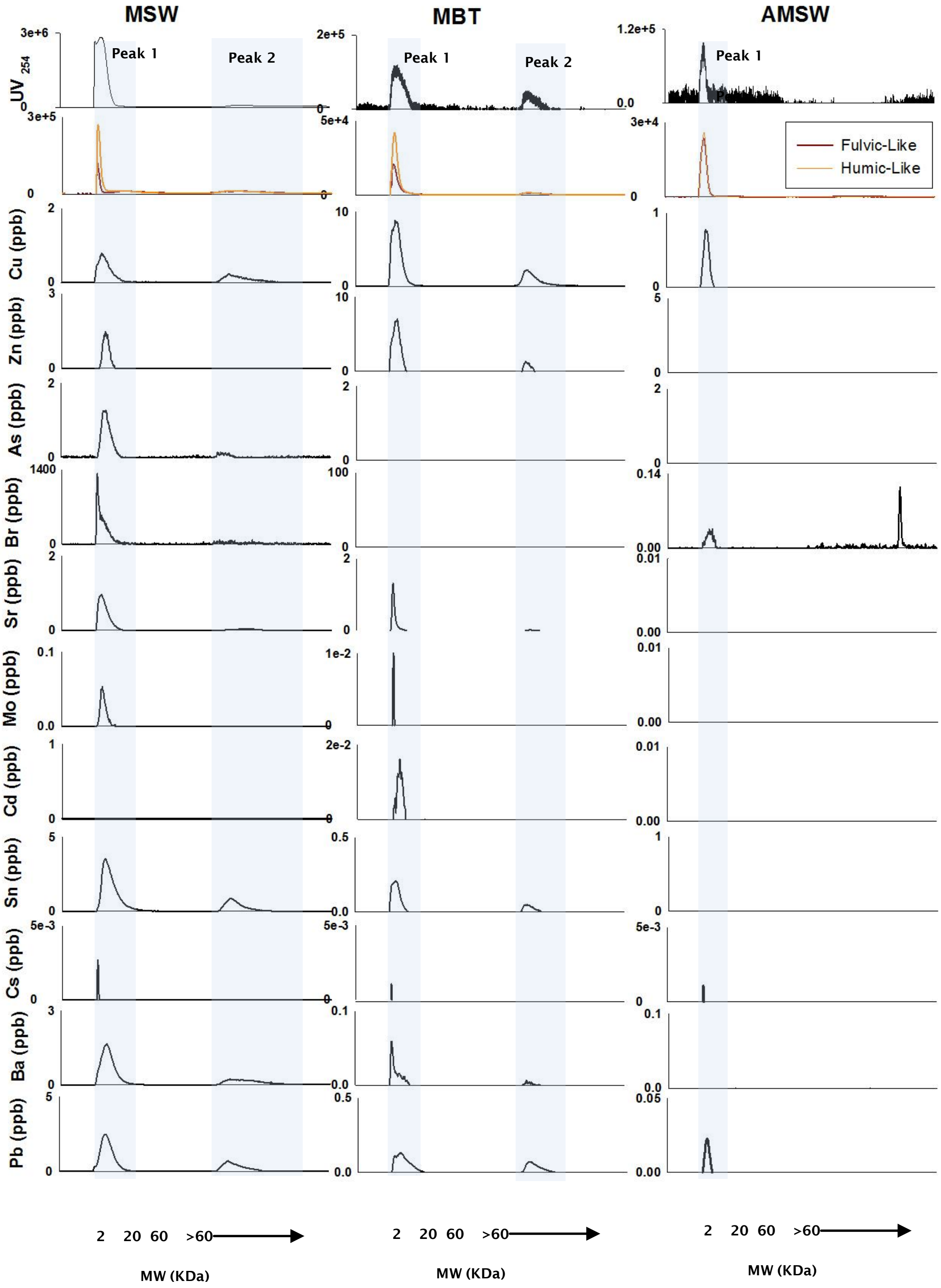


Figure 5.12 Nanocolloidal size distribution of UV_{254}, humic-like, fulvic-like and elements Cu- Pb in mass order for MSW, MBT and AMSW leachates

5.3.7.1 UV and Flu Results for MSW, MBT and AMSW

The distribution of organic nanocolloids in MSW, MBT and AMSW was indicated by the UV_{254} and FLU intensity (

Figure 5.11 (Li-Ni) and Figure 5.12 (Cu-Pb)).

Initial observations include:

- In all three leachates there was a peak in UV_{254} intensity representing low MW organic particles (Peak 1). This was between 2 -20 kDa in MBT and MSW and 2-10 kDa in AMSW.
- A second less intense and broader peak (Peak 2) was present in MBT and MSW leachates indicating the presence of particles in the larger fraction (> 60 kDa). These particles eluted when the cross flow was removed and all remaining material was able to leave the channel, thus this fraction may contain a large variety of colloids of different sizes and compositions.
- The intensity of Peak 2 however, indicates that there was less organic material than in Peak 1 therefore the organic material present was likely to be organic coatings rather than aggregated organic particles (which would have a higher intensity).
- The organic humic-like particles as shown by the fluorescence signal (Excitation: 260 nm, Emission: 450 nm) followed a similar pattern to the UV_{254} signal in all leachates, however in MSW and MBT this peak was narrower than the UV_{254} signal indicating UV_{254} absorbing particles other than humic and fulvic-like in MSW and MBT.
- The organic fulvic-like signal (Excitation: 335 nm, Emission: 450 nm) peak was less intense, narrower and peaked slightly earlier (~ 2 kDa) than the humic- like signal in both MSW and MBT, however the humic-like and fulvic-like signals were synchronous in AMSW and peaked ~ 2 kDa.

5.3.7.2 Element Distributions in MSW, MBT and AMSW

The distribution of elements as revealed by AF4-HR-ICP-MS in the nanocolloidal fraction of MSW, MBT and AMSW is also shown in Figures 5.11 and 5.12 and show two distinct populations of nanocolloids (Peak 1 and Peak 2). Noticeably:

- The distribution profiles of elements in the three leachates match closely with the distribution of organic particles in the leachates and with the exception of Si, all were either present in both the smaller fraction (Peak 1) and the collection of larger unfractionated particles (Peak 2) or present in only the smallest fraction (Peak 1).
- The same distribution patterns between small (Peak 1) and larger (Peak 2) fractions was seen in the three leachates for elements in which there was a detectable concentration in all of the leachates however peak 1 was broader in the MSW leachate for some metals (Fe, Mn,Cr, Ti, V, Sn).
- Larger concentrations of all elements (except Si) were found in the smaller fraction in the MSW, MBT and AMSW leachates which coincides with the largest concentration of organic particles suggesting a strong association between organic nanocolloids and metals.
- The broader Peak 1 in the MSW leachate for some metals may indicate the presence of metal oxide nanocolloids towards the higher MW of Peak 1 which overlap with lower MW organic nanocolloids.
- Si was the only element that was present solely in the large fraction and did not have a distribution similar to any other elements or to the organic particles which indicates that it does not complex with organics or associate with any of the metals.
- Li and Cs showed the same size distribution and peaks were very narrow and appeared at the lowest MW, similar to the fulvic-like signals (only in Peak 1). This suggests they may preferentially bind with fulvic-like particles.
- Mo was only detected in the smallest fraction in MSW and MBT (and was presumably below detection limits in AMSW) and the peak was very narrow and similar to Li and Cs peaks in MBT.
- As was present only in MSW and closely followed the distribution pattern of the organics, with peaks at the same sizes indicating a strong association with organic particles.

- It is difficult to compare the three leachates because concentrations in AMSW were much lower than in MSW and MBT however, the elements that can be detected matched the distribution patterns of that observed in the MSW and MBT Leachates and all detectable elements except Si showed association with organic particles.

5.3.7.3 Percentage Nanocolloidal Distribution

To further illustrate the distribution of elements in the nanocolloidal fraction the colloidal peak areas (µg of recovered mass) were integrated into three fractions. The fractions (< 2 kDa, 2-60 kDa and > 60 kDa) were representative of the fulvic-like particles, the humic-like particles and the larger less organic particles highlighted above. Figure 5.13 displays the integrated areas as a percentage of the total peak area (recovered mass). The integrated areas were calculated using the OriginsPro software.

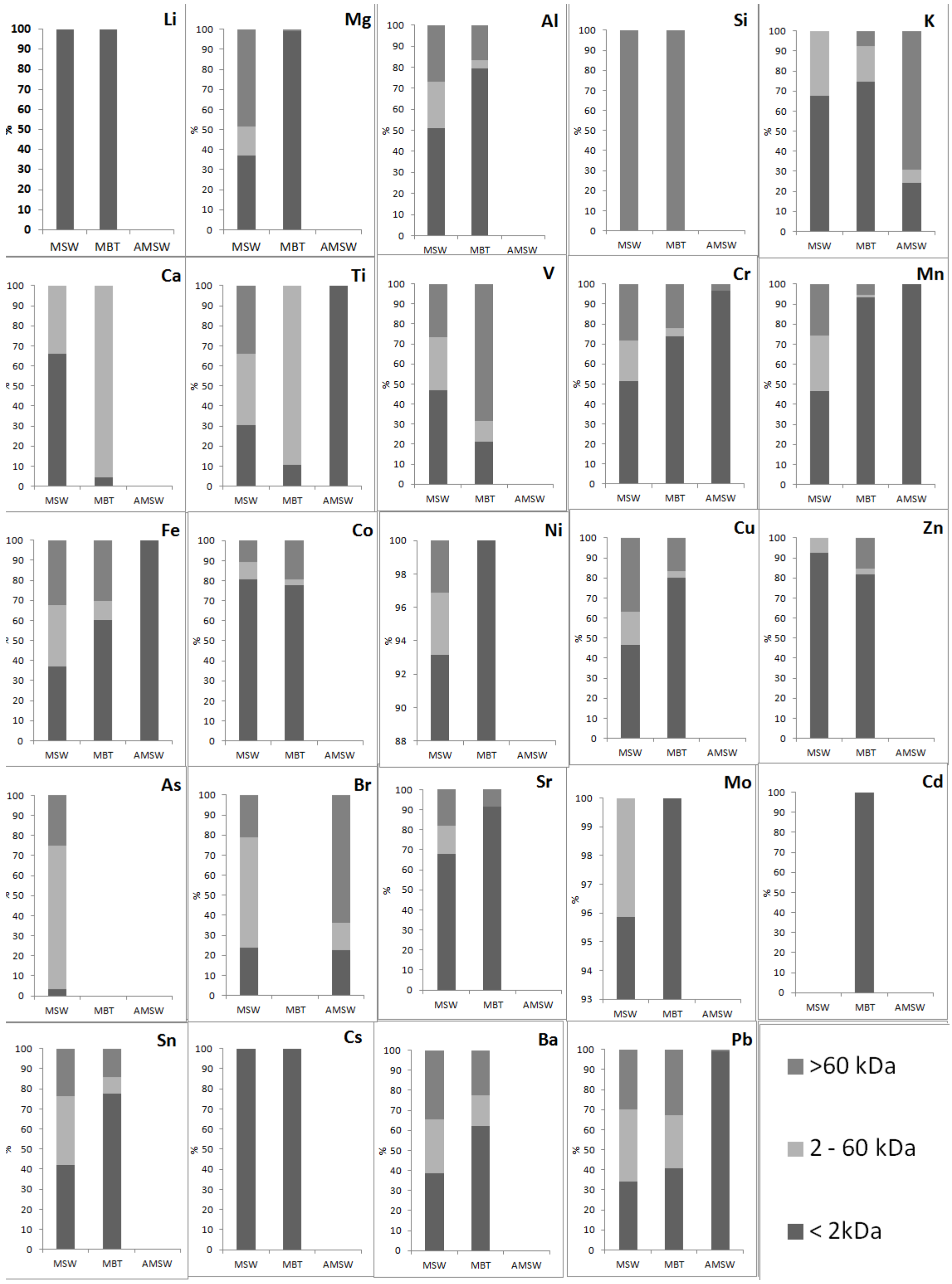


Figure 5.13 Percentage distribution of nanocolloidal fraction determined by integration of total peak areas for MSW, MBT and AMSW as shown in Figures 5.11 and 5.12.

- Li, Cd and Cs were present only in the smallest < 2 kDa fraction which indicates their association with the lowest MW fraction and the fulvic-like particles.
- Si was only present in the largest (> 60 kDa) fraction in MSW and MBT (below detection limits in AMSW) and was the only element with this distribution
- The < 2kDa and 2-60 kDa fractions combined, contained the majority of all the elements apart from K and Br in AMSW which were highest in the > 60 kDa fraction.
- All elements in AMSW were present in the < 2 kDa fraction apart from K and Br.
- MSW had more elements present in the > 60 kDa fraction than MBT and AMSW.
- The partitioning between fractions of Ba and Pb were similar in MSW and MBT.

5.3.8 Leachate Nanocolloid Composition and Distribution

The element distribution results showed that two distinct size populations existed in all leachates, in accord with previous AF4-ICP-MS studies investigating landfill leachates (Dubascoux et al., 2008a) and river studies (Hassellov et al., 1999, Stolpe et al., 2005, Stolpe et al., 2010b). Many landfill studies (Hennebert et al., 2013, Jensen and Christensen, 1999, Jensen et al., 1999, Oygard et al., 2007) have recognised the presence of both organic and inorganic particles in the nanocolloid fraction as well as particles which are a mixture of both however, the filtration methods used in these studies were not able to further fractionate the colloidal fraction to separate the populations.

In this study, a distinct organic-rich fraction was present at lower MW (< 20 kDA), with a less abundant organic fraction also present at higher MW (>60 kDA). The patterns of both the UV and fluorescence peaks were closely matched, indicating that both humic and fulvic components were present in the same type of organic macromolecules as found by (Stolpe et al., 2010b).

It is also worth noting that the fulvic peak appeared at lower molecular weights than the humic peak, which agrees with the findings of Beckett (1987).

Thang et al.,(2001) found that various groundwater humic and fulvic-like substances were distributed in the size ranges of 1.1-1.8 kDa and 1.8-3.3 kDa respectively using FFF which were slightly smaller than those found in this study however, an older FFF version (F-1000, FFFractionation, Inc. (Salt Lake City, USA)) was used and a different carrier solution (Tris buffer). Differing calibration results can however be expected because carrier solution and channel characteristics effect fractionation. The size of the fulvic- like peak in this study at around 2 kDa is typical for that of fulvic- like particles in other studies (Coble, 1996, Buffle et al., 1998) however larger than Beckett (1987) who suggested it was 1.1 kDa.

