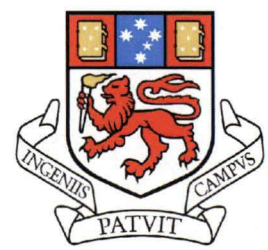
Ultratrace determination of aluminium in seawater and complex samples

by

Juliette Tria

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy



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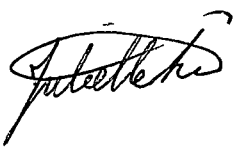

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LIST OF ABBREVIATIONS

HPCIC	high performance chelation chromatography
IC	ion chromatography
ICP-MS	inductively coupled plasma mass spectrometry
PCR	post column reaction
PCV	pyrocatechol violet
CAS	chrome azurol S
ECR	eriochrome cyanine R
IDAS	iminodiacetic acid silica
CTAB	cetyltrimethylammonium bromide
FIA	flow injection analysis
8-HQ	8-hydroxyquinoline
i.d.	internal diameter
M	moles/L
pK_a	negative logarithm of acid dissociation constant
LOD	limit of detection
LOQ	limit of quantification
LDPE	low density polyethylene
ANN	artificial neural network
MES	2-(N-morpholino)ethanesulfonic acid

LIST OF PUBLICATIONS

1. J. Tria, P.R. Haddad and P.N. Nesterenko. Potential applicability of a high performance chelation ion chromatographic method to the determination of aluminium in antarctic surface seawater, *Chemické listy*.102 (2008) s319-s323.

2. J. Tria, P.R. Haddad, P.N. Nesterenko, Determination of alluminium using high performance chelation ion chromatography, *Journal of Separation Science*. 31 (2008) 2231.

3. J. Tria, E.C.V. Butler, P.R. Haddad, A.R. Bowie, Determination of aluminium in natural water samples, *Analytica Chimica Acta*. 588 (2007) 153.

4. J. Tria, P.N. Nesterenko, P. Haddad, Potential applicability of high performance chelation ion chromatography to the determination of aluminium in Antarctic seawater, Oral, *Chemistry and Life 2008*, Brno, Czech Republic.

5. J. Tria, P.N. Nesterenko, P. Haddad, Recent developments in the determination of dissolved aluminium using high performance chelation ion chromatography, Oral, *RACI R&D Topics 2007*, Adelaide, Australia.

6. J. Tria, A.R. Bowie, P.R. Haddad and E.C.V. Butler, Application of shipboard determination of aluminium in seawater to dust deposition studies in the Ross Sea, Antarctica, Oral, *INTERACT 2006*, Perth, Australia.

7. J. Tria, P.N. Nesterenko, P. Haddad, Determination of aluminium in seawater using HPCIC, Poster, *ACROSS Symposium on Advances in Separation Science 2008*, Hobart, Australia.

8. J. Tria, P.N. Nesterenko, P. Haddad, Aluminium as a tracer for dust deposition in the Ross Sea, Antarctica, Poster, *RACI R&D Topics 2006,* Woollongong, Australia

9. J. Tria, P.N. Nesterenko, P. Haddad, Quantifying the impact of dust deposition to the Southern Ocean using dissolved aluminium concentrations, Poster, *RACI R&D Topics 2004,* Melbourne, Australia

ABSTRACT

Oceanographers use surface aluminium concentrations in open-ocean seawater as a tracer to fingerprint the location and magnitude of atmospheric dust deposition. It has become increasingly important to understand the role that such deposition plays in supplying trace elements to surface waters and consequently the effects such episodic supply has on moderating biological processes. For the purpose of real time analysis, quantification must be carried out by a system capable of being deployed shipboard. The most commonly employed technique for this purpose is flow injection analysis (FIA).

This project aimed to develop a method for the onboard quantification of aluminium in seawater, specifically for the analysis of Antarctic surface waters. Initially, the project focussed on the establishment and optimisation of a FIA system incorporating fluorescent detection of the aluminium-lumogallion complex. Significant variables affecting the lumogallion chemistry; including, reaction pH, lumogallion concentration and reaction time were optimised for this specific FIA system. Since aluminium concentrations in Antarctic seawater are expected to be in the nanomolar to subnanomolar range, investigation into the addition of an 8-hydroxyquinoline column to the manifold, for preconcentration purposes, was carried out. Although initial work involving quantification of aluminium in seawater samples appeared promising, complications with the robustness of this technique forced an alternative method to be sought.

High performance chelation ion chromatography (HPCIC) was considered a suitable alternative for development as a technique for the purpose of shipboard quantification of aluminium in seawater. The HPCIC system developed, involved the novel use of iminodiacetic acid functionalised silica for the separation of aluminium. Separation conditions, such as eluent composition and column temperature were optimised. Both photometric and fluorometric detection systems were developed, employing post column reaction (PCR) with a variety of reagents. Of those tested for photometric

detection, Eriochrome® Cyanine R, which was used for the first time for PCR determination of aluminium in a flow system, was found to be the most sensitive. A limit of detection of 100 nM for a 100 μL injection volume was achieved for this particular system.

For the HPCIC system with fluorescence detection, lumogallion was the reagent of choice given its reported high sensitivity. Variables such as buffer type and pH, as well as temperature and lumogallion concentration were optimised. A limit of detection of 0.39 nM for a 500 μL injection volume was obtained, with the performance of the system with a variety of other injection volumes also examined.

Finally, this study presents a discussion on the applicability of the newly developed HPCIC system to the quantification of aluminium in real samples. This work involves the analysis of paper mill process water and seawater from the Ross Sea, Antarctica. Particular attention is given to the topic of aluminium speciation with sample acidification. Conclusions and suggested future direction of studies in this area conclude this project.

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CHAPTER THREE 55

FIA WITH FLUORESCENCE DETECTION

Chapter One - Introduction

1.1 Overview

The focus of this project is on the development of a technique capable of being deployed shipboard for real-time determination of aluminium in seawater. The chosen analytical system was intended for use during a cruise of the Ross Sea, Antarctica in 2005-2006 and for subsequent work thereafter. Aluminium concentrations in seawater are used to trace dust deposition events, which are extremely important to the supply of trace elements and subsequent biological processes. Very little is known about dust supply to the Southern Ocean; however, oceanographers estimate atmospheric deposition to be limited. Subsequently, aluminium concentrations in the region are expected to be in the nanomolar to sub-nanomolar range. The method established during this project consequently had to be not only suitable for use aboard a ship, but also capable of determining extremely low concentrations of aluminium in such a complex matrix as seawater.

The following chapter discusses the biogeochemistry of aluminium in the ocean, its suitability as a tracer for dust deposition, existing methodologies for determination of aluminium in a range of natural water samples, as well as introducing the aims of the project.

1.2 Background

1.2.1 Aluminium in seawater

Aluminium is the third most abundant element in the Earth's crust (8.1% by weight) [1], but exists at only trace (nanomolar) concentrations in seawater. These very low concentrations (e.g. <0.5 nM in deep waters of the North Pacific; Figure 1.1(a)) can in part be explained by a balance between its input and removal processes [2]. Early studies [3] suggested that the concentration of dissolved aluminium in open-ocean was controlled predominantly by fluvial inputs. However, Maring and Duce [4] have since demonstrated that fluvial contributions to the remote ocean are negligible. Although early estimates of global riverine flux of dissolved aluminium were between 15 and 110 Gmol yr^{-1} [3], it is now known that the majority is lost to estuarine sediments [4-8] and through biological processes in the coastal ocean [9-12]. In contrast, aluminium-laden particles of aeolian dust with radii <5 μm are capable of long-range transport and deposition in the open-ocean, where they have atmospheric residence times of up to several days [4]. Aeolian dust, predominantly from the great deserts of the world, is therefore considered to be the likely major source of aluminium to the open ocean. Vertical profiles of aluminium in major oceans have shown that a further minor input occurs in deep waters, most likely from dissolution of sedimentary particles [13] (Figure 1.1 (a)).

Vertical profiles of dissolved aluminium in the Pacific and Atlantic Oceans (Figure 1.1(a)) show similar-shaped profiles, with highest concentrations in the

surface waters, mid-depth minima and an increase at the base of the water column. Aluminium is known to exhibit a scavenged-type distribution; that is, it has strong interactions with particles and a short residence time [14]. Although both oceans follow this type of vertical structure for dissolved aluminium, the concentration ranges are markedly different (8-40 times lower in the central North Pacific than in the central North Atlantic) [15]. This inter-ocean fractionation can be attributed to geographical variations, principally in the atmospheric flux experienced by the different ocean basins.

The Atlantic Ocean is subject to major inputs from the vast Sahara Desert, whereas the majority of the Pacific Ocean does not have such influential terrestrial sources, although the NW Pacific may receive dust from the Gobi desert [16]. These Saharan inputs significantly influence aluminium levels due to the relatively small size of the Atlantic Ocean compared to the Pacific Ocean. High surface aluminium concentrations in the Atlantic are also reflected in deep waters due to dissolution of previously absorbed surface aluminium on sinking particles [17]. The highest levels of aluminium have been observed in the semi-enclosed Mediterranean Sea (Figure 1.1(b)), a body of water that does not typically demonstrate a surface maximum [18].