The fulvic-like peak was smaller than the humic-like peak in MSW and MBT indicating that there were less fulvic-like particles however, this disagreed with a previous study in which NOM was found to be composed of 70 to 80% fulvic acids (Thurman, 1985) although this was not in a landfill but a natural water environment. This suggests that there may be fulvic-like particles in the truly dissolved fraction which could have traversed through the membrane and be lost to the detection system and this is further supported by the size distribution of Li and Cs (discussed further below).

Lyven et al., (2003) investigated freshwater and found that organic-rich fractions were only present at molecular weights < 10 kDa, but this study showed that organic particles were present in fractions > 60 kDa in size. This larger size fraction of organic particles could be caused by the agglomeration of fulvic and humic acids when they were complexed with tri- and tetravalent cations (Bolea et al., 2006) however, due to the lower intensity of the signal it is likely due to coating of organic matter on larger, inorganic particles (Gounaris et al., 1993, Lead and Wilkinson, 2006). This study also suggested that there was a competition between organic and Fe colloids for the binding of trace elements however this was not seen in the results of this study.

Fe-rich particles have been shown to form a distinct class of particles (Stolpe et al., 2010a, Plathe et al., 2013) in river studies. However, Matura et al.,(2012) investigated landfill leachates and concluded that Fe-rich particles only have a minor role in trace element binding in landfill leachates. In this study it appears that there may be Fe-rich nanocolloids in the MSW leachate but they cannot be resolved from the organic rich particles so conclusions cannot be drawn. It appears that in both MBT and AMSW leachates Fe was predominantly found in association with the small organic particles, rather than as a separate inorganic fraction or in free ion form. Benedetti et al.,(2003) also found that Fe distributions had only minor differences from UV_{254} signal intensities, supporting the results in this study and those by Krachler et al.,(2010) where Fe complexed with NOM in the < 10 kDa fraction was observed.

Silica was the only element present in the larger leachate fraction in the absence of organics and the only element that did not appear to completely elute from the system during fractionation, indicating that Si was present in much larger particles than the rest of the elements and did not associate with organic particles or any of the other elements. In a previous landfill leachate study, (Jensen and Christensen, 1999) clay particles were present in a colloidal fraction from 1 to 400 nm which supports the finding here that Si particles may be larger than the majority of nanocolloids in the leachates.

5.3.9 Distribution of Metal(loids) in Nanocolloidal Fraction

It was clear that some elements had significant concentrations associated with the smaller organic-rich fraction, suggesting that they formed organic complexes and thus were colloidal carriers of trace elements. It was apparent, however, that a proportion of some metals were also found in the larger MW fraction that contained a lower organic component. In these cases, the metals appeared to be present in largely inorganic particles, perhaps complexed with organic coatings as mentioned previously.

Li, Cs and Mo showed the strongest association with only the lowest MW organic-rich fraction, which suggests that these elements formed organic complexes with fulvic-like substances rather than humic-like. It is possible that the low proportion of these elements present in this fraction may be due to very low MW < 1 kDa fulvic metal complexes passing through the membrane suggesting that these elements have high dissolved concentrations. This is supported by the high dissolved proportion (70-100%) for these elements shown in Figure 5.4.

Cs was found by Bouby et al.,(2011) to be barely bound to colloids, but this study used a 5 kDa membrane, therefore the fraction present in the smallest peak in this study will have eluted with the cross flow via the membrane making comparison with this study difficult. They suggested however, that metal ions preferentially sorb to smaller colloids because of their higher specific surface area which would support preferential binding to smaller fulvic particles.

The first row transitional metals all showed similar distribution between the two populations in the MSW and MBT leachates however Peak 1 in MSW was broader for Fe, Mn, Cr and Ti,. In addition to finding the association of this group of metals with low MW humic substances, the study suggests that the presence of these metals with much larger particles may be due to organic coatings on larger inorganic NP as discussed by Gounaris et al.,(1993). It appears that this group of elements is largely complexed by low molecular weight organic-rich NP (that may form coatings on larger inorganic NP) however metals existing as a separate inorganic form were also possible.

Bolea et al.,(2006) suggested that polyvalent ions may be more likely to form complex species with humic substances than monovalent ions but they could not explain this influence with only humate/fulvate complex information. The findings of this study support this suggestion with polyvalent elements such as Cu and Pb forming organic complexes with all humic substances whereas monovalent Li and Cs formed complexes with only the lowest MW humic substances and appeared predominantly in truly dissolved form .

Previous studies of landfill leachate have yielded conflicting results. Matura et al., (2010) found that organic-metal complexes formed a very small fraction of the total metal speciation and concluded that most metals were present in inorganic colloid complexes. In contrast, others have found that metal-organic complexes are a major component of landfill leachates (Jensen and Christensen, 1999, Jensen et al., 1999, Li et al., 2009a). The results of this study appear to agree with the findings of the latter and an appreciable amount of all elements is associated with organic complexation however it is possible that within the < 60 kDa organic rich fraction there were both organic and inorganic nanocolloid populations which overlapped.

In previous landfill leachate studies, Pb has been found to be largely affiliated with low MW particles (Jensen and Christensen, 1999, Li et al., 2009a), however the results here showed that Pb was associated with both small and larger particles, both organic and inorganic, as well as a significant amount present in the dissolved fraction. Gounaris et al.,(1993) also found that Pb was either dissolved, or associated with non-humics in landfill leachates using ultrafiltration however, organic complexation was not observed which contrasts with this study. These previous leachate studies however, have not used the same method as in this study making comparisons difficult.

Bolea et al.,(2006) investigated compost leachates rather than landfill leachates, but their characteristics were similar to the leachates used here and they used the same separation methods, hence some comparison with this study can be made. They found that Pb was able to form separate colloidal phases from the organic-rich fraction, however it appears that in this study Pb was closely associated with the organic fraction because in AMSW, with no second peak, no Pb was present in the larger fraction.

Worms et al.,(2010) used wastewater effluent in their study however the results were comparable because of the methods used. They found that whilst Pb was associated with larger NP, it was equally bound with low and high MW particles. A competition for binding of Pb between low and high MW particles was suggested; with low MW particles having a higher affinity for metals which supports the larger, lower MW, peak in this study.

As a divalent cation, Zn would be expected, according to Bolea et al.,(2006), to complex with small organics. This was also shown by it’s distribution in the three leachates in this study, which is in agreement with the findings of Jensen and Christensen (1999).

Cd was only detected in MBT and was present in the low MW fraction which has been seen in a previous landfill leachate study (Lu et al., 2009) as well as a wastewater study (Worms et al., 2010) and thus if Cd was present in higher concentrations in MSW and AMSW it would be expected to show the same size distribution pattern.

Si was present only in the largest size fraction, suggesting that it may be able to exist in a distinct NP form, perhaps forming SiO_2 colloids although it was mostly present (> 90%) in the dissolved fraction. Additionally, Si did not appear to elute completely from the system in the 120 minute fractionation time. It may be that the results for the dissolved: colloidal distribution were not entirely representative of the Si in leachates because not all Si was recovered in the 120 minute period. There are limited studies to compare the Si distribution with, because it has been shown to be difficult to detect using ICP-MS because of it's high background levels (Bouby et al., 2008). Because of this, in their study, Al was therefore chosen as an indicator for clay colloids. However this study showed that Si and Al have a different distribution and therefore that Al cannot be used as a clay colloid indicator in leachates. They also found that there was a clear size separation of humic (3 to 20 nm) and clay colloids (30 to 200 nm) (as indicated by Al) however, this was not seen here and Al was instead complexed with organics. Hennebert et al.,(2013) also found Al and Si particles to be separate and thus were not identified as aluminosilicates but instead were associated with Na, Ca and Mg.

Wu et al., (2012) suggested that humic functional groups e.g. phenols which make up humic substances play an important role in determining metal-particle associations, but these have not been investigated in this study. Indeed, it may be that elements which have a more complicated distribution may benefit from identification of these groups in the different types of particles.

Without a clear pattern from atomic weight or valency to indicate element affiliation to dissolved or other fractions it would be fair to suggest that those elements not present or in low amounts in dissolved fractions were either able to form their own NPs or that when competing with others, these were able to form complexes quicker or easier than other elements and thus are more likely to be present in the colloidal fraction.

5.3.10 Comparison of Elemental Distribution between Leachates

The only difference between the nanocolloidal distributions of the three leachate types was that the size range of the lower MW fraction (Peak 1) in MSW was broader for some metals however the overall distribution of elements between the two populations was similar for all leachates.

The broader low MW peak for some metals in MSW indicated that these metals were not only complexed with organic particles but may have also been able to form their own inorganic fraction which formed the upper range of the low MW fraction. Fe-rich nanocolloids have been observed in river studies (Stolpe et al., 2010a, Plathe et al., 2013) alongside organics and shown to associate with both Mn and Cr (Stolpe et al., 2010b, Stolpe et al., 2014). It is therefore possible that there was a separate Fe and/or Mn population which overlapped with the organic population in the low MW fraction however this cannot be substantiated with the results of this study.

The potential Fe-rich population was only observed in the MSW leachate and could perhaps be explained by the differences in redox chemistry in the leachates. The MSW leachate was the youngest leachate and thus would be less methanogenic than the older leachates (See Figure 1.1) and the environment less reducing (Christensen et al., 2001). Both Fe and Mn can be subject to redox processes so it is possible that the reduction of the nanocolloidal Fe and Mn oxides have resulted in the true dissolution or precipitation of this Fe and Mn in the MBT and AMSW leachates. The MSW leachate did however contain higher concentrations of Fe (Table 5.3) and Mn than MBT and AMSW which could also explain this result.

It is important to also recognise that although the landfill leachate would be anaerobic in situ, these conditions were not maintained when sampling and analysing the leachates and could affect the results.

Noting any similar distribution between the leachates of different types is in contrast to previous studies (Jensen and Christensen, 1999, Li et al., 2009a) in which no distinct behaviour of individual metal distributions were found and thus reinforces the benefits of AF4-HR-ICP-MS compared with filtration methods.

It could be hypothesised that organic nanocolloids within the leachate become saturated as metals and metalloids complex. Therefore if the organic concentration was increased, the amount of metals complexed with organic nanocolloids would increase and the amount of truly dissolved metals reduce. A young leachate (higher organic concentration than an older leachate) would therefore have higher amounts of metals present in the colloidal fraction than an older leachate because there are more humic and fulvic components available for complexation. The AMSW leachate results support this statement because lower amounts of elements (0 to 55%) are found in the colloidal fraction when compared with the younger MSW (0 to 100%). This is also in agreement with the findings of Li et al.,(2009a) in which less colloidal OM was found in an aged MSW leachate compared with a young MSW leachate.

It can therefore be suggested that over time the amount of metals present in larger size fractions of MSW leachate will decrease, as the OM in these fractions degrade. The humic and fulvic-like particles may also change chemically with age as the amounts of O-containing functional groups in humic and fulvic acids have been shown to decrease with age (Xiaoli et al., 2008). Thus, the distribution of metals in MSW will likely become similar to that of AMSW with age as humification and chemical changes occur.

The similarities between MSW and MBT metal/nanocolloidal distributions suggest that whilst MBT literature is limited, MSW leachates may be used to provide data for MBT when investigating colloidal distributions. The similarities also suggest that whilst MBT wastes produce less landfill gas and have a smaller volume than MSW leachates, the regulations imposed on landfilling waste do not have any impact on the potential for colloidal transport in the environment compared with traditional MSW waste.

The comparison of the leachates indicates that element distributions do not change significantly over time and that mechanical biological pre-treatment of MSW waste does not impact significantly on the element distributions within this size fraction

5.3.11 Influence of pH on Element Distribution in MSW

The effect of lowering the pH of MSW from 8.3 to 7, 6 and 5 (Section 5.2.2.3) on the elemental distribution of MSW is illustrated in Figure 5.14 and Figure 5.15. These results are the data from one injection of each sample modification.

In brief:

- No clear effects of the lowering of pH on the elemental distributions were seen at all lowered values.
- The distribution of elements followed the same pattern as the MSW sample in that an initial peak at lower retention times (Peak 1) was visible followed by a much smaller broad peak at higher retention times (Peak 2).

The pH of a leachate may be altered if it escapes in to the subsurface environment or if acid/alkaline water infiltrates into the landfill. However, this study shows that there is little effect on the nanocolloidal distribution of elements when MSW was lowered to pH 5. This may be due to the small effect that the pH change has on the organic content of the sample. As discussed in Section 5.3.9 and 5.3.10, the distribution of elements in the nanocolloidal fraction appears to be controlled by the organic content and therefore little effect of pH on distribution may be observed. A study by Balnois et al.,(1999) investigated the effects of pH between 3- 10 on humic substances using AFM and found no aggregation of humic substances within this range and this supports the results seen here.

Mohd Omar et al., (2014) found that whilst pH did influence the stability of NP, this was reduced with the addition of SRHA and thus the high OM content in the MSW leachate (> 450x higher than the SRHA concentration of 5 mg/L) compared with their study may reduce the influence of pH on aggregation of particles and thus the distribution of elements. It must also be noted that both of these stuides used synthetic environmental samples and therefore it is difficult to compare the findings of the studies.

The shape of humic substances has shown however, to be strongly influenced by pH and at low pH they are fibrous, at neutral more sponge-like and at higher pH they are more plate like (White, 2013). Therefore, although no change in the size distribution with pH is seen, shape changes could have occurred which may impact on transport of these nanocolloids in the environment (Seymour et al., 2013).

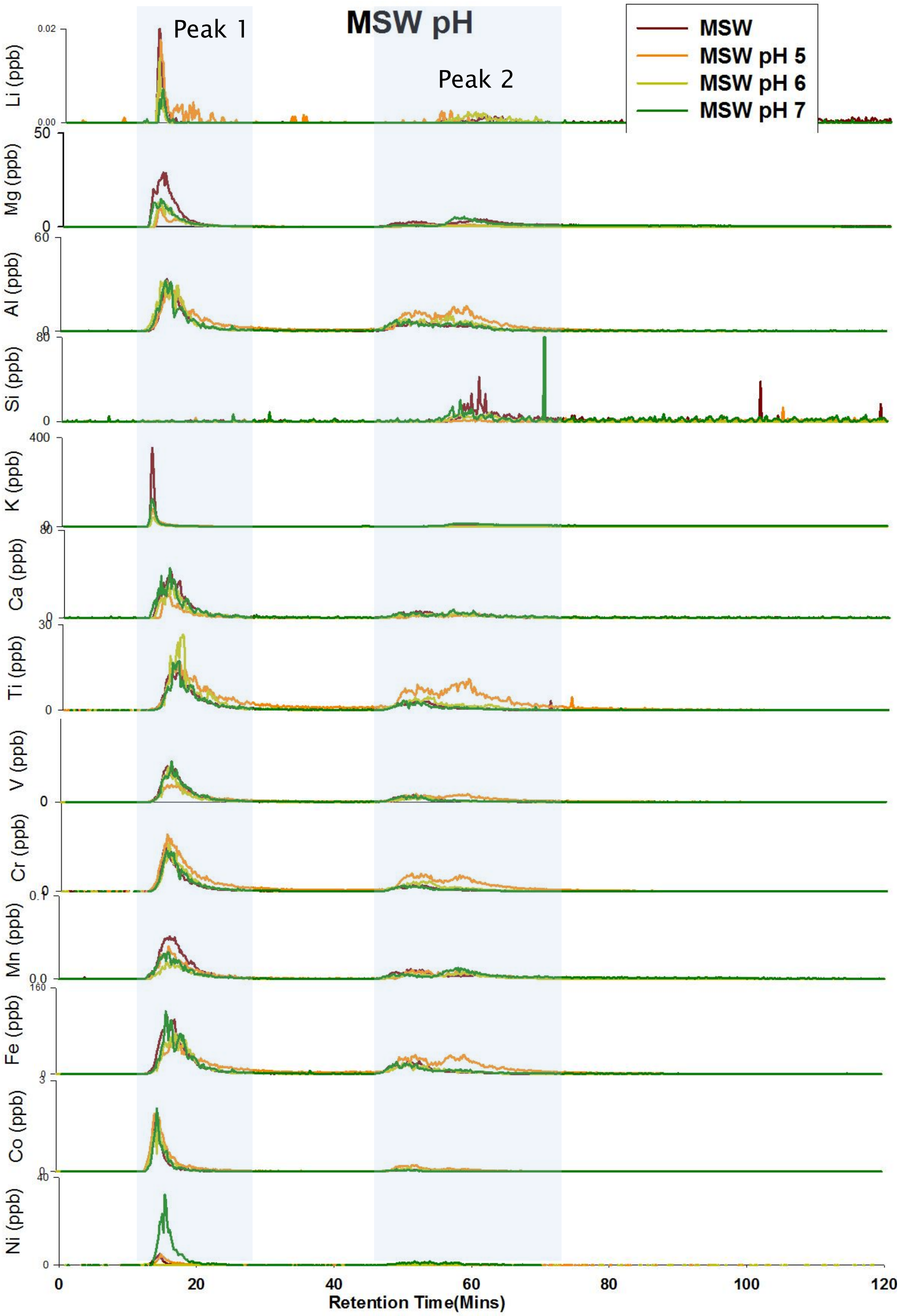


Figure 5.14 Influence of pH change on elemental distribution (Li- Ni in mass order) in MSW leachate.

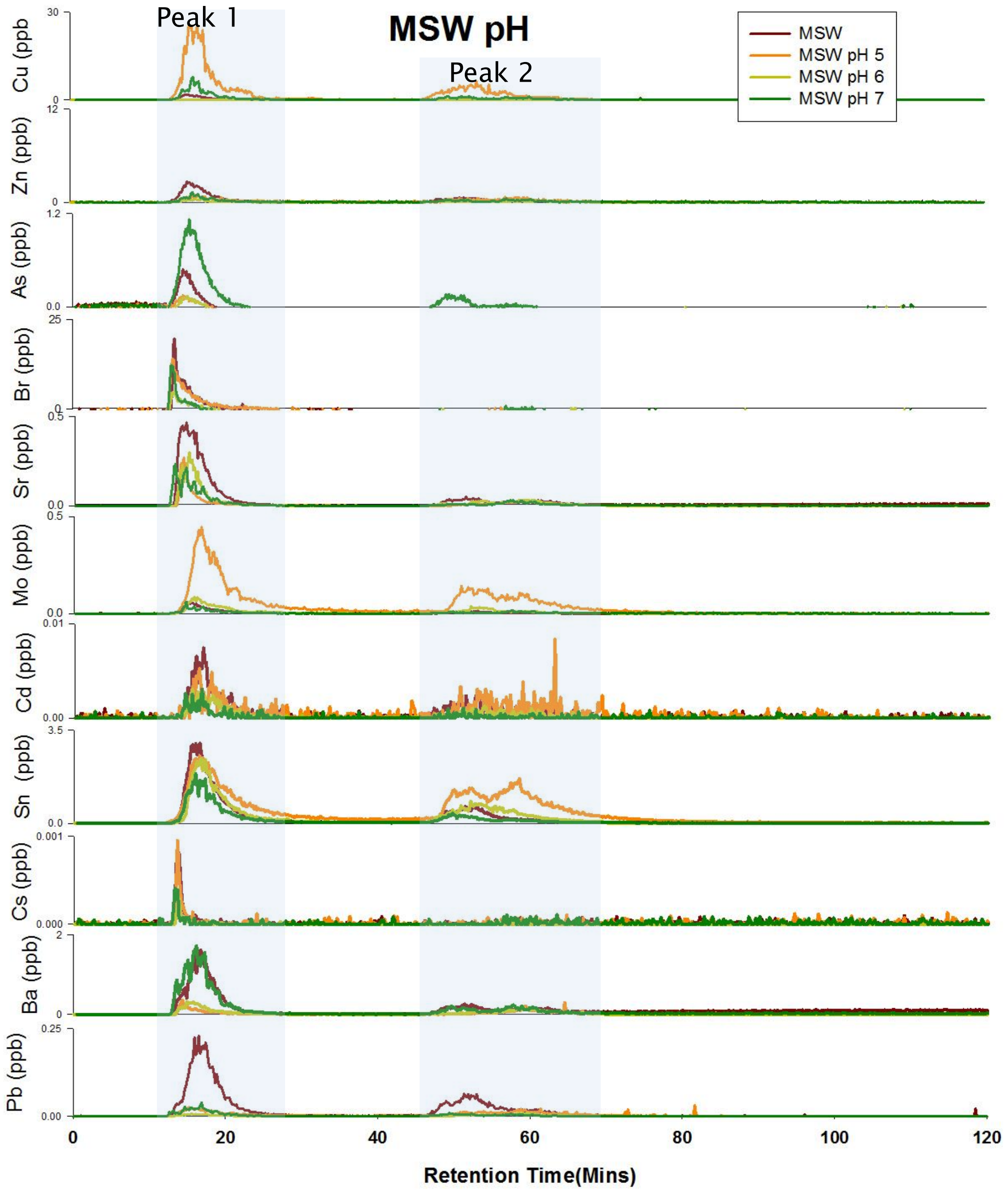


Figure 5.15 Influence of pH change on elemental distribution (Cu- U in mass order) in MSW leachate.

5.3.12 Influence of Ionic Strength on Element Distribution in MSW

The effect of lowering the ionic strength of MSW on the size distribution of elements by diluting with Milli-Q (Section 5.2.2.2) is displayed in Figures 5.16 and Figure 5.17.

- No effect of lowering the ionic strength on the distribution of elements was observed apart from Fe, Mn and Cr at a dilution of 1:50 in which, for these elements, Peak 2 increased in size.
- For most elements the modified samples showed the same distribution as the initial sample with the majority of each element contained in the low MW fraction (Peak 1) and a smaller flatter peak present in the high MW fraction (Peak 2) containing lower concentrations of the element.
- For Fe, Peak 2 was larger than Peak 1 indicating that more of this element was now present in the higher molecular weight fractions than the lower. This was not observed at the 1:100 dilution.
- For Mn and Cr, Peak 2 was not larger than Peak 1 however, it increased proportionally compared with Peak 1 and thus the elements were more evenly distributed between low and higher MW fractions.

The lowering of ionic strength of a leachate may occur when a leachate escapes in to the subsurface environment or is diluted by intrusion of water in to the landfill. The results in this study indicate that as a leachate is diluted, Fe colloids which were most likely previously bound to or coated with OM may be able to form their own population which is associated with Mn and Cr.

This theory is supported by the findings of Baalousha et al., (2008) who suggested that the stability of colloidal Fe is highly dependent on adsorbed OM and therefore in the MSW leachate, the high OM content is supporting the stability of smaller Fe colloids bound with OM and restricting their aggregation. As the MSW is diluted, the OM content decreases and the Fe colloids are therefore less stable and aggregation occurs.

This conflicts with the results of Stolpe and Hassellov (2007) who found that simulated mixing of freshwater with synthetic seawater to increase the ionic strength, induced aggregation of the Fe-rich colloidal matter.

The differences however are the environments, landfill leachates have typically higher TOC contents than rivers and as such the OM is more dominant in controlling the colloidal population than in rivers. Mn and Cr have however, both been found associated with Fe colloids in rivers (Stolpe et al., 2010b, Stolpe et al., 2014).

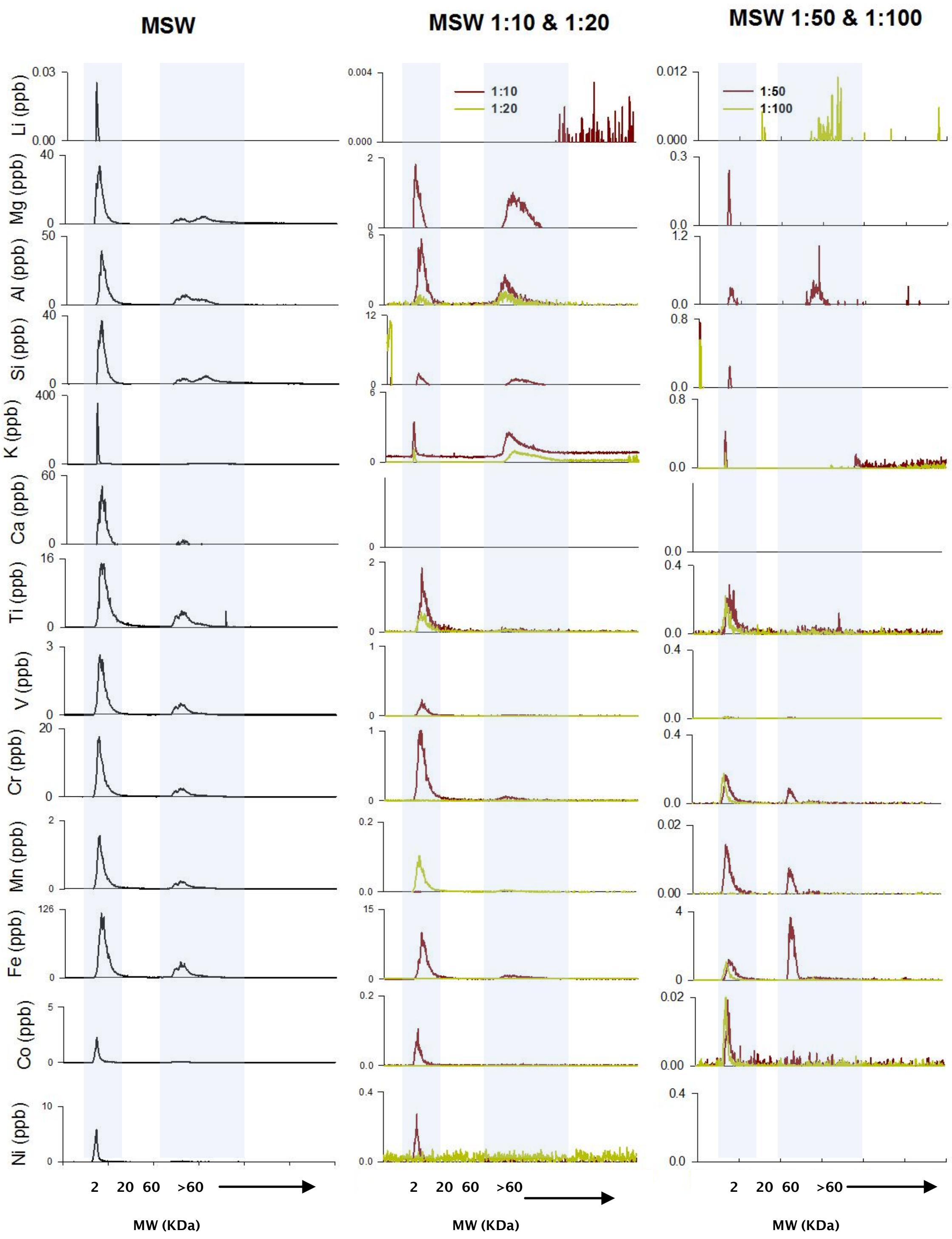


Figure 5.16 Influence of ionic strength on elemental distribution (Li-Ni in mass order) in MSW leachate.