Aluminium is characterised by its relatively short (2-6 yr) residence time in surface seawater [19, 20]. This short residence time can largely be attributed to the element's rapid hydrolysis rate and the extremely low solubility of the hydrolysis products [21]. Furthermore, the hydrolysis products of aluminium at the pH of seawater, namely

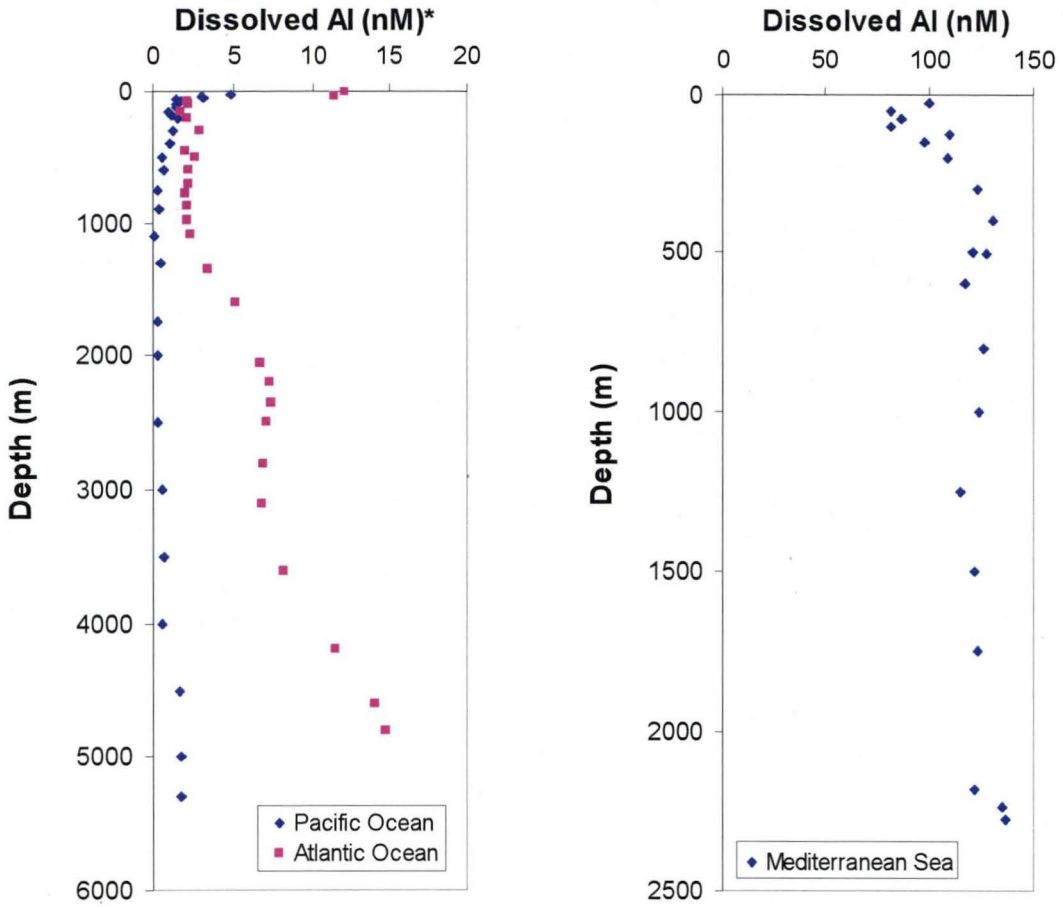

Figure 1.1.

(a) Vertical profiles of dissolved aluminium for the Pacific and Atlantic Oceans [17, 19].

(*Original concentrations of aluminium in the Pacific Ocean were given in nmol kg^{-1})

(b) Vertical profile of dissolved aluminium in the Mediterranean Sea [18].

$Al(OH)_3$ and $Al(OH)_4^-$, are extremely particle-reactive [15]. Removal of aluminium from seawater may occur by either passive or active processes [19]. Passive adsorption or scavenging is believed to be the major form of removal, as demonstrated by the vertical distributions of dissolved aluminium in the major ocean basins, and occurs via interaction with particles, both inorganic or organic in nature [15]. Active biological uptake by plankton has also been demonstrated to occur, although evidence of this is limited to studies on coastal waters, the Mediterranean (a confined marine basin), and in the laboratory [3, 10-12, 22, 23]. Additionally, the mechanism of biological removal is uncertain [24] and in part may be the result of increased adsorptive scavenging due to heightened particle fluxes during periods of increased primary productivity [19, 25], rather than uptake into cellular tissues [23].

A schematic of the biogeochemical cycle of aluminium is given in Figure 1.2. A thorough understanding of the marine geochemistry of aluminium is important for several reasons. Recently the focus has been on the use of aluminium as a tracer to fingerprint the location and magnitude of aeolian dust deposition. Atmospheric dust inputs are a significant source of several trace elements to the surface waters of the open-ocean. Delivery of trace metals (such as iron) to the ocean surface may occur directly by dry deposition (dust) or indirectly by wet deposition (rainfall) [26]. Iron is of particular interest since although it is an element essential for the growth and metabolism of all marine organisms [27-30], its concentration in the surface ocean is extremely low (0.1-0.5 nmol L^{-1}) [31] and limits the growth of marine phytoplankton in about 40% of the World's oceans [32]. Thus, increases in dissolved iron concentrations in the surface oceans may affect global climate through the

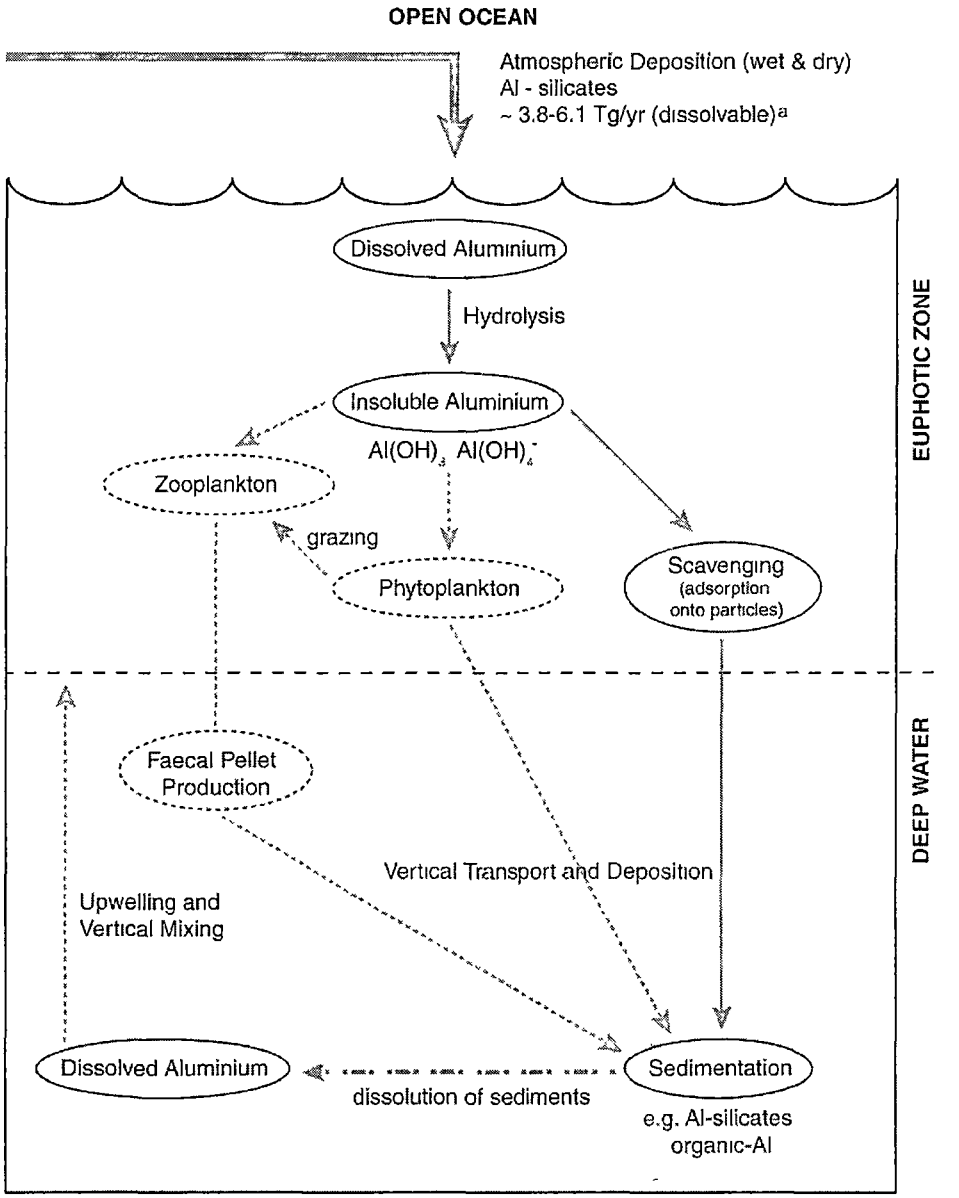

Figure 1.2. Biogeochemical cycle of aluminium. The weighting of the arrows indicates how significant the particular pathway is to the overall cycling of aluminium.

[a] Magnitude of dissolvable aluminium calculated from values given in Maring *et al.* [4].

stimulation of primary phytoplankton production and the subsequent drawdown from the atmosphere of climatically-important gases required for photosynthetic biological processes (e.g. CO_2) [33]. A reliable method for monitoring the atmospheric input of iron-laden dust to the ocean is thus imperative to furthering our understanding of Earth's climate control system.

1.2.2 Use of aluminium for dust deposition calculations

The concentration of dissolved aluminium in surface marine waters has been used successfully as a proxy for dust deposition in the ocean [2, 34]. The MADCOW (Model of Aluminium for Dust Calculation in Oceanic Waters) model first proposed by Measures and Brown in 1996 [2] estimates the annual dust input required to maintain background dissolved aluminium concentrations against an annual scavenging flux of 20% of the existing Al signal. The model assumes an average surface water residence time of 5 yr and a mixed layer depth of 30 m. As a result, comparisons between calculated and measured concentrations for different areas of the global ocean are not all in good agreement. However, Measures and Brown [2] note that local scaling of such parameters is anticipated to improve results.

Estimates of dust deposition to surface waters calculated by the MADCOW model can be used to provide information on the atmospheric delivery of a variety of other trace metals, including iron. Research has been undertaken to calculate the total input of iron (dissolved plus particulate) from the calculated dust fluxes, with the assumption that continentally-derived dust contains 4.3% iron [35]. Based on the molar ratio of Al to Fe in atmospheric dust (~3.5), the soluble fraction of iron in the

total flux has also been investigated. Although it was assumed initially that the Al/Fe molar ratio of the aeolian dust would be reflected in surface ocean waters, much higher than expected ratios have been observed [36, 37]. This indicates that iron solubility, along with its rapid removal by biological uptake, controls the extent of dissolution of aeolian-delivered iron and thus the observed surface water concentrations.

In order to ensure models such as MADCOW are reliable, there is a need for the development of robust and portable instrumentation capable of the precise, rapid and accurate determination of aluminium at extremely low concentrations (detection limit approaching 1 nM) during oceanographic expeditions. Consequently, in recent years much research has been undertaken not only to improve the methods of determination employed for trace elements such as aluminium, but also the entire analytical procedure starting from sample collection and filtration.