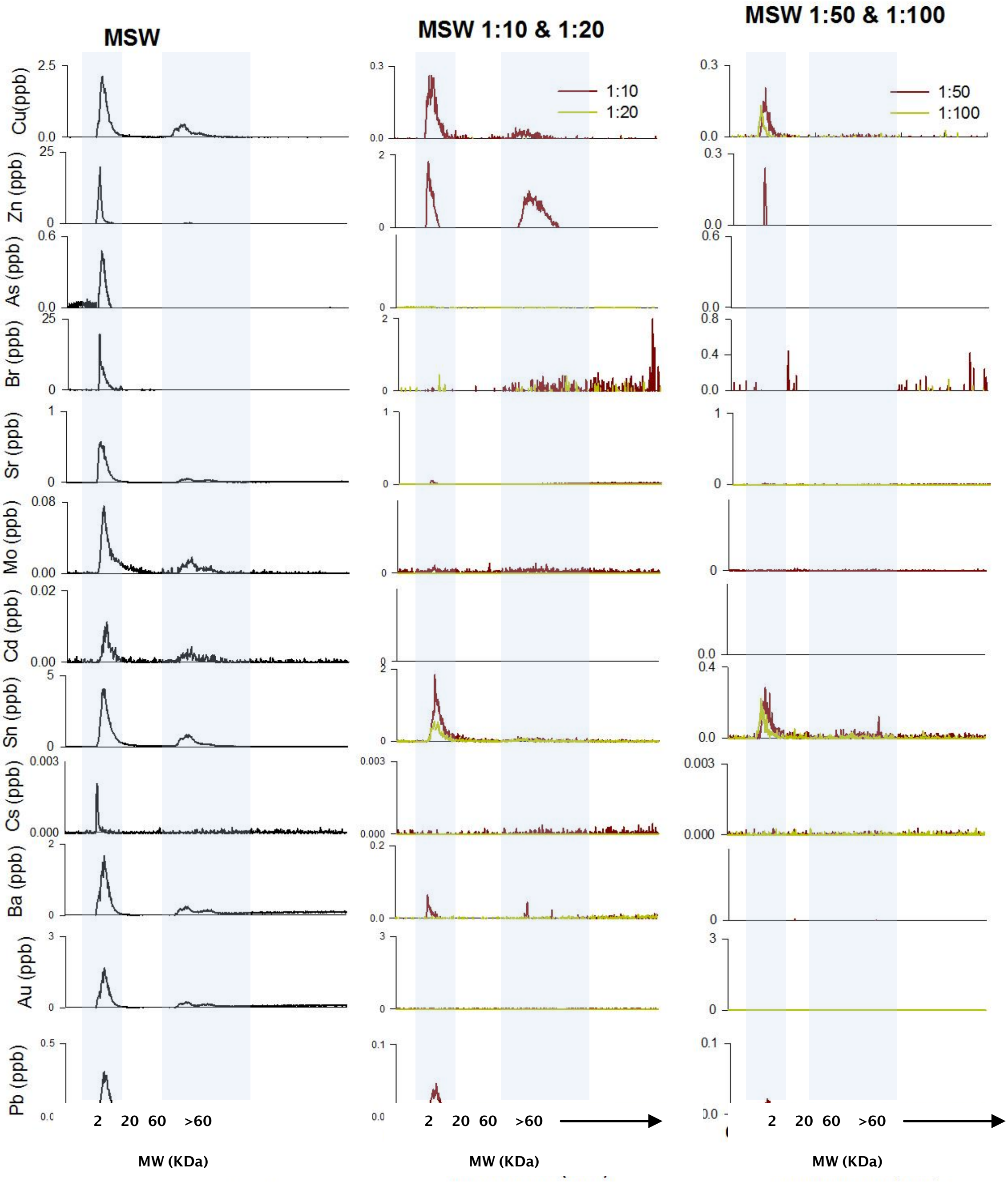


Figure 5.17 Influence of ionic strength on elemental distribution (Cu – Pb in mass order) in MSW leachate.

5.3.13 Implications of nanocolloidal element distributions for the environment

The results of this study clearly show that current risk assessments, in which all elements present below 0.45 μm are assumed to be present as free ions and therefore travel as dissolved components, do not realistically model the subsurface environment.

Humic substances tend to be highly refractory and therefore resist biological decomposition and have a high residence time in water, from weeks to thousands of years (White, 2013). Metals bound to humic substances may therefore be more persistent in the environment than if they were in dissolved form because the OM is not likely to degrade and thus remobilise the metals.

Fulvic-like particles have been shown to be more mobile than humic-like (Weng et al., 2002) due to their smaller size and Persson et al.,(2006) has found that particles with lower MW travel further away from a landfill in a leachate plume. Therefore the finding that a significant proportion of metals are complexed with low MW fulvic and humic-like particles suggests that these potentially toxic metals are likely to be more persistent in the environment and may travel further away from a landfill than metals complexed with larger particles in the subsurface environment .Thus metal/nanocolloidal interactions must be accounted for in risk assessment models.

The finding that the majority of toxic metals are complexed with organic matter also has implications for the treatment of landfill leachates in that removal of organics through processes such as lime precipitation (Renou et al., 2008) would likely also remove contaminants.

5.4 Conclusions

The key findings of this chapter concerning the MSW, MBT and AMSW leachates were:

DLS and AFM both confirmed the presence of nanocolloids in the leachates however they detected a different range of particle sizes within the same leachates and must therefore be used in conjunction. DLS was not able to detect particles below 30 nm in all three leachates, presumably due to masking of smaller particles by larger particles. AMSW showed the highest range of particle sizes compared with MSW and MBT.

HR-ICP-MS analysis of bulk concentrations of the MSW, MBT and AMSW leachate showed that MSW had typically higher element concentrations than the other leachates and that MBT element concentrations were more variable than MSW and AMSW indicating a less homogenous leachate.

AF4-HR-ICP-MS was capable of analysing the distribution of 24 elements in the nanocolloidal fraction of landfill leachates. The fraction collection of cross flow and channel elutants provided mass balance recovery data to compliment AF4-HR-ICPMS fractograms. Although AMSW had significantly lower element concentrations than MSW, this did not affect the recovery results. MSW, MBT and AMSW all showed real losses in the AF4 system for some elements, but there was no apparent pattern between particular elements within the leachates.

The mass balance recovery showed that the majority of metals in the nanocolloidal fraction were in fact truly dissolved (< 1 kDa) however a proportion of metals (1-100%-variable between elements and leachates) were present in the nanocolloidal fraction and thus may be affected by colloid facilitated transport.

The AF4-HR-ICP-MS analysis alongside UV_{254} and FLU revealed that within the nanocolloidal fraction of MSW, MBT and AMSW leachates there were two distinct populations:

1) An organic-rich low MW fraction

   The OM rich low MW can be further divided in to two sub fractions, humic-like and fulvic-like with fulvic-like the smaller of the two.

2) An inorganic-rich higher MW fraction with organic coatings

The OM was found to be a host for most metal(loids) which preferentially bound to OM rather than inorganic nanocolloids and the majority of these metal(loids) were associated with the fulvic-like particle (< 2 kDa in this study).

Si did not interact with other particles or elements and appeared to form its own colloids whilst Li and Cs were bound to fulvic-like particles only.

There was no difference in the element distribution in nanocolloids between the MSW, MBT and AMSW leachates suggesting that the effect of recent regulations, such as the pre-treatment of waste or the age of the waste, do not alter the distribution within the nanocolloidal fraction.

The lowering of MSW leachate pH from pH 8.32 to pH 5 did not alter the distribution of elements in the nanocolloidal fraction of MSW leachate, thus implying that mixing of leachates with lower pH value waters would not impact on the potential for colloid facilitated transport.

The lowering of the ionic strength of MSW leachate did however appear to promote aggregation of Fe nanocolloids (associated with Mn and Cr), likely caused by the decrease in OM content which controls the distribution of elements in the nanocolloidal fraction. Thus nanocolloidal Fe, Mn and Cr may be less mobile at lower ionic strengths due to their larger particle size.

Chapter 6: Conclusions and Future Perspectives

6.1 Conclusions

This thesis aimed to investigate the behaviour of metals and metalloids in the nanocolloidal fraction of leachates in order to obtain quantitative data of the partitioning of these species between the various components of the “dissolved fraction” that may be used to enhance landfill risk assessment models.

To achieve this, the research was conducted in two parts:

1) The first part of the research focused on the optimisation of techniques to enable the quantitative analysis of nanocolloids in landfill leachates. This required extensive method development for the AF4 fractionation and for the coupling of AF4-HR-ICPMS. The main findings from the method optimisation were:

AF4 method parameters must be optimised to consider compatibility with leachate samples (e.g. effects of carrier solution on the sample) and compatibility with HR-ICP-MS analysis (e.g. concentration of elements of interest in the carrier solution compared with sample concentration).

Separating and fraction collecting leachate samples using AF4 for subsequent analysis with HR-ICPMS was shown to be a useful tool for preliminary and complementary experiments prior to the online coupling of AF4-HR-ICP-MS. Whilst the offline method was able to provide information on element distribution, the online coupling of AF4-HR-ICP-MS provided higher size resolution and lower detection limits than the offline fraction collecting method.

The interface between AF4-HR-ICP-MS (diluting AF4 elutant with internal standard spiked HNO_3 and splitting the flow to match HR-ICP-MS uptake) was shown to be integral for optimising and monitoring the performance of the online system. The internal standards were of particular importance for flow monitoring and it was demonstrated that these must be selectively chosen for each sample type to reduce interaction and interference with sample element concentrations, the carrier solution and the AF4 system.

The data transformation steps (flow, sensitivity, baseline and concentration corrections) presented in this study were shown to be successful in quantifying element concentration and the internal standard was integral for these corrections. The sensitivity baseline sample was particularly useful for correcting for sensitivity variations. Accurate reporting of the data transformation was recommended so that the data can be assessed with these transformations in consideration.

The introduction of a baseline measurement provided information about sample carryover and showed that the baseline concentrations in the AF4 system were in flux due to increasing system inputs. A baseline measurement was recommended between each sample injection so that a new baseline was established for use with the next sample measurement and thus to ensure that representative results of sample concentrations were obtained.

Repeatability assessments using peak retention times and the total colloidal fraction concentration were shown to be useful for method validation. Fraction collecting the cross flow and channel elutants provided an indication of the mass balance in the system and was an improvement on previous recovery assessment approaches because it identified that real sample loss occurred in the system. It was also able to infer the split between colloidal and dissolved fractions and therefore it was recommended to be conducted for each sample and set of AF4 parameters.

The first part of this thesis presented the first approach to quantifying multiple elements associated with colloidal size fractions in a landfill leachate using AF4-HR-ICP-MS considering the baseline, mass balance of the system and repeatability of the method.

2) The second part of this research was to apply the techniques developed to landfill leachate samples, thus validating the method and providing knowledge on the distribution and interaction of metals and nanocolloids in leachates. This part of the research showed that:

.DLS and AFM can both be used to investigate the presence of nanocolloids within leachates however they detect different ranges of particle sizes within the same leachates because of the different principle used to determine particle size. They must therefore be used in conjunction.

DLS was not able to detect particles below 30 nm in all three leachates, presumably due to masking of smaller particles by larger particles. AMSW showed the highest range of particle sizes compared with MSW and MBT.

HR-ICP-MS analysis of bulk concentrations of the MSW, MBT and AMSW leachate showed that MSW had typically higher element concentrations than the other leachates and that MBT element concentrations were more variable than MSW and AMSW indicating a less homogenous leachate.

AF4-HR-ICP-MS was capable of analysing the distribution of 24 elements in the nanocolloidal fraction of landfill leachates. The fraction collection of cross flow and channel elutants provided mass balance recovery data to compliment AF4-HR-ICP-MS fractograms. Although AMSW had significantly lower element concentrations than MSW, this did not affect the recovery results. MSW, MBT and AMSW all showed real losses in the AF4 system for some elements, but there was no apparent pattern between particular elements within the leachates.

The mass balance recovery showed that the majority of metals in the nanocolloidal fraction were in fact truly dissolved (< 1 kDa) however a proportion of metals (1-100%-variable between elements and leachates) were present in the nanocolloidal fraction and thus may be affected by colloid facilitated transport.

The AF4-HR-ICP-MS analysis alongside UV_{254} and FLU revealed that within the nanocolloidal fraction of MSW, MBT and AMSW leachates there were two distinct populations:

1) An organic-rich low MW fraction

The OM rich low MW can be divided in to two sub fractions, humic-like and fulvic-like, with fulvic-like the smaller of the two.

2) An inorganic-rich higher MW fraction with organic coatings

The leachate OM was found to be a host for most metal(loids) which preferentially bound to OM rather than inorganic nanocolloids and the majority of these metal(loids) were associated with the fulvic-like particle (< 2 kDa in this study).

Si did not interact with other particles or elements and appeared to form its own colloids whilst Li and Cs were bound to fulvic-like particles only.

There was no difference in the element distribution in nanocolloids between the MSW, MBT and AMSW leachates suggesting that the effect of recent regulations such as the pre-treatment of waste and the age of the waste do not alter the distribution within the nanocolloidal fraction.

The lowering of MSW leachate pH from pH 8.32 to pH 5, did not alter the distribution of elements in the nanocolloidal fraction of MSW leachate thus implying that mixing of leachates with lower pH value waters would not impact on the potential for colloid facilitated transport

The lowering of the ionic strength of MSW leachate did however appear to promote aggregation of Fe nanocolloids (associated with Mn and Cr), which was likely caused by the decrease in OM content which controls the distribution of elements in the nanocolloidal fraction. This aggregation may reduce the mobility of Fe, Mn and Cr in the nanocolloidal fraction.

Thus this thesis has shown that current risk assessments may not accurately predict the potential transport of pollutants in the environment because they do not account for metal/nanocolloid interactions in landfill leachates and therefore these must be incorporated.

6.2 Future Perspectives

Whilst the methods applied in this study have shown to be successful in providing quantitative data about nanocolloid behaviour, there are some alterations to the analytical techniques and additional data analysis which would benefit landfill risk assessment models.

Injecting larger sample volumes would likely achieve more repeatable results and overcome precision issues which stem from low CPS values. Larger volumes may also reduce detection limits, allowing for an increased number of elements to be measured. This would however require longer focusing times and may increase sample carryover and baseline concentrations.

This study has focused on the low MW colloids in leachates because as expected, and as shown, the majority of metals and metalloids are present in this fraction. Fractionation runs with lower cross flow to separate higher MW colloids would provide further information on inorganic colloids and complexes as well as the behaviour of Si in the leachate.

Additional data analysis may provide supplementary information for incorporation into risk assessment models.

Correlation analysis of the interactions between elements in the nanocolloidal fraction may highlight interactions that were not obvious from visual observation and would also quantify them. This would require a powerful statistical software programme due to the large number of data points.

Further data analysis could also be undertaken to determine the types of clays and minerals present in the leachate fractions by examining ratios e.g. Al:Si. Furthermore, chemical speciation software could predict compounds.

Investigation of a larger number of leachate types would also provide more substantive data for incorporation into risk assessment models.

6.2.1 Complementary Research

To complement the research undertaken in this study, experiments to examine sorption and transport of nanocolloids in landfill leachates and the surrounding environment could also be undertaken.

AF4-HR-ICP-MS analysis before and after batch sorption experiments (as described in Environment Agency (2003)) consisting of leachate and landfill lining material (e.g. clay) would provide insight in to how the nanocolloids interact with lining materials and provide further data to be incorporated into risk assessments.

Column transport studies (also described in Environment Agency (2003)) in conjunction with AF4-HR-ICP-MS could further provide data for risk assessment models, by indicating the transport behaviour of nanocolloids. This approach could also be used to explore the transport of manmade NPs in a landfill environment. NPs such as Au or Ag (which are of low concentrations in the leachates presented here) could be added to leachate and thus information on the transport of natural and manmade NPs could be ascertained.

Although this study has focused on the association of metals with nanocolloids within landfill leachates, there are other contaminants e.g. pesticides which may be present in the nanocolloid fraction and thus influenced by colloid facilitated transport. These could also be examined using AF4-HR-ICP-MS however alternative detectors (GC and HPLC-MS) would be required. The method could also be applied to other environmental samples e.g. wastewater for which multi-elemental analysis is required.

Appendix

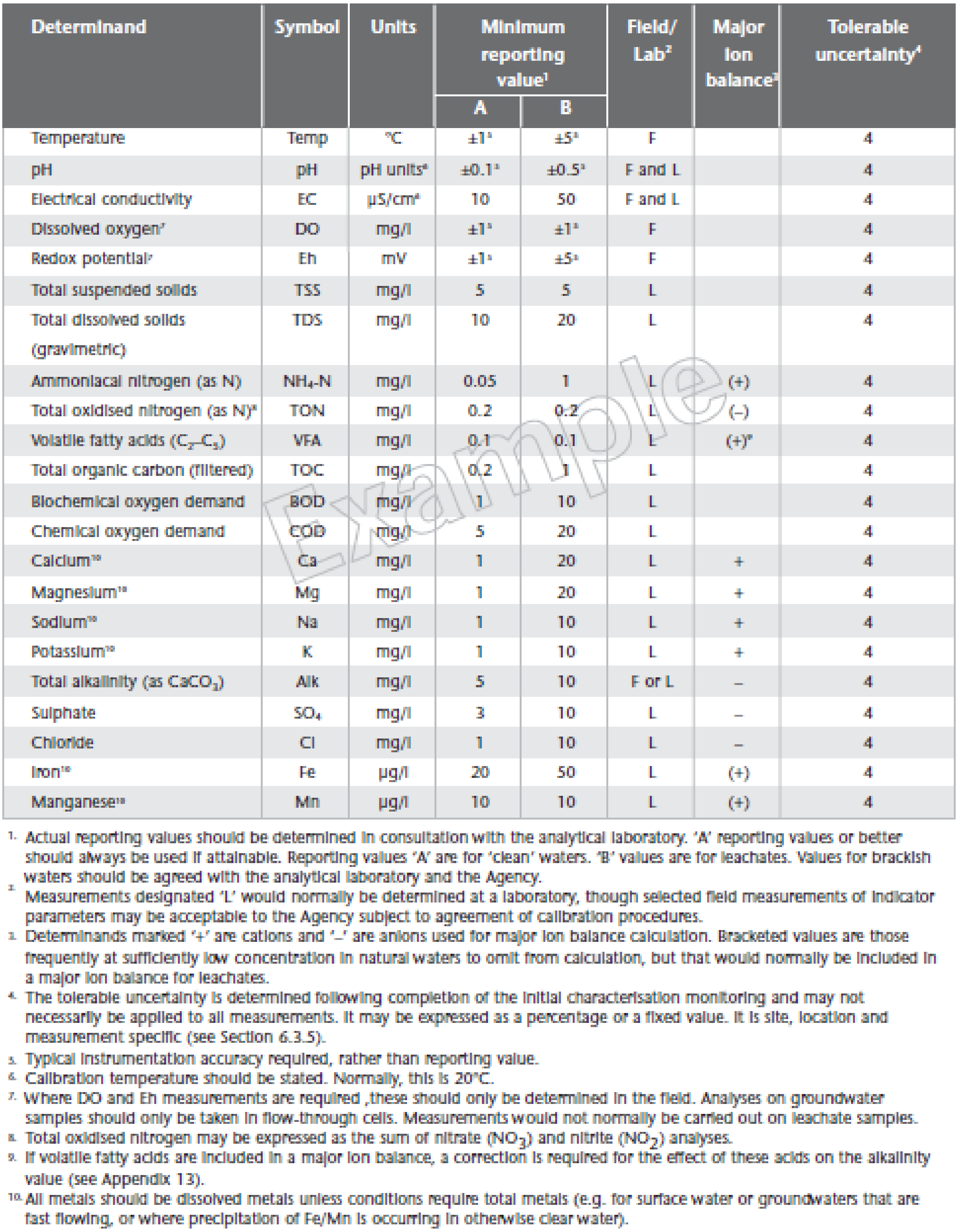
Appendix 1.1 UK Regulations related to landfills (Butt et al., 2014)

Regulation	Details of relevant legislation.
1. Waste Management Licensing Regulations (SI, 1994a, 2005):	Under these regulations, waste management licences are issued by the Environment Agency to ensure that the authorized activities do not cause pollution to the environment, harm to human health or serious detriment to local amenities.
2. Groundwater Regulations (SI, 1998, 2009):	This environmental legislation is an environmental protection measure which completes transposition of the environmental protection Groundwater Directive (80/68/EEC) and provides enhanced protection for measure which completes transposition of the groundwater.
3. EU Directive on IPPC (Integrated Pollution Control and Prevention) (EU, 1996):	This Directive ('the IPPC Directive') enforces a requirement for industrial and agricultural activities with a high pollution potential to have a permit which can only be issued if certain environmental conditions are met, so that the companies will bear the responsibility for preventing and reducing any pollution they may cause.
4. EC Directive on EIA (Environmental Impact Assessment) (EC, 1985):	The Directive has been applied to the assessment of environmental effects of those public and private projects which are likely to have significant effects on the environment.
5. Environmental Protection Act, 1990 and Environmental Act, 1995:	The 1990 Act places certain obligations on businesses to ensure that their waste is suitably contained and disposed of in a proper wide and abandoned mines; to make further provision in relation to National Parks; environment. This Act is to make provision with respect to contaminated land range of issues which are related directly and/or indirectly to the manner. The 1995 Act covers a to make further provision for the control of pollution, the conservation of natural resources and the conservation or enhancement of the environment; to make provision for imposing obligations on certain persons in respect of certain products or materials; to make provision in relation to fisheries; to make provision for certain enactments to bind the Crown; to make provision with respect to the application of certain enactments in relation to the Isles of Scilly; and for connected purposes.
6. Strategic Environmental Assessment (SEA) Directive (ODPM, 2003):	The Directive ensures environmental effects to be taken into account by authorities during the preparation of plans and programmes in the fields of land-use, transport, waste and water management, energy, and a range of other sectors. Thus, this legislation enhances the degree of integration between various sectors rather than each sector being treated on its own as a separate entity.
7. EC Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora (The Habitats Directive) (EC, 1992)	: The EC Habitats Directive promotes the maintenance of biodiversity by requiring Member States to take measures to maintain or restore natural habitats and wild species at a favourable conservation status. The Directive introduces robust protection for those habitats and species that are of European importance. In applying these measures Member States are required to take account of economic, social and cultural requirements and regional and local characteristics.
8. Water Framework Directive — WFD (EC, 2000):	The 1980 Groundwater Directive (80/68/EEC) aims to protect only groundwater whereas the WFD lead to a major overhaul of water protection legislation (Burges Salmon LLP, 2009). The WFD commits European Union member states to protecting and making all water bodies (rivers and lakes), transitional waters (estuaries), coastal waters and groundwater of good qualitative and quantitative status by 2015. Thus, this introduces integrated approach on much larger scale.

Appendix

9. Landfill Directive (EC, 1999) and Landfill Regulations (Scottish Executive et al.,2005; SI, 2002):	The overall aim of this environmental legislation is to prevent or reduce as far as possible negative effects on the environment, in particular the pollution of surface water, groundwater, soil and air, and on the global environment, including the greenhouse effect, as well as any resulting risk to human health, from the landfilling of waste, during the whole life-cycle of the landfill. This legislation also has important implications for waste handling and waste disposal. Principles of the Landfill Directive and WFD together aim for: · The minimum requirement of ‘no deterioration’ for all waters,· Achieving good ecological and chemical quality status for inland and coastal waters. Good ecological status can be defined as only a slight departure from the biological community that would be expected in conditions of minimal anthropogenic impact. Good chemical status fulfils all the standards set by EU legislation for the concentration of chemicals in water. Additionally, more stringent requirements for ‘protected zones’ such as drinking waters, bathing waters, designated areas for the protection of habitats or species (including Natura 2000 sites), also other zones may be designated for the protection of economically significant species or recreational activities. · Achieving international agreements such as OSPAR and eliminate emissions of priority hazardous substances, such as heavy metals and PAHs.

Appendix 1.2 Principle Chemical Compositions for Routine Monitoring(Environment Agency, 2014b)



Determinand	Symbol	Units	Minimum reporting value[1]		Field/ Lab[2]	Major ion balance[3]	Tolerable uncertainty[4]
			A	B			
Temperature	Temp	°C	±1[5]	±5[5]	F		4
pH	pH	pH units[6]	±0.1[5]	±0.5[5]	F and L		4
Electrical conductivity	EC	µS/cm[6]	10	50	F and L		4
Dissolved oxygen[7]	DO	mg/l	±1[5]	±1[5]	F		4
Redox potential[7]	Eh	mV	±1[5]	±5[5]	F		4
Total suspended solids	TSS	mg/l	5	5	L		4
Total dissolved solids (gravimetric)	TDS	mg/l	10	20	L		4
Ammoniacal nitrogen (as N)	NH_4-N	mg/l	0.05	1	L	(+)	4
Total oxidised nitrogen (as N)[8]	TON	mg/l	0.2	0.2	L	(–)	4
Volatile fatty acids (C_2–C_5)	VFA	mg/l	0.1	0.1	L	(+)[9]	4
Total organic carbon (filtered)	TOC	mg/l	0.2	1	L		4
Biochemical oxygen demand	BOD	mg/l	1	10	L		4
Chemical oxygen demand	COD	mg/l	5	20	L		4
Calcium[10]	Ca	mg/l	1	20	L	+	4
Magnesium[10]	Mg	mg/l	1	20	L	+	4
Sodium[10]	Na	mg/l	1	10	L	+	4
Potassium[10]	K	mg/l	1	10	L	+	4
Total alkalinity (as $CaCO_3$)	Alk	mg/l	5	10	F or L	–	4
Sulphate	SO_4	mg/l	3	10	L	–	4
Chloride	Cl	mg/l	1	10	L	–	4
Iron[10]	Fe	µg/l	20	50	L	(+)	4
Manganese[10]	Mn	µg/l	10	10	L	(+)	4

[1] Actual reporting values should be determined in consultation with the analytical laboratory. 'A' reporting values or better should always be used if attainable. Reporting values 'A' are for 'clean' waters. 'B' values are for leachates. Values for brackish waters should be agreed with the analytical laboratory and the Agency.

[2] Measurements designated 'L' would normally be determined at a laboratory, though selected field measurements of indicator parameters may be acceptable to the Agency subject to agreement of calibration procedures.

[3] Determinands marked '+' are cations and '–' are anions used for major ion balance calculation. Bracketed values are those frequently at sufficiently low concentration in natural waters to omit from calculation, but that would normally be included in a major ion balance for leachates.

[4] The tolerable uncertainty is determined following completion of the initial characterisation monitoring and may not necessarily be applied to all measurements. It may be expressed as a percentage or a fixed value. It is site, location and measurement specific (see Section 6.3.5).

[5] Typical instrumentation accuracy required, rather than reporting value.

[6] Calibration temperature should be stated. Normally, this is 20°C.

[7] Where DO and Eh measurements are required ,these should only be determined in the field. Analyses on groundwater samples should only be taken in flow-through cells. Measurements would not normally be carried out on leachate samples.

[8] Total oxidised nitrogen may be expressed as the sum of nitrate (NO_3) and nitrite (NO_2) analyses.

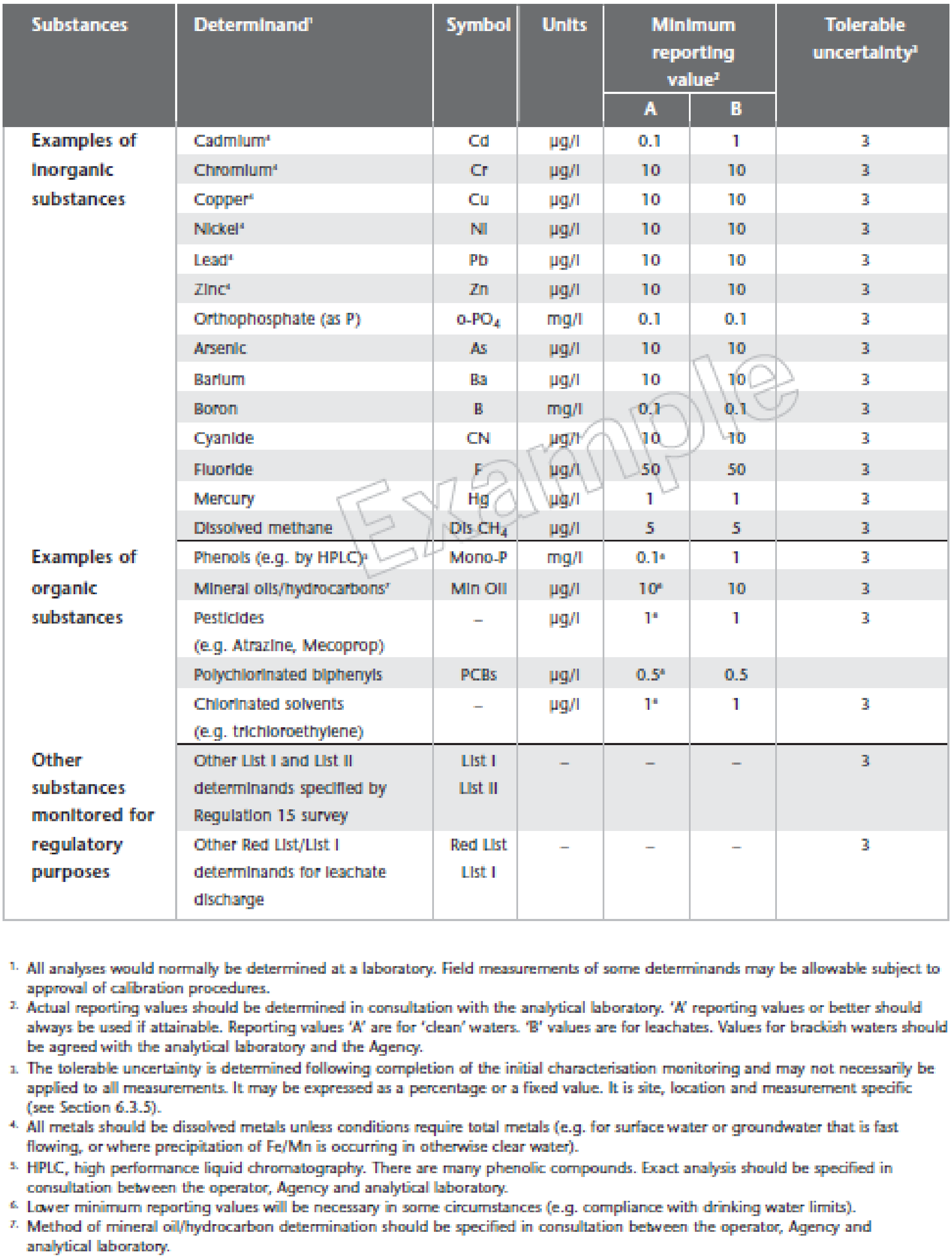
[9] If volatile fatty acids are included in a major ion balance, a correction is required for the effect of these acids on the alkalinity value (see Appendix 13).