1.2.3 Aluminium speciation

It is important to consider the speciation of aluminium when selecting particular quantification techniques. Fractionation methods have developed primarily in response to the realisation that toxicity effects of aluminium depend largely on its chemical forms. Labile positively charged aqua- and hydroxy-mononuclear aluminium complexes have been reported as the most toxic to aquatic organisms and plants, with further toxicity effects recognised in crops and humans [38].

Aluminium speciation is somewhat difficult in aquatic systems, in which aluminium exists in numerous forms including: free Al^{3+}, Al-hydroxide complexes, monomeric fluoride complexes and various organic complexes. This difficulty arises due to several factors including: the participation of aluminium species in dynamic reactions, their low concentration and the presence of complex matrices that have the potential to interfere with analytical detection systems [39]. Distribution of aluminium species is dependent on factors such as pH, total concentrations of specific ligands and dissolved organic carbon [40] and it must be realised that inappropriate sample manipulation, storage and separation processes can alter the true distribution of species [41, 42].

The fractionation of aluminium is normally defined operationally since the content of real samples is very difficult to determine exactly. Fractions are given terms such as 'total reactive' and 'total monomeric' and generally contain multiple species, e.g for 'total monomeric', all inorganic and organic monomeric complexes. Aluminium speciation typically involves either a theoretical or experimental approach. The theoretical procedure involves the use of thermodynamic data together with the concentration of total aluminium and significant ligands, determined analytically. The experimental approach involves separation of species based on different reaction kinetics with a complexing reagent and/or separation based on size or charge of the species.

Clarke and co-workers [43] give a thorough review of methods published up until 1994 for the determination of aluminium fractions in natural waters. However, this

review focuses on fresh water examples only. This work classifies the main fractionation principles as: (1) kinetic or binding strength discriminations; (2) ion chromatographic separations; (3) size exclusion; (4) non-invasive methods; (5) ion mobility in an electric field; (6) minimised disturbance.

Pyrzyńska *et al.* [39] also present a review on aluminium speciation in natural waters. This review pays particular attention to the specific problems associated with aluminium speciation analysis and highlights some of the more applicable methods that have been developed. The same authors present a subsequent review of aluminium speciation in natural waters with particular focus on flow-injection methodologies [44]. The analytical performance of several separation procedures based on flow-injection analysis, as well as the detection methods are discussed and compared.

Five methods used in different laboratories in Norway and Finland for the fractionation and subsequent determination of aluminium were compared and the results presented in a paper by Wickstrom and co-workers [45]. Different fractionation principles, types of cation exchanger, reaction time, flow systems and determination techniques were tested. It was reported that of the procedures studied, determination of the labile fraction was best achieved using ICP-AES with an Amberlite column. The authors also present a discussion on the influence of various parameters on the distribution of the species and the effects of filtration and sample storage.

In more recent years, Bi *et al.* [40] and Ščančar and Milačič [38] have published reviews regarding aluminium speciation in environmental samples. Bi and co-workers concentrate on presenting advances in analytical methodologies for both environmental and biological samples in the preceding five years to publication. Their review includes concerns about specific problems of aluminium speciation, such as interference issues experienced by many techniques for samples with complex matrices, and also advantages and applications of particular methods. Ščančar and Milačič present a comprehensive review of the most important analytical methodologies of the last decade and new trends for the speciation of aluminium in environmental samples.

1.3 Analytical Techniques:

Separation/Preconcentration and Detection Methods for Trace Aluminium

1.3.1 Overview

Numerous methods for the separation, preconcentration and detection of trace concentrations of aluminium have been developed in order to suit a wide range of applications. There are many factors, such as sample matrix, potential interferences, required detection limit, and robustness and portability of the instrumentation that must be considered when choosing an appropriate procedure. In the following

sections, several established analytical methods for the determination of aluminium in natural waters will be discussed critically. These are summarised in Table 1.1.

1.3.2 Review of Current Literature

1.3.2.1 Atomic spectrometry

Atomic absorption spectrometry (AAS), atomic emission spectrometry (AES) and mass spectrometry (MS) can all be used for the determination of aluminium, and these methods are often coupled advantageously with chromatographic separation techniques, such as high-performance liquid chromatography (HPLC). Although flame AAS has been used routinely for the detection of many metals in a variety of matrices, it has insufficient sensitivity for samples containing ultra-trace levels of aluminium.

Graphite furnace (GF)-AAS has several advantages over flame AAS, the most important being increased sensitivity due to the sample residence time being greater, and a smaller required sample size. GF-AAS has been used successfully to determine trace aluminium levels in natural waters (seawater, river, soil water) [46-48]. Detection limits for this technique for trace metals are generally low and for aluminium have been reported to be as low as 0.1 nM in a seawater matrix, following preconcentration, with precision at 5% at the 1.0 nM level [19]. The primary disadvantages of GF-AAS are serious matrix interferences and the formation of

Table 1.1. An overview of analytical methods used for the determination of aluminium in natural water matrices.
[a]Concentrations reported in the original articles have been converted to μM or nM here for comparison purposes.

Method type	Preconcentration	Sample matrix	LOD[a]	Precision[a]	Ref.
GF-AAS	Solvent extraction (8-HQ)	Seawater	0.1 nM	5% at 1.0 nM	[19]
ICP-AES	HPLC (Chromazurol S immobilised silica gel)	River and seawater	10.4 nM	Not reported	[49]
ICP-MS	Single drop microextraction (SDME)	Lake water (and synthetic)	0.12 nM (synthetic)	10% at 37 nM	[50]
	None reported	Lagoon, lake water	3.7 nM	Not reported	[51]
Voltammetry (CSV)	Hg drop electrode using 1,2-dihydroxyanthraquinone-3-sulphonic acid (DASA)	Seawater	1.0 nM	2% at 15 nM	[52]
ECD-GC	Solvent extraction (HTFA)	Seawater	0.6 nM	3.8% at 19 nM	[53]
UV-Vis (absorption)	HPLC (Kromasil C_{18}, Spherisorb ODS-2, LiChrosorb RP-18, Nova-Pak C_{18})	River, stream water	51.9 nM	1% at 5.2 μM	[54]
Fluorometry					
Lumogallion	HPLC (LiChrosorb RP-18)	Seawater (and tap)	1.85 nM	2.4% at 1.9 μM	[55, 56]
	Solid phase extraction (8-HQ)	Seawater	0.15 nM	1.7% at 2.6 nM	[57]
8-HQS	IE (Amberlite IR-120)	Salt water (and fresh)	3.7 nM	2% at 0.37 μM	[58]
8-HQ	Solvent extraction (chloroform)	River (drinking and waste)	7.4 nM	4.9% at 1.9 μM	[59]
SAPH	None	Seawater	11.1 nM	1.9% at 7.4 nM	[60]
Morin	rp-HPLC (Spherisorb ODS)	Natural water (e.g. lake, river)	1.85 nM	1.8% at 1.0 μM	[61]

refractory carbides. The use of chemical modifiers has become routine during GF-AAS in order to overcome these interferences [62-64]. These chemicals act by helping the analyte to be retained at higher temperatures during pyrolysis, thereby ensuring that matrix interferences in the vapour stage are minimised. The modifiers also remove unwanted contaminants and aid in the separation of the analyte signal from background noise [64]. Nitrates of metals, such as magnesium and calcium, have been employed commonly as chemical modifiers, but with only a moderate improvement in sensitivity. More successful approaches have been found in the use of hydrogen peroxide with nitric acid [62] and β-diketones such as acetylacetone [63], with the latter having shown to improve the absorption signal intensity approximately 3-fold.

The plasma in inductively coupled plasma – atomic emission spectrometry (ICP-AES) efficiently atomises the sample before exciting the resulting atoms for detection. The primary advantage AES has over AAS is that emission modes can handle multi-element analysis, since all atoms are excited simultaneously. Additionally, AES is more capable of handling analyses in which the element has formed stable complexes that need to be broken down. The robustness of the method allows it to analyse all kinds of dissolved samples from dilute acids to those containing a high salt content. The detection limits of elements using ICP-AES have been recorded in the low ng g^{-1} range. However, the technique does suffer from the problem of spectral overlap from various elements present in the sample [65]. Aluminium is one such element, with its emission beginning at 212 nm and

continuing to below 190 nm [66]. This continuum emission makes simultaneous detection of cadmium, boron and tungsten extremely difficult with aluminium since the wavelengths of these three elements lie within the same region [67]. Chemical separation, for example using solid-phase extraction, is one method used to overcome this form of spectral interference. Successful applications of the technique for the detection of aluminium have been carried out for the analysis of natural waters (e.g. reservoir, spring, river and seawater) [49, 68].

Inductively coupled plasma – mass spectrometry (ICP-MS) provides an alternative to ICP-AES for multi-element analysis and is often the preferred method because of its much larger elemental scope and greater sensitivity. The technique is able to provide semi-quantitative data for samples in aqueous or organic media in only a few minutes [65], as well as the ability to measure individual isotopes of the analyte of interest. Typically, ions are separated in a quadrupole, with heightened performance having been attained with changes to the RF power source used for ICP generation and the introduction of high-resolution magnetic sector mass spectrometers. Detection limits for ICP-MS are now quoted in the ng L^{-1} range (part per trillion) with specific limits for aluminium between 2.6 pM-0.4 nM depending on the type of instrumentation used [69]. Although a relatively new technique, ICP-MS has been highly successful for the analysis of trace metals in a variety of samples including seawater, lake, spring and forest soil waters [50, 51, 68, 70, 71]. Table 1.1 gives specifics for two ICP-MS methods for the determination of aluminium in aqueous medium. Although the first method [50], successfully applies the system to lake water, the detection limit

(0.12 nM) is only reported for synthetic water. The second reference, Prendez *et al.* [51], gives a detection limit of 3.7 nM for lake and lagoon water matrices.

Despite the advantages of the atomic spectrometric methods discussed above, it is generally impossible to analyse a sample directly because of interfering species in the surrounding matrix, or the concentration of the analyte being below the detection limit of the instrument. HPLC (using ion-exchangers) is the most common technique used for sample separation and preconcentration coupled to the atomic spectrometric instrumentation. An important operational criterion is that the selected mobile phase must allow for adequate separation within a realistic time frame for the detection method.