[10] All metals should be dissolved metals unless conditions require total metals (e.g. for surface water or groundwaters that are fast flowing, or where precipitation of Fe/Mn is occurring in otherwise clear water).

Appendix

Appendix 1.3 Minor Chemical Composition Measurements for Routine Monitoring

(Environment Agency, 2014b)



Substances	Determinand[1]	Symbol	Units	Minimum reporting value[2]		Tolerable uncertainty[3]
				A	B	
Examples of inorganic substances	Cadmium[4]	Cd	µg/l	0.1	1	3
	Chromium[4]	Cr	µg/l	10	10	3
	Copper[4]	Cu	µg/l	10	10	3
	Nickel[4]	Ni	µg/l	10	10	3
	Lead[4]	Pb	µg/l	10	10	3
	Zinc[4]	Zn	µg/l	10	10	3
	Orthophosphate (as P)	o-PO_4	mg/l	0.1	0.1	3
	Arsenic	As	µg/l	10	10	3
	Barium	Ba	µg/l	10	10	3
	Boron	B	mg/l	0.1	0.1	3
	Cyanide	CN	µg/l	10	10	3
	Fluoride	F	µg/l	50	50	3
	Mercury	Hg	µg/l	1	1	3
	Dissolved methane	Dis CH_4	µg/l	5	5	3
Examples of organic substances	Phenols (e.g. by HPLC)[5]	Mono-P	mg/l	0.1[6]	1	3
	Mineral oils/hydrocarbons[7]	Min Oil	µg/l	10[6]	10	3
	Pesticides (e.g. Atrazine, Mecoprop)	–	µg/l	1[6]	1	3
	Polychlorinated biphenyls	PCBs	µg/l	0.5[6]	0.5	
	Chlorinated solvents (e.g. trichloroethylene)	–	µg/l	1[6]	1	3
Other substances monitored for regulatory purposes	Other List I and List II determinands specified by Regulation 15 survey	List I List II	–	–	–	3
	Other Red List/List I determinands for leachate discharge	Red List List I	–	–	–	3

[1] All analyses would normally be determined at a laboratory. Field measurements of some determinands may be allowable subject to approval of calibration procedures.

[2] Actual reporting values should be determined in consultation with the analytical laboratory. 'A' reporting values or better should always be used if attainable. Reporting values 'A' are for 'clean' waters. 'B' values are for leachates. Values for brackish waters should be agreed with the analytical laboratory and the Agency.

[3] The tolerable uncertainty is determined following completion of the initial characterisation monitoring and may not necessarily be applied to all measurements. It may be expressed as a percentage or a fixed value. It is site, location and measurement specific (see Section 6.3.5).

[4] All metals should be dissolved metals unless conditions require total metals (e.g. for surface water or groundwater that is fast flowing, or where precipitation of Fe/Mn is occurring in otherwise clear water).

[5] HPLC, high performance liquid chromatography. There are many phenolic compounds. Exact analysis should be specified in consultation between the operator, Agency and analytical laboratory.

[6] Lower minimum reporting values will be necessary in some circumstances (e.g. compliance with drinking water limits).

[7] Method of mineral oil/hydrocarbon determination should be specified in consultation between the operator, Agency and analytical laboratory.

Appendix 1.4 PSS standard Retention times and Molecular Weights

MW	log (MW)	Retention Time (minutes)	Retention Time (Seconds)	Log (RT)
1,000	3	14	824.5002	2.916191
4000	3.602059991	15.24167	914.5002	2.961184
6000	3.77815125	16.24167	974.5002	2.988782
14900	4.173186268	17.9	1075.5	3.03161
33290	4.522313795	22.30833	1338.5	3.126618
63000	4.819543936	30.4	1824	3.261025

List of References

ABBAS, A. A., GUO, J., LIU ZHI, P., PAN YING, Y. & AL-REKABI, W. S. 2009. Review on landfill leachate treatments. *American Journal of Applied Sciences,* 6, 672-684684.

ALASONATI, E., SLAVEYKOVA, V. I., GALLARD, H., CROUÉ, J.-P. & BENEDETTI, M. F. 2010. Characterization of the colloidal organic matter from the Amazonian basin by asymmetrical flow field-flow fractionation and size exclusion chromatography. *Water Research,* 44, 223-231.

ALASONATI, E., STOLPE, B., BENINCASA, M. A., HASSELLOV, M. & SLAVEYKOVA, V. I. 2006. Asymmetrical flow field flow fractionation - Multidetection system as a tool for studying metal-alginate interactions. *Environmental Chemistry,* 3, 192-198.

BAALOUSHA, M., KAMMER, F. V. D., MOTELICA-HEINO, M., HILAL, H. S. & LE COUSTUMER, P. 2006a. Size fractionation and characterization of natural colloids by flow-field flow fractionation coupled to multi-angle laser light scattering. *Journal of Chromatography A,* 1104, 272-281.

BAALOUSHA, M., KAMMER, F. V. D., MOTELICA-HEINO, M. & LE COUSTUMER, P. 2005. Natural sample fractionation by F1FFF-MALLS-TEM: Sample stabilization, preparation, pre-concentration and fractionation. *Journal of Chromatography A,* 1093, 156-166.

BAALOUSHA, M. & LEAD, J. R. 2007. Characterization of natural aquatic colloids (< 5 nm) by flow-field flow fractionation and atomic force microscopy. *Environmental Science & Technology,* 41, 1111-1117.

BAALOUSHA, M. & LEAD, J. R. 2012. Rationalizing Nanomaterial Sizes Measured by Atomic Force Microscopy, Flow Field-Flow Fractionation, and Dynamic Light Scattering: Sample Preparation, Polydispersity, and Particle Structure. *Environmental Science & Technology,* 46, 6134-6142.

BAALOUSHA, M., LEAD, J. R., JU-NAM, Y. & WILDERER, P. 2011a. 3.05 - Natural Colloids and Manufactured Nanoparticles in Aquatic and Terrestrial Systems. *Treatise on Water Science.* Oxford: Elsevier.

BAALOUSHA, M., LEAD, J. R., VON DER KAMMER, F. & HOFMANN, T. 2009. Natural Colloids and Nanoparticles in Aquatic and Terrestrial Environments. *Environmental and Human Health Impacts of Nanotechnology.* John Wiley & Sons, Ltd.

BAALOUSHA, M., MANCIULEA, A., CUMBERLAND, S., KENDALL, K. & LEAD, J. R. 2008. Aggregation and surface properties of iron oxide nanoparticles: Influence of pH and natural organic matter. *Environmental Toxicology and Chemistry,* 27, 1875-1882.

BAALOUSHA, M., MOTELICA-HEINO, M. & COUSTUMER, P. L. 2006b. Conformation and size of humic substances: Effects of major cation concentration and type, pH, salinity, and residence time. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 272, 48-55.

BAALOUSHA, M., STOLPE, B. & LEAD, J. R. 2011b. Flow field-flow fractionation for the analysis and characterization of natural colloids and manufactured nanoparticles in environmental systems: A critical review. *Journal of Chromatography A,* 1218, 4078-4103.

BALNOIS, E., WILKINSON, K. J., LEAD, J. R. & BUFFLE, J. 1999. Atomic Force Microscopy of Humic Substances: Effects of pH and Ionic Strength. *Environmental Science & Technology,* 33, 3911-3917.

BARTH, H. G. & BOYES, B. E. 1992. Size exclusion chromatography. *Analytical Chemistry,* 64, 428R-442R.

BAUN, D. L. & CHRISTENSEN, T. H. 2004. Speciation of heavy metals in landfill leachate: a review. *Waste Management & Research,* 22**,** 3-23.

BECKETT, R. 1987. THE APPLICATION OF FIELD-FLOW FRACTIONATION TECHNIQUES TO THE CHARACTERIZATION OF COMPLEX ENVIRONMENTAL-SAMPLES. *Environmental Technology Letters,* 8**,** 339-354.

BECKETT, R., JUE, Z. & GIDDINGS, J. C. 1987. Determination of molecular weight distributions of fulvic and humic acids using flow field-flow fractionation. *Environmental Science & Technology,* 21**,** 289-295.

BEDNAR, A. J., PODA, A. R., MITRANO, D. M., KENNEDY, A. J., GRAY, E. P., RANVILLE, J. F., HAYES, C. A., CROCKER, F. H. & STEEVENS, J. A. 2013. Comparison of on-line detectors for field flow fractionation analysis of nanomaterials. *Talanta,* 104**,** 140-148.

BENEDETTI, M. F., RANVILLE, J. F., ALLARD, T., BEDNAR, A. J. & MENGUY, N. 2003. The iron status in colloidal matter from the Rio Negro, Brasil. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 217**,** 1-9.

BHATTACHARYA, P., MUKHERJEE, A. & MUKHERJEE, A. B. 2014. Groundwater Arsenic in India: Source, Distribution, Effects and Alternate Safe Drinking Water Sources. *Reference Module in Earth Systems and Environmental Sciences.* Elsevier.

BOLEA, E., GORRIZ, M. P., BOUBY, M., LABORDA, F., CASTILLO, J. R. & GECKEIS, H. 2006. Multielement characterization of metal-humic substances complexation by size exclusion chromatography, asymmetrical flow field-flow fractionation, ultrafiltration and inductively coupled plasma-mass spectrometry detection: A comparative approach. *Journal of Chromatography A,* 1129**,** 236-246.

BOLEA, E., LABORDA, F. & CASTILLO, J. R. 2010. Metal associations to microparticles, nanocolloids and macromolecules in compost leachates: Size characterization by asymmetrical flow field-flow fractionation coupled to ICP-MS. *Analytica Chimica Acta,* 661**,** 206-214.

BOUBY, M., GECKEIS, H. & GEYER, F. W. 2008. Application of asymmetric flow field-flow fractionation (AsFlFFF) coupled to inductively coupled plasma mass spectrometry (ICPMS) to the quantitative characterization of natural colloids and synthetic nanoparticles. *Analytical and Bioanalytical Chemistry,* 392**,** 1447-1457.

BOUBY, M., GECKEIS, H., LÜTZENKIRCHEN, J., MIHAI, S. & SCHÄFER, T. 2011. Interaction of bentonite colloids with Cs, Eu, Th and U in presence of humic acid: A flow field-flow fractionation study. *Geochimica Et Cosmochimica Acta,* 75**,** 3866-3880.

BRADFORD, S. A. & BETTAHAR, M. 2006. Concentration dependent transport of colloids in saturated porous media. *Journal of Contaminant Hydrology,* 82**,** 99-117.

BUESSELER, K. O., BAUER, J. E., CHEN, R. F., EGLINTON, T. I., GUSTAFSSON, O., LANDING, W., MOPPER, K., MORAN, S. B., SANTSCHI, P. H., VERNONCLARK, R. & WELLS, M. L. 1996. An intercomparison of cross-flow filtration techniques used for sampling marine colloids: Overview and organic carbon results. *Marine Chemistry,* 55**,** 1-31.

BUFFLE, J., WILKINSON, K. J., STOLL, S., FILELLA, M. & ZHANG, J. 1998. A Generalized Description of Aquatic Colloidal Interactions: The Three-colloidal Component Approach. *Environmental Science & Technology,* 32**,** 2887-2899.

BUTT, T. E., GOUDA, H. M., BALOCH, M. I., PAUL, P., JAVADI, A. A. & ALAM, A. 2014. Literature review of baseline study for risk analysis - the landfill leachate case. *Environ Int,* 63, 149-62.

CALACE, N., LIBERATORI, A., PETRONIO, B. M. & PIETROLETTI, M. 2001. Characteristics of different molecular weight fractions of organic matter in landfill leachate and their role in soil sorption of heavy metals. *Environmental Pollution,* 113, 331-339.

CAMPAGNA, M., ÇAKMAKCI, M., BÜŞRA YAMAN, F. & ÖZKAYA, B. 2013. Molecular weight distribution of a full-scale landfill leachate treatment by membrane bioreactor and nanofiltration membrane. *Waste Management,* 33, 866-870.

CHEN, B. & SELEGUE, J. P. 2002. Separation and Characterization of Single-Walled and Multiwalled Carbon Nanotubes by Using Flow Field-Flow Fractionation. *Analytical Chemistry,* 74, 4774-4780.

CHRISTENSEN, J. B. & CHRISTENSEN, T. H. 2000. The effect of pH on the complexation of Cd, Ni and Zn by dissolved organic carbon from leachate-polluted groundwater. *Water Research,* 34, 3743-3754.

CHRISTENSEN, T. H., KJELDSEN, P., ALBRECHTSEN, H. J., HERON, G., NIELSEN, P. H., BJERG, P. L. & HOLM, P. E. 1994. ATTENUATION OF LANDFILL LEACHATE POLLUTANTS IN AQUIFERS. *Critical Reviews in Environmental Science and Technology,* 24, 119-202.

CHRISTENSEN, T. H., KJELDSEN, P., BJERG, P. L., JENSEN, D. L., CHRISTENSEN, J. B., BAUN, A., ALBRECHTSEN, H. J. & HERON, C. 2001. Biogeochemistry of landfill leachate plumes. *Applied Geochemistry,* 16, 659-718.

CHRISTIAN, P., KAMMER, F., BAALOUSHA, M. & HOFMANN, T. 2008. Nanoparticles: structure, properties, preparation and behaviour in environmental media. *Ecotoxicology,* 17, 326-343.

COBLE, P. G. 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry,* 51, 325-346.

CUSS, C. W. & GUÉGUEN, C. 2012. Determination of relative molecular weights of fluorescent components in dissolved organic matter using asymmetrical flow field-flow fractionation and parallel factor analysis. *Analytica Chimica Acta,* 733, 98-102.

DALTON, C. 2014. *Influences of landfill leachate Dissolved organic carbon on the attenuation of toluene and naphthalene by oxford clay.* PhD, Southampton.

DEFRA 2007. Characterising the Potential Risks posed by Engineered Nanoparticles LONDON.

DEFRA 2011. Environmental Permitting Guidance: Radioactive Substances Regulation LONDON: DEFRA.