1.3.2.2 Voltammetry

The direct determination of aluminium using classical voltammetric techniques is difficult, owing to the highly negative reduction potential of aluminium. This potential (approximately -1.75 V vs. SCE; saturated calomel electrode) is very close to that of major cations, including sodium and potassium [72]. Nevertheless, two types of stripping voltammetry - anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV) - do have the scope to determine aluminium in natural waters. Since the CSV method involves the complexation of aluminium in a preliminary reaction in solution, and adsorption of the complex in the concentration step at the electrode surface, it is often described as adsorption stripping voltammetry. The most commonly used ligand for complexation with aluminium in CSV is 1,2-

dihydroxyanthraquinone-3-sulfonic acid (DASA) [52, 72, 73], with the detection limit being recorded as low as 1.0 nM for a seawater matrix [52]. However, other compounds, such as solochrome violet [74, 75] and pyrocatechol violet [76, 77], have also been employed. The method has been applied successfully to determination of aluminium in natural waters other than seawater, e.g. river, lake and reservoir waters.

1.3.2.3 Electron capture detection - gas chromatography

Trace levels of aluminium and other metal ions have been detected successfully by electron capture detection – gas chromatography (ECD-GC), through the formation and extraction of volatile complexes. The technique is highly sensitive and subsequently allows for the use of small sample volumes. The basics of ECD (DC or pulsed mode) have been described elsewhere [78, 79]. The determination of aluminium by ECD-GC was reported by Measures and Edmond in 1986 [80], adapting a method first developed for the determination of beryllium in natural waters (seawater, river and rain water). However, the two methods differ in both the handling protocols and the type of solvent used for extraction. For aluminium analyses, 15 mL samples were buffered with sodium acetate and the metal reacted with 1,1,1-trifluoro-2,4-pentanedione (HTFA). This fluorinated volatile derivative was then extracted using toluene and back-washed using a sodium hydroxide solution. ECD-GC was carried out on 3 μL aliquots of the extracts using a Ni^{63} ionisation source. The method obtained a detection limit of 0.6 nM, with a precision of 3.8% at 18.5 nM in seawater [53]. Despite these impressive results, the use of this

method is limited by the considerable amount of sample handling, increasing the risk of contamination, and has not been used widely in recent years.

1.3.2.4 UV-Vis spectrophotometry and fluorometry

The absorption of UV-Vis light may be used to selectively determine trace metal ions, especially after formation of metal-organic complexes. The spectrophotometric determination of aluminium is typified by the use of quercetin (3,5,7,3′,4′-penatahydroxyflavone) as the colour-forming reagent. Quercetin selectively forms a stable complex with aluminium and detection is relatively free from interfering species [54]. Unlike many other complexing reagents, quercetin may be used for *in vivo* determination [54]. However, limits of detection for its general use are usually reported in the mg L^{-1} to μg L^{-1} range. Other typical chelating reagents include morin [81], pyrocatechol violet (PCV) [82] and eriochrome cyanine [83, 84]. UV-Vis spectrophotometry has been applied to the analysis of natural waters, including river, stream, spring, pond, lake and sea water and can be coupled to such techniques as flow injection analysis (FIA) and HPLC. The addition of masking agents is often required for the spectrophotometric determination of aluminium to increase selectivity. Masking is achieved by forming a stable complex from the potentially interfering species so that it can no longer react with the colour-forming reagent. Common masking agents used in the analysis of aluminium include hydrogen peroxide and cyanide [85]. However, many masking agents require a specific, and often narrow, pH range in which to complex the interferent of interest.

Photoluminescence, which incorporates fluorescence, is the emission of radiation resulting from the excitation of a sample by the absorption of photons. It has the advantages of high sensitivity, selectivity and linearity compared to other methods for the determination of aluminium, such as colorimetric spectrophotometry. The majority of fluorescence applications involve the use of extrinsic fluorescent reagents; that is, chromophoric molecules that react with, or adsorb onto, the analyte of interest. Since aluminium is not fluorescent itself, it must first react with a ligand to produce a fluorescing complex. Lumogallion (4-chloro-6-[(2,4-dihydroxyphenyl)azo]-1-hydroxybenzene-2-sulfonic acid; Figure 1.3), a tetradentate ligand that coordinates with aluminium to produce a fluorescent complex, is the most common, although a number of other compounds have also been investigated with varying degrees of selectivity, sensitivity and suitability for various applications. Several fluorescence-based analytical methods for aluminium, ordered by the particular fluorescent reagent employed, are discussed below.

(i). Lumogallion

The aluminium-lumogallion complex offers excellent fluorescence sensitivity and minimal interferences, and as such has been used successfully for the determination of aluminium in matrices with a high salt content, such as body fluids [55] and seawater [56]. In a method described by Wu and co-workers [52] for the determination of aluminium in human blood serum, the sample was reacted with lumogallion, with the resulting complex separated by HPLC (LiChrosorb RP-18

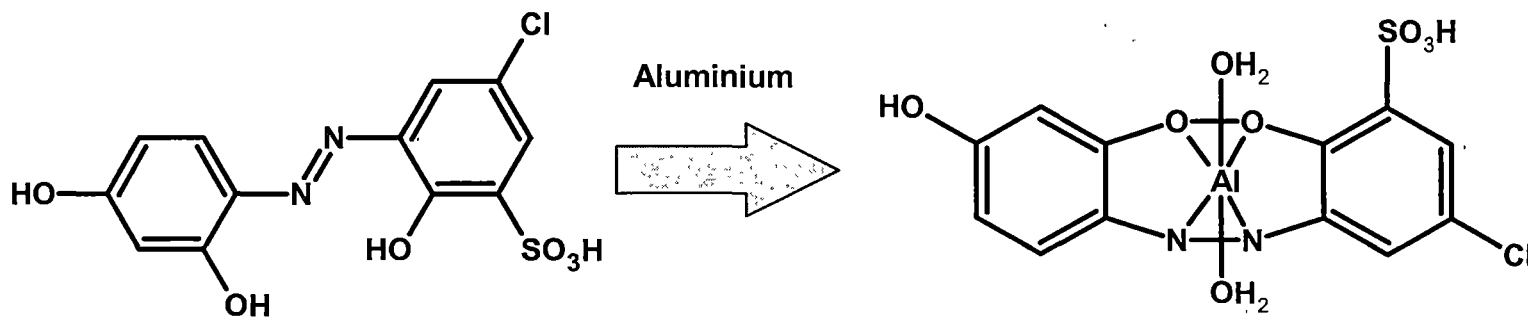

Figure 1.3. Chemical structure of lumogallion and reaction with aluminium.

column) and detected by fluorescence. A detection limit of 1.9 nM for aqueous solutions was achieved. The authors used the same procedure for the determination of aluminium in both tap and coastal seawater [56]. The results from this HPLC method were compared against those determined by GFAAS. For tap water, the results were in good agreement; 1.04 ± 0.04 μM (HPLC) compared with 1.04 ± 0.06 μM (GFAAS). However, differences were observed for seawater; 7.8 ± 1.5 μM (HPLC) compared with 9.3 ± 0.52 μM (GFAAS). The authors attributed this difference to inadequate background correction for the high salt content in seawater for GFAAS.

Capillary electrophoresis is another technique that has been used for the separation of the aluminium-lumogallion complex. He *et al.* [86] used a fused-silica capillary for separation after preparation of the complex by a batch method and theyreported a detection limit of approximately 0.70 μM with a precision of 3.1% at 3.7 μM (sample solution). It was found that by using this technique only one peak was observed in sample analyses, suggesting that the method was free from interference from common species, such as iron. The newly developed procedure was applied successfully to the determination of aluminium in river, reservoir and spring water samples, with relatively good agreement of results compared to those obtained by ICP-MS.

Hara and co-workers [87] developed a method for the fluorometric detection of the total concentration and individual species of aluminium using lumogallion. Determination of the free form of aluminium (Al^{3+}) was obtained directly using

gradient elution cation-exchange chromatography where the separation was based predominantly on the charge on the analyte. Aluminium fluoride complexes were estimated mathematically using a fluoride ion-selective electrode to measure the free and total amount of fluoride (assuming that all 2+ charged aluminium species were of the form AlF^{2+}) and complexation constants for the various fluoride complexes. The concentration of total dissolved aluminium was obtained using the same HPLC system as for the free and total fluoride-complexed species, but without the use of a separation column. Although the speciation of dissolved aluminium was carried out on 15 rainwater samples, Hara *et al.* [88] concluded in further investigation that the elution of dissolved aluminium from the column was not quantitative when using this method.

In a recent article by Fuse *et al.* [89], the use of a 5′-chloro-5-dodecyl-2,4,2′-trihydroxyazobenzene-impregnated XAD-4 resin (for the preconcentration of aluminium) coupled with fluorometric detection using lumogallion was investigated. The authors found that iron and other common ions caused no interference, and that successful speciation could be achieved without any change of pH by separating the aluminium species on ion-exchange resins. A detection limit of 2.2 nM in environmental water samples, with a precision of 7.3% at 3.7 nM was obtained and the method was tested on several lake and river samples with satisfactory results.

This section has highlighted the use of lumogallion for the detection of aluminium by fluorescence in many natural waters However, its application specifically to seawater analysis will be discussed further in sections 1.4.2 and 1.4.3.

(ii) 8-hydroxyquinoline

8-hydroxyquinoline (oxine or 8-HQ) and its sulfonated derivative, 8-hydroxyquinoline-5-sulfonic acid (8-HQS), are strong chelators for aluminium and give rise to fluorescent complexes.

Bloom and co-workers [90] applied the reaction of aluminium with 8-HQ in a batch method using butyl acetate as the solvent for extraction of the resulting complex. Detection was made by both spectrophotometric and fluorometric techniques, with the latter demonstrating the best detection limit (11.1 nM in distilled water). Sugimura and Suzuki [91] also utilised the fluorescent complex formed between aluminium and 8-HQ for the analysis of aluminium in seawater, after adsorption on a XAD-2 resin. Iron was initially removed by adsorption of the resultant complex with 4,7-diphenyl-1,10-phenanthroline on XAD-4 resin, and magnesium and zinc were prevented from interfering through sequential washes of ammoniacal solution of EDTA and acetate buffer solution. The reported detection limit was 3.0 nM in seawater (per 20 mL of chloroform eluent).