DEPARTMENT OF THE ENVIRONMENT 1995. *Landfill Design, Construction and Operational Practice - Draft for consultation.* UK: Department of the Environment Waste Management

DONISA, C., MOCANU, R. & STEINNES, E. 2003. Distribution of some major and minor elements between fulvic and humic acid fractions in natural soils. *Geoderma,* 111, 75-84.

DOUCET, F. J., LEAD, J. R. & SANTSCHI, P. H. 2006. Colloid–Trace Element Interactions in Aquatic Systems. *Environmental Colloids and Particles.* John Wiley & Sons, Ltd.

DRINKING WATER INSPECTORATE 2010. What are the drinking water standards? London: Drinking Water Inspectorate.

DRURY, D., HALL, D. & DOWLE, J. 2003. The Deveoplemt of LandSim 2.5. The Environment Agency.

DUBASCOUX, S., HEROULT, J., LE HÉCHO, I., POTIN-GAUTIER, M. & LESPES, G. 2008a. Evaluation of a combined fractionation and speciation approach for study of size-based distribution of organotin species on environmental colloids. *Analytical and Bioanalytical Chemistry,* 390, 1805-1813.

DUBASCOUX, S., LE HÉCHO, I., HASSELLÖV, M., VON DER KAMMER, F., POTIN GAUTIER, M. & LESPES, G. 2010. Field-flow fractionation and inductively coupled plasma mass spectrometer coupling: History, development and applications. *Journal of Analytical Atomic Spectrometry,* 25, 613.

DUBASCOUX, S., LE HÉCHO, I., POTIN GAUTIER, M. & LESPES, G. 2008b. On-line and off-line quantification of trace elements associated to colloids by As-Fl-FFF and ICP-MS. *Talanta,* 77, 60-65.

DUBASCOUX, S., LESPES, G., DENAIX, L. & GAUTIER, M. P. 2008c. Kinetic monitoring of trisubstituted organotins in soil after sewage sludge application. *Applied Organometallic Chemistry,* 22, 481-487.

DUBASCOUX, S., VON DER KAMMER, F., LE HECHO, I., GAUTIER, M. P. & LESPES, G. 2008d. Optimisation of asymmetrical flow field flow fractionation for environmental nanoparticles separation. *Journal of Chromatography A,* 1206, 160-165.

DUFRÊNE, Y. F. 2009. Atomic force microscopy: A powerful molecular toolkit in nanoproteomics. *PROTEOMICS,* 9, 5400-5405.

EBENSTEIN, Y., NAHUM, E. & BANIN, U. 2002. Tapping Mode Atomic Force Microscopy for Nanoparticle Sizing: Tip−Sample Interaction Effects. *Nano Letters,* 2, 945-950.

ENVIRONMENT AGENCY 2003. Attenuation of organic contaminants in leachates by mineral landfill liners.

ENVIRONMENT AGENCY 2009a. Landfill (EPR 5.02). Bristol: Environment Agency.

ENVIRONMENT AGENCY 2009b. LFE5 - Using geomembranes in landfill engineering. Bristol: Environment Agency,.

ENVIRONMENT AGENCY 2010a. Harvesting rainwater for domestic uses: an information guide. Bristol, UK: Environment Agency.

ENVIRONMENT AGENCY 2010b. Understanding the Landfill Directive. Bristol: Environment Agency.

ENVIRONMENT AGENCY 2012. Strategy for the management of solid low level radioactive waste from the non-nuclear industry in the United Kingdom.

ENVIRONMENT AGENCY. 2014a. *Groundwater source protection zones* [Online]. Available: http://apps.environment-agency.gov.uk/wiyby/37833.aspx [Accessed 29.11.14.

ENVIRONMENT AGENCY 2014b. Guidance on Monitoring of Landfill Leachate, Groundwater and Surface Water.

ENVIRONMENTAL PROTECTION AGENCY 2006. Voluntary Estuary Monitoring Manual.

ENVIRONMENTAL PROTECTION AGENCY. 2012. *Current Drinking Water Regulations* [Online]. [Accessed 12/2014.

EUROSTAT. 2014. *-Environment in the EU27-Municipal waste generation and treatment, by type of treatment method* [Online]. Available: http://ec.europa.eu/eurostat/tgm/refreshTableAction.do?tab=table&plugin=1&pcode=tsdpc240&language=en [Accessed 28/12/14 2014].

FAN, H.-J., CHIU, T., HSIN-SIN, Y., CHEN, W.-C. & FURUYA, E. 2007 REMOVAL OF HUMIC ACIDS, FULVIC ACIDS AND NON HUMIC SUBSTANCEFROM LANDFILL LEACHATE. *J. Environ. Eng. Manage,* 17(5), 325-331

FANG, J., XU, M.-J., WANG, D.-J., WEN, B. & HAN, J.-Y. 2013. Modeling the transport of TiO2 nanoparticle aggregates in saturated and unsaturated granular media: Effects of ionic strength and pH. *Water Research,* 47, 1399-1408.

FATTA, D., PAPADOPOULOS, A. & LOIZIDOU, M. 1999. A study on the landfill leachate and its impact on the groundwater quality of the greater area. *Environmental Geochemistry and Health,* 21, 175-190.

FILELLA, M., WILKINSON, K. J. & LEAD, J. R. 2007. *Environmental colloids and particles: Behaviour separation and characterisation,* Chichester, John Wiley & Sons Ltd.

FILELLA, M., ZHANG, J., NEWMAN, M. E. & BUFFLE, J. 1997. Analytical applications of photon correlation spectroscopy for size distribution measurements of natural colloidal suspensions: capabilities and limitations. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 120, 27-46.

FLURY, M. & QIU, H. 2008. Modeling Colloid-Facilitated Contaminant Transport in the Vadose Zone. *Vadose Zone Journal,* 7, 682-697.

FREDERICK, P. & STEPHEN, H. 1985. Critical Review and Summary of Leachate and Gas Production from Landfills. US: Enviornmental Protection Agency.

GECKEIS, H., NGO MANH, T., BOUBY, M. & KIM, J. I. 2003. Aquatic colloids relevant to radionuclide migration: characterization by size fractionation and ICP-mass spectrometric detection. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 217, 101-108.

GEISS, O., CASCIO, C., GILLILAND, D., FRANCHINI, F. & BARRERO-MORENO, J. 2013. Size and mass determination of silver nanoparticles in an aqueous matrix using asymmetric flow field flow fractionation coupled to inductively coupled plasma mass spectrometer and ultraviolet–visible detectors. *Journal of Chromatography A,* 1321, 100-108.

GIDDINGS, J., C 1966 New separation concept based on a coupling of concentration and flow non-uniformities. *Separation Science,* 1, 123-125.

GIDDINGS, J. C. & CALDWELL, K. D. 1989. Field-Flow Fractionation. *In:* ROSSITER, B. W. & HAMILTON, J. F. (eds.) *Physical Methods of Chemistr.* New York: John Wiley and Sons.

GOUNARIS, V., ANDERSON, P. R. & HOLSEN, T. M. 1993. CHARACTERISTICS AND ENVIRONMENTAL SIGNIFICANCE OF COLLOIDS IN LANDFILL LEACHATE. *Environmental Science & Technology,* 27, 1381-1387.

GUEGUEN, C. & CUSS, C. W. 2011. Characterization of aquatic dissolved organic matter by asymmetrical flow field-flow fractionation coupled to UV-Visible diode array and excitation emission matrix fluorescence. *Journal of Chromatography A,* 1218, 4188-4198.

HASSELLOV, M., LYVEN, B., HARALDSSON, C. & SIRINAWIN, W. 1999. Determination of continuous size and trace element distribution of colloidal material in natural water by on-line coupling of flow field-flow fractionation with ICPMS. *Analytical Chemistry,* 71, 3497-3502.

HASSELLOV, M., READMAN, J. W., RANVILLE, J. F. & TIEDE, K. 2008. Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. *Ecotoxicology,* 17, 344-361.

HASSELLÖV, M., DER KAMMER, F. V. & BECKETT, R. 2006. Characterisation of Aquatic Colloids and Macromolecules by Field-Flow Fractionation. *Environmental Colloids and Particles.* John Wiley & Sons, Ltd.

HENNEBERT, P., AVELLAN, A., YAN, J. & AGUERRE-CHARIOL, O. 2013. Experimental evidence of colloids and nanoparticles presence from 25 waste leachates. *Waste Manag,* 33, 1870-81.

HOCHELLA, M. F., LOWER, S. K., MAURICE, P. A., PENN, R. L., SAHAI, N., SPARKS, D. L. & TWINING, B. S. 2008. Nanominerals, mineral nanoparticles, and Earth systems. *Science,* 319, 1631-1635.

HUANG, P.-R. 2014. *The influence of solid phase organic carbon on the sorption/desorption of hydrophobic organic pollutants in landfill liners.* 2014, Southampton.

JENSEN, D. L. & CHRISTENSEN, T. H. 1999. Colloidal and dissolved metals in leachates from four Danish landfills. *Water Research,* 33, 2139-2147.

JENSEN, D. L., LEDIN, A. & CHRISTENSEN, T. H. 1999. Speciation of heavy metals in landfill-leachate polluted groundwater. *Water Research,* 33, 2642-2650.

JIMENEZ, M. S., GOMEZ, M. T., BOLEA, E., LABORDA, F. & CASTILLO, J. 2011. An approach to the natural and engineered nanoparticles analysis in the environment by inductively coupled plasma mass spectrometry. *International Journal of Mass Spectrometry,* 307, 99-104.

KANG, K. H., SHIN, H. S. & PARK, H. 2002. Characterization of humic substances present in landfill leachates with different landfill ages and its implications. *Water Research,* 36, 4023-4032.

KIM, S. Y., TANAKA, N. & MATSUTO, T. 2002. Solubility and adsorption characteristics of Pb in leachate from MSW incinerator bottom ash. *Waste Management & Research,* 20, 373-381.

KJELDSEN, P., BARLAZ, M. A., ROOKER, A. P., BAUN, A., LEDIN, A. & CHRISTENSEN, T. H. 2002. Present and Long-Term Composition of MSW Landfill Leachate: A Review. *Critical Reviews in Environmental Science and Technology,* 32, 297-336.

KRACHLER, R., KRACHLER, R. F., VON DER KAMMER, F., SÜPHANDAG, A., JIRSA, F., AYROMLOU, S., HOFMANN, T. & KEPPLER, B. K. 2010. Relevance of peat-draining rivers for the riverine input of dissolved iron into the ocean. *Science of The Total Environment,* 408, 2402-2408.

KÖRDEL. W., D. M., LINTELMANN. J., PADBERG. S. 1997. The importance of natural organic material for environmental processes in waters and soils (Technical Report). *Pure and Applied Chemistry,* 69, 1571–1600.

LABORDA, F., BOLEA, E., GORRIZ, M. P., MARTIN-RUIZ, M. P., RUIZ-BEGUERIA, S. & CASTILLO, J. R. 2008. A speciation methodology to study the contributions of humic-like and fulvic-like acids to the mobilization of metals from compost using size exclusion chromatography-ultraviolet absorption-inductively coupled plasma mass spectrometry and deconvolution analysis. *Anal Chim Acta,* 606, 1-8.

LABORDA, F., RUIZ-BEGUERIA, S., BOLEA, E. & CASTILLO, J. R. 2011. Study of the size-based environmental availability of metals associated to natural organic matter by stable isotope exchange and quadrupole inductively coupled plasma mass spectrometry coupled to asymmetrical flow field flow fractionation. *Journal of Chromatography A,* 1218, 4199-4205.

LAPWORTH, D. J., STOLPE, B., WILLIAMS, P. J., GOODDY, D. C. & LEAD, J. R. 2013. Characterization of Suboxic Groundwater Colloids Using a Multi-method Approach. *Environmental Science & Technology,* 47, 2554-2561.

LEAD, J. R., MUIRHEAD, D. & GIBSON, C. T. 2005. Characterization of Freshwater Natural Aquatic Colloids by Atomic Force Microscopy (AFM). *Environmental Science & Technology,* 39**,** 6930-6936.

LEAD, J. R. & WILKINSON, K. J. 2006. Environmental Colloids and Particles: Current Knowledge and Future Developments. *Environmental Colloids and Particles.* John Wiley & Sons, Ltd.

LESHER, E. K., PODA, A., BEDNAR, A. J. & RANVILLE, J. 2012. Field Flow Fractionation coupled to inductively coupled plasma mass spectrometry (FFF-ICP-MS): Methodology and Application to Environmental Nanoparticle Research. *In:* WILLIAMS, S. K. R. & CALDWELL, K. D. (eds.) *Field Flow Fractionation in Biopolymer Analysis.* Springer Vienna.

LI, R., YUE, D., LIU, J. & NIE, Y. 2009a. Size fractionation of organic matter and heavy metals in raw and treated leachate. *Waste Management,* 29**,** 2527-2533.

LI, R., YUE, D. B., LIU, J. G. & NIE, Y. F. 2009b. Size fractionation of organic matter and heavy metals in raw and treated leachate. *Waste Management,* 29**,** 2527-2533.

LOFTS, S. & TIPPING, E. 1998. An assemblage model for cation binding by natural particulate matter. *Geochimica et Cosmochimica Acta,* 62**,** 2609-2625.

LOPEZ-SERRANO, A., OLIVAS, R. M., LANDALUZE, J. S. & CAMARA, C. 2014. Nanoparticles: a global vision. Characterization, separation, and quantification methods. Potential environmental and health impact. *Analytical Methods,* 6**,** 38-56.

LU, F., CHANG, C.-H., LEE, D.-J., HE, P.-J., SHAO, L.-M. & SU, A. 2009. Dissolved organic matter with multi-peak fluorophores in landfill leachate. *Chemosphere,* 74**,** 575-582.

LU, J. C. S., EICHENBERGER, B. & STEARNS, R. J. 1985. *Leachate from Municipal Landfills: Productionand Management,* New Jersey, Noyes Publications.

LYVEN, B., HASSELLOV, M., HARALDSSON, C. & TURNER, D. R. 1997. Optimisation of on-channel preconcentration in flow field-flow fractionation for the determination of size distributions of low molecular weight colloidal material in natural waters. *Analytica Chimica Acta,* 357**,** 187-196.

LYVEN, B., HASSELLOV, M., TURNER, D. R., HARALDSSON, C. & ANDERSSON, K. 2003. Competition between iron- and carbon-based colloidal carriers for trace metals in a freshwater assessed using flow field-flow fractionation coupled to ICPMS. *Geochimica Et Cosmochimica Acta,* 67**,** 3791-3802.