Zhu and co-workers [92] reported on the sensitising effect of a cetyltrimethylammonium bromide (CTAB) microemulsion on the determination of aluminium using fluorescence detection of its 8-HQ complex. It was found that the CTAB microemulsion gave a higher sensitivity than both CTAB micelles and water as media. The method gave a limit of detection of 0.15 μM in distilled water with

precision at 2.4%. The technique was applied successfully to the determination of aluminium in both tap and lake water.

Alonso and co-workers [59] compared three forms of liquid-liquid extraction in FIA systems and coupled them with fluorometric determination of the Al-8HQ complex. Of the three procedures investigated, it was found that injecting a single segment of organic solution into an aqueous stream of buffered sample without phase separation gave the lowest detection limit of 7.4 nM in distilled water. Each method was also trialled on real samples, including river and waste waters.

8-HQS has been acknowledged as one of the most sensitive organic ligands used for the determination of aluminium [93]. It forms a highly fluorescent complex, without showing any intrinsic fluorescence itself. Alonso and co-workers [58] reported a method using 8-HQS that was applied to the determination of aluminium in fresh and saline waters. Continuous determination of aluminium was possible with the use of FIA. Their method made use of the cationic surfactant cetyltrimethylammonium bromide (CTAB), which greatly enhanced the fluorescence intensity and accelerated the reaction rate. Many of the interferences inherent to batch methods were largely overcome, since post-column reaction after HPLC separation was employed. The detection limit for the method was 3.7 nM (in distilled water), with a precision of 2% at the 0.37 μM level. This system was shown by Fairman and Sanz-Medel [94] to be superior to the conventional batch methods using pyrocatechol violet. Their report

also detailed the importance of the separation step for natural water samples (lake) using an ion-exchange column of Amberlite IR-120 cation-exchange resin.

(iii) Salicylaldehyde picolinoylhydrazone (SAPH)

Salicylaldehyde picolinoylhydrazone (SAPH) forms a fluorescent chelate with aluminium in a stoichiometric ratio of 1:3. The complex exhibits a blue-green fluorescence at excitation and emission wavelengths of 384 and 468 nm, respectively [60, 95]. The method is very sensitive, has minimal interferences, and has been applied satisfactorily to the determination of trace amounts of aluminium in seawater and spring water.

Manuel-Vez and co-workers [60] applied the method to a batch determination of aluminium by reacting the metal with SAPH in an acetate buffer solution and measuring the resultant fluorescence intensity. The lowest detection limit achieved was 9.8 nM, with a precision of 1.85% at 166 nM in synthetic seawater. Canizares *et al.* [95] coupled the method to both a conventional FIA system and a flow-through sensor. The manifold of the sensor design involved injection of sample into an acetic acid/sodium acetate buffer and subsequent merging with the SAPH solution to form the fluorescent complex. The complex was retained in the flow cell by interaction with a C_{18} solid support and flushed to waste by injection of hydrochloric acid. The flow-through sensor method achieved a detection limit of 0.30 μM (6.7% precision) compared to 0.57 μM (8.1% precision) for the conventional FIA system (deionised water matrix) [61].

(iv) Morin

A particularly sensitive fluorometric reagent for the determination of trace aluminium is 3,5,7,2′,4′-pentahydroxyflavone (morin). Morin can selectively form a highly fluorescent complex with aluminium and has been used widely as a reagent for both fluorometric and spectrophotometric determinations. Various investigations have been undertaken to improve both the extraction efficiency of the fluorescent complex into isobutyl methyl ketone (IBMK) [96, 97], and the sensitivity via the addition of non-ionic surfactants [98]. Despite continued research, the application of morin remains limited since the very long reaction time with aluminium makes automation difficult [99].

A recent paper by Lian *et al.* [100] describes a reversed-phase high-performance liquid chromatographic method (Spherisorb ODS column), with pre-column complexation of morin and aluminium and fluorometric detection. A detection limit of 2.0 nM in a distilled water matrix and precision of 1.8% at the 1.0 μM level [100] was achieved and a wide linear range for detection was possible due to the unreactive morin being separated from the fluorescent Al-morin complex. The method was applied to the analysis of a substantial number of natural water samples including canal, river, stream, cave, pond, spring and lake waters. Lian and co-workers [61] later developed a novel strategy for the speciation of aluminium using selective analytical reagents, including morin, under specific pH conditions. They reported considerable advantages to this fluorometric method for the fractionation of

aluminium in natural waters, namely high sensitivity, easy manipulation and the exclusion of a separation step.

(v) Other fluorescent reagents

Various other fluorescent reagents have been utilised for the determination of aluminium in natural waters, such as chromotropic acid [101] and 2,6-bis[(*o*-hydroxy)phenyliminomethyl]-1-hydroxybenzene (BPhH) [102]. Although some satisfactory results have been obtained, the use of these reagents is not widespread and consequently details will not be discussed further.

1.3.2.5 Chelation ion chromatography

The use of ion chromatography (IC) for the quantification of aluminium has been previously restricted to the determination of Al^{3+} [103]. However, the scope of this technique has since been broadened to include separation and determination of multiple Al complexes (e.g. fluoro, oxalate, citrate) [87, 104-106]. Both anion- and cation-exchange modes of IC may be utilised in order to determine positively or negatively charged species of aluminium [107]. The obvious restriction of using common ion-exchangers in IC separations is their high sensitivity to the presence of simple electrolytes (KCl, NH_4Cl, $CaCl_2$ and others) which are used frequently for the extraction of aluminium from samples such as soil, sediment and different plant materials [108]. Chelating ion-exchangers are therefore of particular interest for the separation and determination of aluminium as an alternative to traditional ion-exchange materials. They function by retaining metal ions according to the stability

of the corresponding complexes with chelating groups on the stationary phase and allow for the separation and preconcentration of aluminium in complex samples having a high content of alkali- and alkaline-earth metal salts.

The determination of aluminium can take into account three categories of species. These groups have been described as labile weakly bound monomeric (free aluminium, aluminium sulfate, fluoride, and hydroxide complexes), non-labile thermodynamically stable monomeric (complexes of aluminium with organic ligands) and kinetically inert thermodynamically stable polymeric type complexes and colloids [109]. Usually the differentiation of aluminium species is based on competitive complexation and/or acid reactivity [110]. Recently, competitive chelation with the chelating Chelex 100 resin (which carries iminodiacetic acid functional groups) has been used in a resin titration method proposed by Pesavento *et al.* [111]. So, another possible advantage of chelating ion-exchangers is their ability to discriminate between kinetically-labile complexes and stable, inert complexes of aluminium, which provides additional information on the bioavailability and ecotoxicity of this element in natural samples.

High performance chelation ion chromatography (HPCIC), or other IC modes in which chelation is the dominant retention mechanism, offers several advantages over ion-exchange separation [112, 113]. Firstly, it allows for the possibility of using only one type of functionalised resin for both preconcentration and separation. This has obvious consequences in terms of the simplicity of a system for an application requiring both processes, since the same eluent can be used. Secondly, chelation acts

in such a way as to convert all species of aluminium into uniform surface complexes. While an ion-exchange chromatogram may show multiple peaks for largely unidentified aluminium species, a chromatogram using chelation will show only one or two; corresponding to total soluble and more strongly bound species. This is beneficial if full speciation of aluminium is not required.

There are few known attempts to use HPCIC for the separation and determination of aluminium. Jones *et al.* used different neutral polystyrenedivinylbenzene (PS-DVB) microspherical resins impregnated with Chrome Azurol S dye, which has two salicylic acid groups in the molecule selective to aluminium [114]. Isocratic separation of aluminium, indium and gallium was achieved on Benson BPI-10 resin with 1 M KNO_3 at pH 2.25 as the eluent. This separation was repeated on PS-DVB resin (Polymer Labs PRLP-S) [115] and a slightly different elution order (Al(III) < Ga(III) < In(III)) was observed. Two-step pH gradient elution from 2.2 to 1.0 in 1 M KNO_3 was used for the separation of aluminium, gallium, indium and iron(III) on a similar chromatographic column. Finally, this same HPCIC system was used for the determination of aluminium in seawater [116]. In all of these studies, photometric detection after post-column reaction (PCR) with 0.004% Pyrocatechol Violet in 0.5 M hexamine adjusted to pH 6 was used.

The chromatographic behaviour of aluminium on Hamilton PRP-1 neutral PS-DVB resin dynamically modified with 4-chlorodipicolinic acid was investigated by Shaw *et al.* [117]. Aluminium was retained by this chelating substrate using an eluent comprising 1 M KNO_3 – 0.25 mM 4-chlorodipicolinic acid only when the eluent pH

was higher than 2.0. The retention order Al(III) < La(III) < Lu(III) < Fe(III) < U(VI) was observed. The separation of aluminium and lead on a short (50 mm.) column packed with aminocarboxylated polymethacrylate Bio-Rad HRLC-MA7C resin was also reported [118].

The most significant problem of the above-mentioned works was very poor column efficiency which is associated with the use of relatively coarse 7-10 μm polymer-based chelating resins and their slow kinetics of complexation with aluminium. This problem can potentially be overcome by the use of iminodiacetic acid functionalized silica (IDAS), which not only has a similar selectivity to Chelex 100 but also exhibits remarkable column efficiency in the separation of metal ions by HPCIC.

1.4 Shipboard Determination of Trace Aluminium in Seawater

1.4.1 Overview

Although many low-cost, sensitive and rapid methods have been developed for the determination of trace levels of aluminium, few are suitable for the analysis of seawater samples and fewer still for shipboard determinations. The major concern for seawater analysis is the matrix effects from interfering ions. Considerations that must be made regarding shipboard instrumentation include size, mass, portability, robustness against shocks and vibrations, and ease of automation. The move towards

finding a suitable method for shipboard trace aluminium analysis is in response to recent progress made in understanding the contamination risks, problems associated with sample storage and the need for high resolution real-time data to aid in tactical cruise planning (e.g. following dust deposition events).

The different types of atomic spectrometric techniques may have advantages such as high sensitivity and multi-element analysis, but the size and shape of such instruments precludes their use at sea. Additional shortcomings include the inability to cope with matrix interferences and vibrations encountered on an underway research vessel. Furthermore the purchase and running costs of such instruments are relatively high.

Voltammetry and FIA with fluorescence detection are two techniques that have been shown to be successful for the onboard determination of aluminium in seawater. The instrumentation needed for these techniques is such that transportation may be achieved relatively easily and disruptions to the operation by any shocks or vibrations that may occur whilst at sea are minimal. In addition, the techniques may be fully automated and operated in a flow-analysis mode, ensuring minimisation of contamination and an efficient analysis time. Initial equipment and ongoing running costs are also significantly lower than for most other techniques. FIA with fluorescence detection using the reagent lumogallion has been used most comprehensively by oceanographers in recent years, notably due to the suitability of the instrumentation for shipboard work and the ability to obtain particularly low detections limits. Consequently, it will be discussed in further detail here.

1.4.2 Fluorescence detection with lumogallion

In the 1960s, Nishikawa *et al.* [119] first described the technique for the determination of aluminium in seawater using the fluorescent reagent lumogallion (Figure 1.3). Hydes and Liss [120] later lowered the detection limit to nanomolar levels using a batch method in which 50 mL samples were reacted with a lumogallion solution and buffered with a sodium acetate/acetic acid solution. The reaction mixture was heated at 80 °C in a water bath for 1.5 h before fluorescence of the samples was measured at an excitation wavelength of 465 nm and emission wavelength of 555 nm. This procedure was reported to detect all forms of aluminium, except that which is incorporated in stable mineral structures (e.g. clay particles) [120]. Interferences from iron were insignificant below 100 $\mu g\ L^{-1}$, and the authors suggested that UV irradiation of samples would overcome competition for the Al-lumogallion complex by naturally-occurring organic ligands. The detection limit for the method was reported as 1.9 nM with a precision of 5% at the 37 nM level [120].

Subsequently, Howard *et al.* [121] achieved a 5-6 fold increase in the fluorescence intensity of the aluminium-lumogallion complex by adding the non-ionic detergent Triton X-100 after the reaction between the aluminium and lumogallion, and immediately before fluorescence detection. Multiple surfactants were tested, and although cationic surfactants gave initial significant enhancements, only non-ionic surfactants showed sustained enhancement. Triton X-100 was chosen, based on its ease of use and high performance with regard to increasing fluorescence intensity.

The detection limit was reduced to 0.74 nM in fresh and saline water, with a precision of 5% at the 3.7 nM level [121].

1.4.3 FIA with fluorescence detection using lumogallion

The lumogallion method for the detection of aluminium in seawater was first incorporated into a FIA system by Resing and Measures in 1994 [57]. The method involved on-line preconcentration of seawater samples on a column of resin-immobilised 8-hydroxyquinoline (R8-HQ), and post-column reaction of the eluted aluminium with the lumogallion reagent. In order to develop a procedure that gave accurate and precise results in only a few minutes, optimisation of each component of the manifold was undertaken. The optimisation was divided into five parts, including the reaction between aluminium and lumogallion, efficiency of the 8-HQ column, surfactant selection, detection parameters and minimisation of interferences. Variables that were adjusted included; reaction and sample pH, column length and type of surfactant used. The optimised system gave a detection limit of ~0.15 nM in seawater, with a precision of 1.7% at the 2.4 nM level. The suitability of this method for shipboard determination of aluminium in seawater was excellent due to the minimal amount of sample handling, speed and ease of use, as well as an extremely low detection limit. Despite being proven to be a successful shipboard method, careful and time-consuming preparation of clean seawater for the system's carrier stream was a requirement and problems with preconcentration existed. These issues have since been further investigated and the method subsequently modified in a paper by Brown and Bruland in 2008 [122].

The use of an 8-HQ column was an integral part of the method by Resing and Measures [57], not only ensuring an adequate preconcentration of the analyte in the sample prior to Al-lumogallion fluorescence detection, but also aiding in eliminating potential interfering species (e.g. iron and copper). This latter outcome results because the interfering species are either only partially retained on the resin, or if retained, are separated from the aluminium during the elution process [57]. Resing and Measures synthesised R8-HQ by a modification of the method of Landing *et al.* [123]. This synthesis is a multi-step process taking at least 15 h. Dierssen *et al.* [124] have recently reported a simplified one-step approach that reacts an epoxy-activated resin directly with 5-amino-8-hydroxyquinoline. The entire synthesis takes less than 7 h and resin functionalised via this method has been used successfully for the preconcentration of a variety of trace metals from acidified seawater samples [124].

Various other adaptations of the original lumogallion method have been made in an attempt to increase the sensitivity and selectivity of the system. Obata *et al.* [125] omitted the sample preconcentration step, but still reported sub-nanomolar detection limits for aluminium. Their method involved the selective removal of iron as an interfering ion through incorporation of a metal alkoxide glass immobilized 8-hydroxyquinoline (MAF-8HQ) column in the manifold. The column, originally designed for the measurement of trace amounts of iron in seawater, was used at a pH of 3.2 so that iron was removed selectively from the sample. The sample was then adjusted to a higher pH by post-column reaction so that optimal reaction with the lumogallion could occur. Using this set-up, 1 μM of iron did not interfere with the

detection of 1 nM aluminium. The detection limit for the method was found to be 0.17 nM for 10 mL of seawater sample, with a precision of 2.7% at the 2 nM level.

Ren and co-workers [126] endeavoured to overcome interferences of both iron and fluoride, based on the work of Zhang *et al.* [127]. Both groups investigated the addition of *o*-phenanthroline and Be^{2+} to mask the interferences of iron and fluoride, respectively, during fluorometric determination of dissolved aluminium. The method of Zhang and co-workers involved tedious extraction of the lumogallion complex into *n*-hexanol and achieved a detection limit of 0.25 nM with a precision of 5% at the 40 nM level. Ren *et al.* developed a more 'operator-friendly' technique that no longer required liquid-liquid extraction. Although interferences were minimised successfully, a considerable amount of sample manipulation was still required, making the method lengthy and contamination risks higher. In addition, the detection limit was higher than that of Zhang *et al.*, with a value of 0.7 nM in distilled water and precision of 3.6% at the 5.0 nM level.

In a more recent paper, Kramer and co-workers [20] successfully applied the technique developed by Resing and Measures [57] to determine the distribution of dissolved aluminium in the North Atlantic Ocean. Their procedure involved only minor changes to the original report, with a deionised water wash of the loaded column before elution. A detection limit of 0.7 nM in seawater with a precision of 2.3% at the 14.2 nM level was achieved.

Although several publications have focused on basin-scale aluminium distributions during long-range transects of the major oceans, high-resolution information available presently for localised and confined regions is both scarce and conflicting, highlighting the continued need for reliable shipboard methods.

1.5 Aims of Project

The overall objective of this project was the establishment of a robust system capable of determining ultra-trace levels of aluminium in seawater. FIA has been discussed at length here and is undoubtedly the most widely accepted technique for the shipboard determination of aluminium in seawater. Consequently, the first approach of accomplishing the project was the set-up of a FIA system. It was envisaged that optimisation of operating parameters, e.g. reaction pH of aluminium-lumogallion complexation, would be beneficial in order to achieve the lowest LOD specific to this system.

In the event that a FIA system capable of determining nanomolar concentrations of aluminium in seawater could not be established satisfactory to requirements, development of an alternative technique would have to be undertaken. HPCIC was considered a viable option if the need arose.

The applicability of the ensuing system to the determination of aluminium in seawater would be assessed by the analysis of surface seawater samples taken from both the Pacific Ocean and the Ross Sea, Antarctica.

Chapter Two -
Methods and Materials

2.1 Practical Considerations

2.1.1. Contamination risks

The detection limits of analytical methods for the determination of trace metals have decreased significantly in recent years. This is as much a result of improved sample handling techniques as it is the development of new detection technologies. The most frequent problem encountered when analysing a sample for trace metal content is the risk of contamination. The extent to which this risk affects final results is dependent on the initial amount of analyte, the handling procedures employed, and the degree to which the analyte is present in the potential contamination source [128]. When analysing ocean seawater, for example, the risk of contamination is high, since most of the metals of interest are at nanomolar concentrations, yet are ubiquitous in the atmosphere, and are universally associated with many manufactured sampling and filtration materials, the research vessel itself and any human activity. Atmospheric contamination can be minimised by applying various levels of controls, with the most stringent being the use of a class-100 laminar flow hood or a full clean room of similar classification [129]. Reagent grade and the type of materials used in equipment manufacture must also be considered. Often, typical materials used in the

manufacture of apparatus for classical chemical analysis and for storage vessels are not suitable for ultra-trace metal work as they may either introduce trace levels of contaminants or provide a surface on which the analyte under investigation may adsorb. Common materials, such as glass, often have to be replaced with alternatives that reduce these problems, such as low-density polyethylene (LDPE) or perfluoroalkoxy (PFA) fluorocarbon polymers.

2.1.2. Storage bottles

The preferred type of plastic and the cleaning protocol for storage bottles for trace metal analysis is not universally agreed upon. In fact, the necessity of cleaning bottles by acid washing is still in debate. Reimann and co-workers [130] reported that acid washing had no systematic beneficial effect on the analytical results for bottles made of high density polyethylene (HDPE) or polypropylene (PP). Suggestions by Reimann regarding why acid washing may be detrimental to sample storage include the introduction of contaminates through the use of an "unclean" acid, and an increased adsorptive capacity or heightened availability of incorporated trace elements to leaching due to damage of the bottle's interior walls caused by acid soaking [130]. Conversely, Kremling and Streu [131] state that sample bottles made of Teflon, HDPE, PP and quartz must be cleaned very carefully before use and they describe a 8-9 step cleaning procedure involving detergent and acid washes, with copious amounts of rinsing with the purest distilled water. Achterberg *et al.* [132] and various other researchers also agree with this approach, although the cleaning protocol does differ somewhat. For example, the type and strength of acid used varies

between groups, with some using HNO_3, others choosing HCl and others preferring aqua regia. Moody and Lindstrom [133] showed that these acids tend to leach various elements with different efficiencies and recommended the use of both HCl and HNO_3, one after another, to ensure maximum cleaning.