MALVERN. 2014. *Dynamic Light Scattering: An Introduction in 30Minutes* [Online]. Available: http://www.malvern.com/en/pdf/secure/TN101104DynamicLightScatteringIntroduction.pdf.

MATURA, M., ETTLER, V., JEŽEK, J., MIHALJEVIČ, M., ŠEBEK, O., SÝKORA, V. & KLEMENTOVÁ, M. 2010. Association of trace elements with colloidal fractions in leachates from closed and active municipal solid waste landfills. *Journal of Hazardous Materials,* 183**,** 541-548.

MATURA, M., ETTLER, V. & KLEMENTOVA, M. 2012. Transmission electron microscopy investigation of colloids and particles from landfill leachates. *Waste Management & Research,* 30**,** 530-541.

MCCARTHY, J. F. & MCKAY, L. D. 2004. Colloid Transport in the Subsurface: Past, Present, and Future Challenges. *Vadose Zone Journal,* 3**,** 326-337.

MOHD OMAR, F., ABDUL AZIZ, H. & STOLL, S. 2014. Aggregation and disaggregation of ZnO nanoparticles: Influence of pH and adsorption of

Suwannee River humic acid. *Science of The Total Environment,* 468–469, 195-201.

MONTASER, A. 1998. *Inductively coupled plasma mass spectrometry,* New York, Wiley.

MOON, M. H., PARK, I. & KIM, Y. 1998. Size characterization of liposomes by flow field-flow fractionation and photon correlation spectroscopy: Effect of ionic strength and pH of carrier solutions. *Journal of Chromatography A,* 813, 91-100.

MUELLER, N. C. & NOWACK, B. 2008. Exposure Modeling of Engineered Nanoparticles in the Environment. *Environmental Science & Technology,* 42, 4447-4453.

MURPHY, E. M. & ZACHARA, J. M. 1995. The role of sorbed humic substances on the distribution of organic and inorganic contaminants in groundwater. *Geoderma,* 67, 103-124.

NELSON EBY, G. 2004. *Principles of Environmental Geochemistry,* USA, Brooks Cole.

NEUBAUER, E., V.D. KAMMER, F. & HOFMANN, T. 2011. Influence of carrier solution ionic strength and injected sample load on retention and recovery of natural nanoparticles using Flow Field-Flow Fractionation. *Journal of Chromatography A,* 1218, 6763-6773.

OADES, J. M. 1989. An introduction to organic matter in mineral soils. *In:* DIXON, J. B. A. W., S.B. (ed.) *Minerals in soil environments.* Madison, WI: Soil Science Society of America.

OYGARD, J. K., GJENGEDAL, E. & ROYSET, O. 2007. Size charge fractionation of metals in municipal solid waste landfill leachate. *Water Res,* 41, 47-54.

PERSSON, L., ALSBERG, T., LEDIN, A. & ODHAM, G. 2006. Transformations of dissolved organic matter in a landfill leachate—A size exclusion chromatography/mass spectrometric approach. *Chemosphere,* 64, 1093-1099.

PLATHE, K. 2010. *Nanoparticle- Heavy metal associations in River Sediments.* Doctor of Philosophy in Geosciences, Virginia Polytechnic Institute and State University.

PLATHE, K. L., VON DER KAMMER, F., HASSELLOV, M., MOORE, J., MURAYAMA, M., HOFMANN, T. & HOCHELLA, M. F. 2010. Using FlFFF and aTEM to determine trace metal-nanoparticle associations in riverbed sediment. *Environmental Chemistry,* 7, 82-93.

PLATHE, K. L., VON DER KAMMER, F., HASSELLÖV, M., MOORE, J. N., MURAYAMA, M., HOFMANN, T. & HOCHELLA JR, M. F. 2013. The role of nanominerals and mineral nanoparticles in the transport of toxic trace metals: Field-flow fractionation and analytical TEM analyses after nanoparticle isolation and density separation. *Geochimica et Cosmochimica Acta,* 102, 213-225.

POHLAND, F. & HARPER, S. 1985. Critical Review and Summary of Leachate and Gas Production from Landfills. US: Enviornmental Protection Agency.

PORNWILARD, M., M & SIRIPINYANOND, A. 2014. Field-flow fractionation with inductively coupled plasma mass spectrometry: past, present, and future. *Journal of Analytical Atomic Spectrometry.*

POWRIE, W., SMALLMAN, D., MARSHALL, J. & PALMER, M. 2010. Proposal: Transport of toxic metals in clay mineral barriers:Influences of mobile sorbent nanoparticles. EPSRC.

PRESTEL, H., SCHOTT, L., NIESSNER, R. & PANNE, U. 2005. Characterization of sewage plant hydrocolloids using asymmetrical flow field-flow fractionation and ICP-mass spectrometry. *Water Research,* 39**,** 3541-3552.

QUASIM, S. & CHIANG, W. 1994. *Sanitary Landfill Leachate: Generation, Control and Treatment,* US, CRC Press.

RANVILLE, J. F., CHITTLEBOROUGH, D. J., SHANKS, F., MORRISON, R. J. S., HARRIS, T., DOSS, F. & BECKETT, R. 1999. Development of sedimentation field-flow fractionation-inductively coupled plasma mass-spectrometry for the characterization of environmental colloids. *Analytica Chimica Acta,* 381**,** 315-329.

RANVILLE, J. F., HENDRY, M. J., RESZAT, T. N., XIE, Q. L. & HONEYMAN, B. D. 2007. Quantifying uranium complexation by groundwater dissolved organic carbon using asymmetrical flow field-flow fractionation. *Journal of Contaminant Hydrology,* 91**,** 233-246.

REINHART, D. R. 1993. A Review of Recent Studies On the Sources of Hazardous Compounds Emitted From Solid Waste Landfills: a U.S. Experience. *Waste Management & Research,* 11**,** 257-268.

RENOU, S., GIVAUDAN, J. G., POULAIN, S., DIRASSOUYAN, F. & MOULIN, P. 2008. Landfill leachate treatment: Review and opportunity. *J Hazard Mater,* 150**,** 468-93.

ROBINSON, H. D., KNOX, K., BONE, B. D. & PICKEN, A. 2005. Leachate quality from landfilled MBT waste. *Waste Management,* 25**,** 383-391.

SCHMITT, D., TAYLOR, H. E., AIKEN, G. R., ROTH, D. A. & FRIMMEL, F. H. 2002. Influence of Natural Organic Matter on the Adsorption of Metal Ions onto Clay Minerals. *Environmental Science & Technology,* 36**,** 2932-2938.

SEYMOUR, M. B., CHEN, G., SU, C. & LI, Y. 2013. Transport and Retention of Colloids in Porous Media: Does Shape Really Matter? *Environmental Science & Technology,* 47**,** 8391-8398.

SIDDIQUI, A. A., RICHARDS, D. J. & POWRIE, W. 2013. Biodegradation and flushing of MBT wastes. *Waste Management,* 33**,** 2257-2266.

SIRIPINYANOND, A. & BARNES, R. M. 1999. Flow field-flow fractionation-inductively coupled plasma mass spectrometry and metal speciation in proteins: A feasibility study. *Journal of Analytical Atomic Spectrometry,* 14**,** 1527-1531.

SIRIPINYANOND, A. & BARNES, R. M. 2002. Flow field-flow fractionation-inductively coupled plasma mass spectrometry of chemical mechanical polishing slurries. *Spectrochimica Acta Part B-Atomic Spectroscopy,* 57**,** 1885-1896.

SLACK, R. J., GRONOW, J. R. & VOULVOULIS, N. 2005. Household hazardous waste in municipal landfills: contaminants in leachate. *Science of The Total Environment,* 337**,** 119-137.

STEVENSON, F. J. 1994. *Humus Chemistry: Genesis, Composition, Reactions, 2nd Edition,* New York, John Wiley.

STOLPE, B., GUO, L. & SHILLER, A. 2010a. Nanoscale colloidal iron-binding organic matter in marine waters. *Geochimica et Cosmochimica Acta,* 74**,** A996-A996.

STOLPE, B., GUO, L. & SHILLER, A. M. 2014. Binding and transport of rare earth elements by organic and iron-rich nanocolloids in Alaskan rivers, as revealed by field-flow fractionation and ICP-MS. *Geochimica et Cosmochimica Acta.*

STOLPE, B., GUO, L., SHILLER, A. M. & AIKEN, G. R. 2012. Abundance, size distributions and trace-element binding of organic and iron-rich nanocolloids in Alaskan rivers, as revealed by field-flow fractionation and ICP-MS. *Geochimica et Cosmochimica Acta.*

STOLPE, B., GUO, L. D., SHILLER, A. M. & HASSELLOV, M. 2010b. Size and composition of colloidal organic matter and trace elements in the Mississippi River, Pearl River and the northern Gulf of Mexico, as characterized by flow field-flow fractionation. *Marine Chemistry,* 118, 119-128.

STOLPE, B. & HASSELLOV, M. 2007. Changes in size distribution of fresh water nanoscale colloidal matter and associated elements on mixing with seawater. *Geochimica et Cosmochimica Acta,* 71, 3292-3301.

STOLPE, B., HASSELLOV, M., ANDERSSON, K. & TURNER, D. R. 2005. High resolution ICPMS as an on-line detector for flow field-flow fractionation; multi-element determination of colloidal size distributions in a natural water sample. *Analytica Chimica Acta,* 535, 109-121.

STURROCK, A. M., HUNTER, E., MILTON, J. A. & TRUEMAN, C. N. 2013. Analysis methods and reference concentrations of 12 minor and trace elements in fish blood plasma. *Journal of Trace Elements in Medicine and Biology,* 27, 273-285.

TAN, X., WANG, X., CHEN, C. & SUN, A. 2007. Effect of soil humic and fulvic acids, pH and ionic strength on Th(IV) sorption to TiO2 nanoparticles. *Applied Radiation and Isotopes,* 65, 375-381.

TAYLOR, R. & ALLEN, A. 2006. Waste disposal and landfill: Information needs. *In:* SCHMOLL, O., HOWARD, G., CHILTON, J. & CHORUS, I. (eds.) *Protecting Groundwater for Health: Managing the Quality of Drinking-water Sources, WHO Drinking Water Quality Series Monograph.* IWA PUBLISHING.

THANG, N. M., GECKEIS, H., KIM, J. I. & BECK, H. P. 2001. Application of the flow field flow fractionation (FFFF) to the characterization of aquatic humic colloids: evaluation and optimization of the method. *Colloids and Surfaces a-Physicochemical and Engineering Aspects,* 181, 289-301.

THOMAS, R. 2013. *Practical Guide to ICP-MS: A Tutorial for Beginners*, CRC Press.

THURMAN, E. M. 1985. *Organic geochemistry of natural waters,* New York, John Wiley and Sons.

TIPPING, E., LOFTS, S. & SONKE, J. E. 2011. Humic Ion-Binding Model VII: a revised parameterisation of cation-binding by humic substances. *Environmental Chemistry,* 8, 225-235.

WAGNER, S., GONDIKAS, A., NEUBAUER, E., HOFMANN, T. & VON DER KAMMER, F. 2014. Finde den Unterschied: synthetische und natürliche Nanopartikel in der Umwelt – Freisetzung, Verhalten und Verbleib. *Angewandte Chemie,* 126, 12604-12626.

WAHLUND, K.-G. 2013. Flow field-flow fractionation: Critical overview. *Journal of Chromatography A,* 1287, 97-112.

WANG, H., QI, J., KELLER, A. A., ZHU, M. & LI, F. 2014. Effects of pH, ionic strength and humic acid on the removal of TiO2 nanoparticles from aqueous phase by coagulation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 450, 161-165.

WENG, L., FEST, E. P. M. J., FILLIUS, J., TEMMINGHOFF, E. J. M. & VAN RIEMSDIJK, W. H. 2002. Transport of Humic and Fulvic Acids in Relation to Metal Mobility in a Copper-Contaminated Acid Sandy Soil. *Environmental Science & Technology,* 36, 1699-1704.

WHITE, W. 2013. *Geochemistry,* New Jersey, John Wiley & Sons

WHITLEY, A. 2012. *Method Development for Detecting and Characterizing Manufactured Silver Nanoparticles in Soil Pore WaterUsing Asymmetrical Flow Field-Flow Fractionation.* Masters, Kentucky.

WIESNER, M. R., LOWRY, G. V., CASMAN, E., BERTSCH, P. M., MATSON, C. W., DI GIULIO, R. T., LIU, J. & HOCHELLA, M. F. 2011. Meditations on the Ubiquity and Mutability of Nano-Sized Materials in the Environment. *ACS Nano,* 5, 8466-8470.

WOODMAN, N. D., STRINGFELLOW, A. M., POWRIE, W., POTTER, H. A. B., SIMOES, A., MARCOSANTI, A., LAZZARINI, F. & PAVANI, C. 2011. Transport of Mecoprop through Mercia Mudstone and Oxford Clay at the laboratory scale. *Quarterly Journal of Engineering Geology and Hydrogeology,* 44, 331-344.

WORLD HEALTH ORGANISATION 2004. Inorganic Tin in Drinking-water.

WORMS, I. A. M., AL-GORANI SZIGETI, Z., DUBASCOUX, S., LESPES, G., TRABER, J., SIGG, L. & SLAVEYKOVA, V. I. 2010. Colloidal organic matter from wastewater treatment plant effluents: Characterization and role in metal distribution. *Water Research,* 44, 340-350.

WU, J., ZHANG, H., SHAO, L.-M. & HE, P.-J. 2012. Fluorescent characteristics and metal binding properties of individual molecular weight fractions in municipal solid waste leachate. *Environmental Pollution,* 162, 63-71.

XIAOLI, C., SHIMAOKA, T., QIANG, G. & YOUCAI, Z. 2008. Characterization of humic and fulvic acids extracted from landfill by elemental composition, 13C CP/MAS NMR and TMAH-Py-GC/MS. *Waste Management,* 28, 896-903.

YANG, X., FLYNN, R., VON DER KAMMER, F. & HOFMANN, T. 2011. Influence of ionic strength and pH on the limitation of latex microsphere deposition sites on iron-oxide coated sand by humic acid. *Environmental Pollution,* 159, 1896-1904.

ÖMAN, C. & ROSQVIST, H. 1999. Transport fate of organic compounds with water through landfills. *Water Research,* 33, 2247-2254.

ÖMAN, C. B. & JUNESTEDT, C. 2008. Chemical characterization of landfill leachates – 400 parameters and compounds. *Waste Management,* 28, 1876-1891.