Reimann's work also investigated the advantage of using expensive plastic bottle types, fluorinated ethylene-propylene co-polymer (FEP) and PFA, over the more affordable LDPE, HDPE and PP. He reported that bottle type was of no importance for the majority of the 62 elements tested. Exceptions to this were Al, Cr, Hf, Hg, Pb and Sn to varying degrees, with these elements showing better results for bottles made from PFA and FEP [130].

For this work, a rigorous bottle cleaning protocol for LDPE bottles was used. This included rinsing with deionised water followed by a 2% Decon (a surfactant with anionic and non-ionic surface active agents) soak for a week, a rinse using water purified on a Milli-Q system (henceforth referred to as Milli-Q water), acid soaking in a 10% HCl bath for the duration of a week, followed by further Milli-Q water rinses (x5), and lastly, acid refluxing using a specially produced glass bottle cleaning "tree" and high purity HCl, followed by final Milli-Q water rinses (x5). Bottles were then filled with a 3-5% high purity HCl solution under trace metal clean conditions until use.

2.1.3. Oceanographic sampling

The risk of contamination during the sampling process has been of great interest in recent years. Much of our current understanding and appreciation of the need for ultra-clean procedures can be attributed to the work of C.C. Patterson in the 1970s on lead. The accepted concentration of lead in seawater decreased three orders of magnitude over the three decades following his work due to the reduction of contamination during sampling storage and analysis [134]. Such work has led to the development of sampling methods aimed specifically at obtaining trace-metal clean seawater. Among the most common samplers used for this purpose are 'Go-Flo' and 'Niskin' bottles (both General Oceanics). The sample-containing tube section of Go-Flos designed for trace metal analysis is made of poly-vinyl chloride (PVC) with a Teflon coating. The bottles are designed to enter the ocean in a closed state, open by a pressure-release mechanism at ~10 m to allow flushing, and close at the desired depth when tripped by a hydroline messenger [135]. Niskins are also constructed from PVC and can be triggered to shut either by a hydroline messenger or simply by pulling a lanyard loop.

Contamination by the various types of hydrowires used for deployment of the sampler bottles has also been investigated in recent years. Whereas traditionally these wires have been made of steel or nylon for strength and durability, new non-contaminating materials (such as Kevlar or Spectra) are now being used by many laboratories around the world. This prevents contamination arising from rusty hydrowires, which will give erroneously high trace metal concentrations. Kevlar, now

widely used for trace metal hydrocasts, is a non-metallic wire with a high strength-to-weight ratio. Plastic-coated steel wire has also been successful in providing a 'non-metallic' option. An early 1980s intercomparison of three types of both sampling devices and hydrowires concluded that modified Go-Flo samplers coupled with plastic-coated steel hydrowires provided the least contaminated samples for the analysis of Cd, Cu, Fe, Mn, Ni, and Zn [136].

In order to obtain surface water trace metal concentration data with a high spatial resolution, techniques for continuous underway sampling have been developed. A towed 'fish' device is typically deployed at 1-10 m below the sea-surface and water is supplied to the ship's deck using a high-volume pumping system. Several variations of underway sampling systems have been reported, based on an original design by Boyle *et al.* in 1982 [137], which involved pumping water to the ship's deck using a vacuum pump and polyethylene tubing connected to a brass bathythermograph which is towed 5 m away from the side of the vessel. Recent modifications to materials used in the construction (e.g. all Teflon PFA tubing), design (e.g. PVC depressor vane 1 m above a 20 kg PVC fish) and pumping (e.g. Teflon PTFE double-diaphragm pump) of towed fish systems have improved oceanographers' ability to collect large volumes of trace metal clean surface seawater.

A towed fish device was employed for all surface seawater samples collected for use during this project. The fish employed was constructed entirely of PVC with Teflon PFA tubing and a Teflon PTFE pump was also utilised (See Figure 2.1).

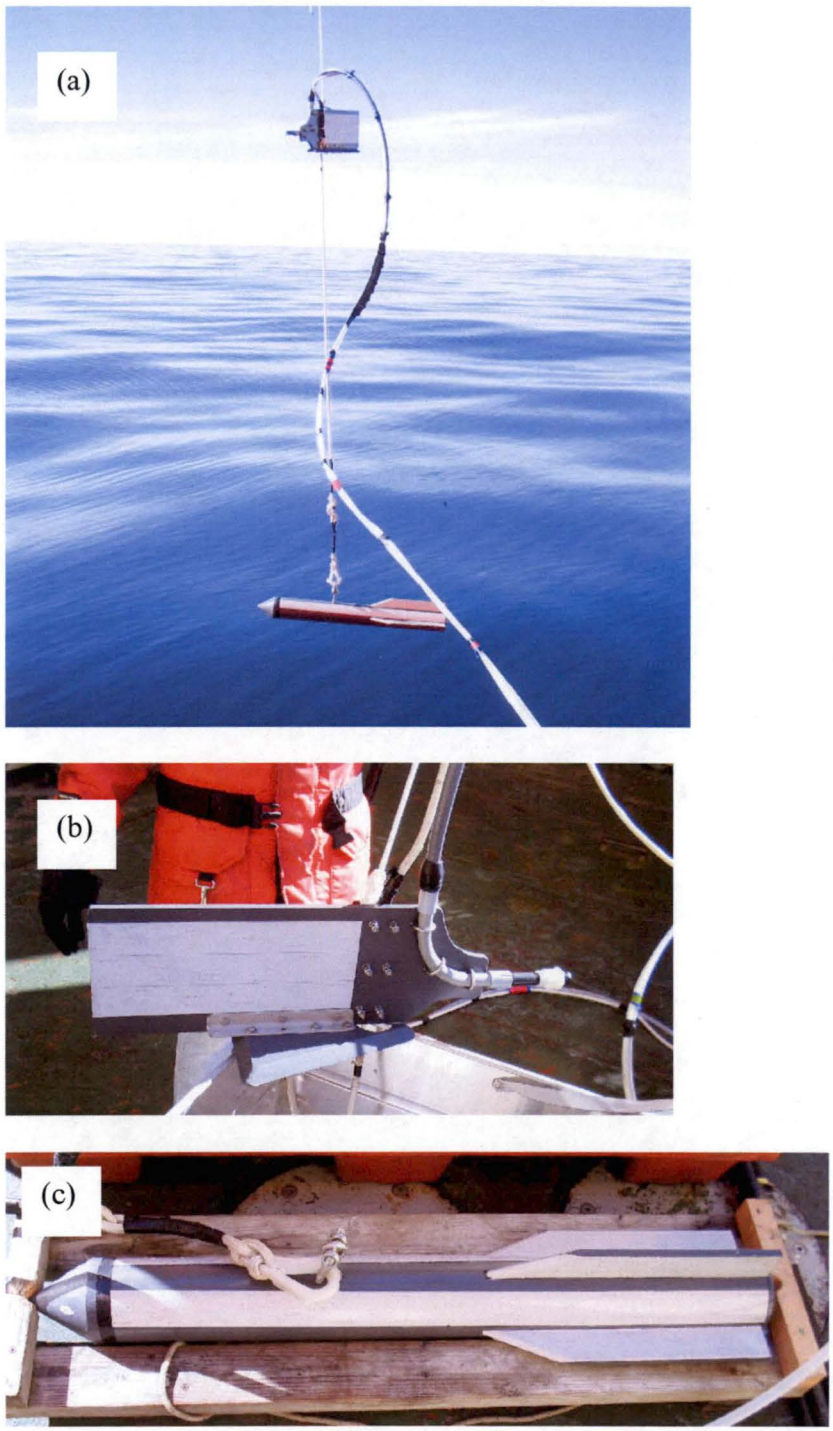

Figure 2.1. Towed fish used aboard R/V Nathanial B. Palmer
(a) System being deployed (b) PVC depressor vane (c) PVC fish.

2.1.4. Filtration

Oceanographers are now beginning to standardise the materials, devices and nominal cut-offs used for filtration. Filtration may be achieved either using cartridges (typically used for collecting dissolved samples) or membranes (typically used for collecting particulate samples). Polycarbonate is widely regarded as the best material for filtration, although others, for example polyethersulfone, have shown particular suitability for applications such as the retention of cell material [138]. Most workers either use 0.2 or 0.4-0.45 μm as their nominal cut-off for operationally differentiating between dissolved and particulate trace metal species. It is also possible to use ultrafiltration (e.g. <200 kDa) to obtain "truly soluble" species, or add higher size-classes (e.g. 2, 5, 20, 55, 210 μm) to investigate the association of trace metals with different biological size classes.

Filtration can be achieved by various means. Filtration carried out by suction under aspirator vacuum is one common method. Although the apparatus is more usually constructed of sintered glass or ceramic, all-plastic units are available for trace metal analysis. There are, however, several disadvantages associated with this technique, namely the potential contamination risks from the amount of sample handling required. Pressure filtration can overcome such problems. It involves pressurising the sample container by supply of a gas, forcing the sample through an inline filter to a collection vessel. An inert gas, such as nitrogen, is used to pressurise samples to be analysed for trace metals to ensure precipitation of compounds, such as iron hydroxide, is prevented [139]. Bewers *et al.* [140] gives a good intercomparison of

eight pressure-driven systems for the determination of several trace elements including iron and manganese. Recently, interest has grown in syringe filtration methods as an alternative to the more common filtration techniques. A thorough description of one such method is given by Shiller [141]. Although this work concentrates on the sampling of small volumes of river water, a similar procedure has been followed for seawater samples [142].

2.1.5. Sample enrichment

Despite advances having been made in detection instrumentation, trace metal analysis most often requires some form of separation and preconcentration methods to remove interfering matrices and ensure the level of analyte is detectable. Some of the major methods of separation and preconcentration involve evaporation, volatilisation, co-precipitation, solvent extraction or solid-phase extraction. Any preconcentration process involves additional sample handling and thus increases the potential risk of contamination. Therefore it is desirable to choose a technique that requires the minimal amount of sampling handling (e.g. on-line methods) and minimises the use of additional reagents.

2.2 FIA

2.2.1 Reagents

All reagents were of an analytical-reagent grade unless specified. A 2 M NH_4OAc buffer was prepared from trace metal grade concentrated acetic acid (GFS Chemicals; Powell, Ohio, USA) and ammonia solution (isopiestic distilled concentrated NH_4OH) and pH adjusted with either ammonia or acetic acid depending on the desired pH. A stock 1 g/L lumogallion solution was prepared and stored for up to two months. Working lumogallion/buffer reagent was prepared daily. A carrier of 0.1 M HCl was prepared using twice distilled concentrated HCl. A 5% Brij-35 solution was prepared by diluting commercially available 30% Brij-35 (Sigma-Aldrich; Castle Hill, NSW, Australia). Acidified 50 nM aluminium standards were prepared daily from a 1000 mg L^{-1} stock solution of aluminium in nitric acid. All solutions were prepared using deionised water from a Milli-Q Gradient water purification system, (Millipore; North Ryde, NSW, Australia).

2.2.2 Apparatus

The FIA manifold (see Figure 2.2) consisted of a Gilson (Middleton, WI, USA) Minipuls 3 eight-channel peristaltic pump for the delivery of all reagents and the sample; a six-port VICI (Houston, TX, USA) Cheminert® injection valve fitted with a two position microelectric actuator. Pump tubing used for the reagent and sample streams was standard flow-rated PVC tubing (Pro-tech Group; Coolum Beach, QLD, Australia). The remainder of the manifold was constructed from 1/16" O.D. x 0.03"

I.D. Teflon tubing (Alltech Associates Australia; Baulkam Hills, NSW, Australia). The reaction coil was constructed from 2 m of the same Teflon tubing knitted by way of a knitting apparatus (equivalent to a "Knitting Nancy") with spools set 1 cm apart. The resultant coil was ~ 25 cm long. The reaction coil was heated by way of a silicon heating pad (RS components; Smithfield, NSW, Australia), which was wrapped in glass tape and secured with a sticky back glass tape. The heater had an accuracy of ± 0.5 °C. A 1 m reaction coil (not heated) was knitted in the same way for mixing between the carrier and surfactant streams.

The preconcentration column was packed with 8-HQ functionalised Toyopearl AF-Epoxy-650 M resin (65 μm polystyrene divinylbenzene beads) (Supelco; Castle Hill, NSW, Australia). The column was constructed from PTFE tubing and had an inside diameter of 2.4 mm and a length of 25 mm. A sample loop of 30 cm of manifold tubing replaced the column when preconcentration was not required.

Detection was carried out using a Varian (Palo Alto, CA, USA) Prostar 363 fluorescence detector fitted with a xenon lamp. The excitation and emission wavelengths were set to 484 and 552 nm respectively.

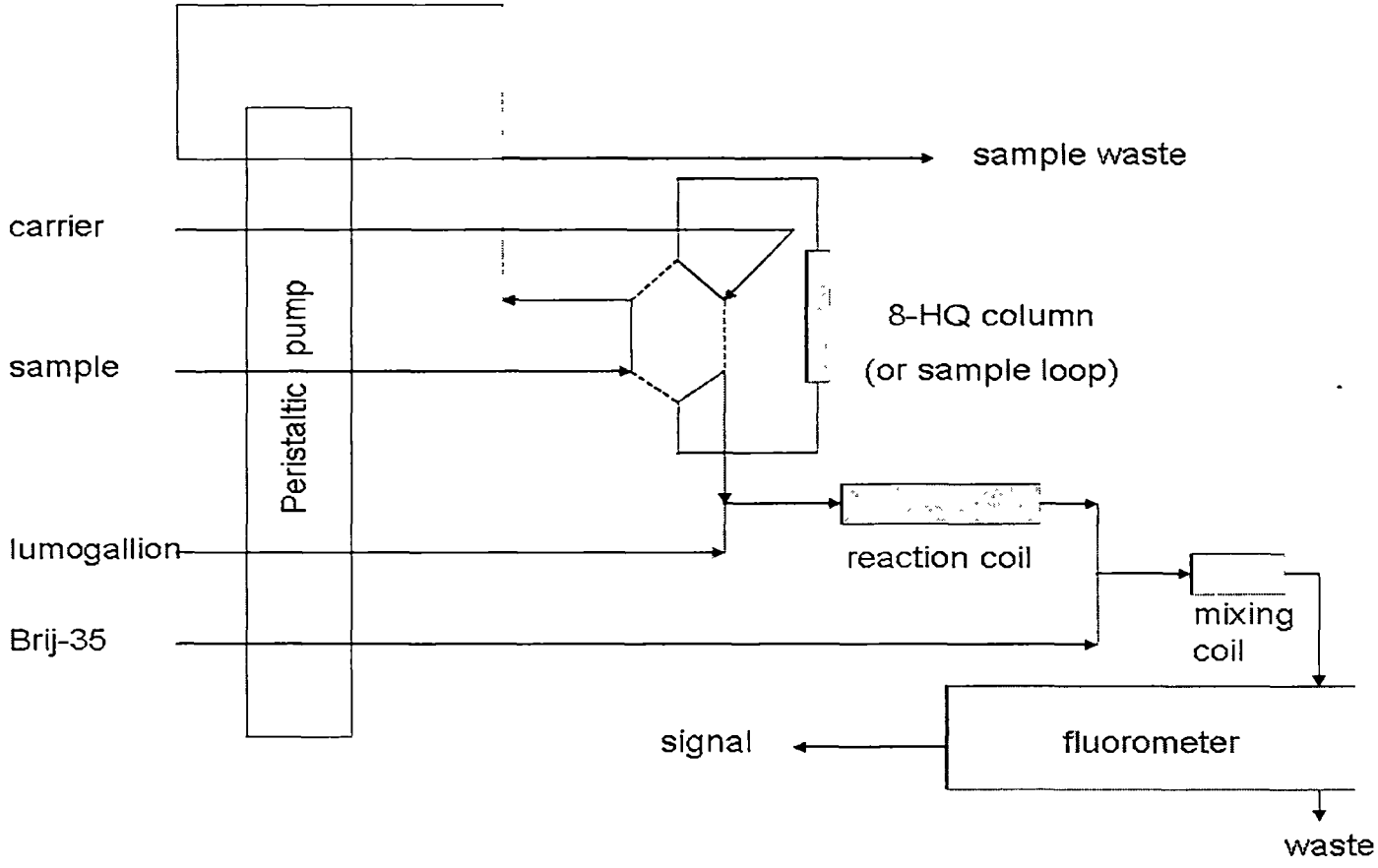

Figure 2.2. FIA manifold used for the determination of aluminium.

2.2.3 Design of experiments

Due to the problems with univariate experimental designs (discussed in section 3.2.2) a multivariate, full factorial design was implemented. This was deemed feasible since only three variables were considered significant enough to optimise based on literature reports of aluminium-lumogallion chemistry. The three variables investigated were pH, lumogallion concentration and reaction time, and all were tested at three levels producing a 3 factor, 3 level (3^3) design. Table 2.1 shows the levels at which the variables were tested. Ranges for both pH and reaction time were selected based on previous literature findings [57, 121]. The concentrations at which lumogallion was tested were based on a preliminary investigation in which the lumogallion concentration was increased against a standard 50 nM aluminium solution and the fluorescence intensity recorded.

The number of experiments performed was 27. The level at which each variable was held for individual experiments is given in the following chapter, in Table 3.1.

2.2.4 Experimental procedure

All optimisation experiments were carried out using a 30 cm sample loop and 50 nM acidified aluminium standard made in Milli-Q water. The carrier for this series of experiments was also Milli-Q water. The aluminium standard was flushed through the sample loop for 30 sec. This ensured sufficient rinsing and complete filling of the sample loop at each pump speed tested. Reaction times were controlled by pump

speed and varied from 4.5 to 12.5 rpm to achieve the required reaction times. Each experiment was run in triplicate.

Table 2.1. Specifications of levels of pH, lumogallion concentration and reaction time tested in optimisation experiments

Factor	**Level**		
	–	**0**	+
pH	4.5	5.5	6.5
Lumogallion concentration (μM)	5.90	1.18	23.6
Reaction time (min)	1	2	3

2.2.5 Statistical modelling and neural network simulation

Results from the optimisation of lumogallion chemistry experiments were analysed by two methods. Firstly, statistical modelling, using principles of general linear regression, was carried out in order to derive a function that represented if, and to what extent, experimental factors and their interactions influenced fluorescence intensity. From this function, optimum levels of the most influential factors could be determined. Statistical analysis was performed using SYSTAT®10.2.05 (Systat Software, Richmond, CA, USA). Model suitability was tested using analysis of estimates and tests of fit.

Optimum levels of the experimental factors were also determined using Trajan Neural Network Simulator 6.0 (Trajan Software, Horncastle, UK). The Intelligent Problem Solver (IPS) analysis option was utilised for this. Predictions, residuals, response graphs and response surfaces were generated using the 'run existing model' option in the analysis menu. The most suitable neural network was chosen primarily based on the selection performance, with the training and selection errors also taken into consideration.

2.3 HPCIC with Photometric Detection

2.3.1 Reagents

All chemicals were of analytical-reagent grade. Potassium chloride solutions and all buffers underwent filtration (0.45 μm). KCl-HNO_3 eluent was prepared from stock 1 M KCl and 1 M HNO_3 solutions. Other eluents investigated were similarly prepared from stock solutions. Aluminium standards were prepared daily from a 1000 mg L^{-1} stock solution of aluminium in nitric acid. All solutions were prepared using deionised water from a Milli-Q Gradient water purification system, (Millipore; North Ryde, NSW, Australia). PCR reagents were Tiron (disodium salt of 4,5-dihydroxybenzenedisulfonic acid monohydrate) (TCI; Taren Point, NSW, Australia); Pyrocatechol Violet, (Aldrich; Castle Hill, NSW, Australia); Chrome Azurol S and Eriochrome® Cyanine R, (both from Riedel-de Haën; Castle Hill, NSW, Australia).

2.3.2 Apparatus

A Metrohm 844 UV/Vis Compact IC with built-in photodiode array UV/Vis detection was used for all analyses. The system allowed for the delivery of eluent at 0.2 - 2.5 mL min^{-1} and was set up with a column heater (up to 75 °C) and a post-column reactor, consisting of a 2m PTFE reaction coil (1/16" x 0.02"). Peristaltic pump tubing delivered the PCR reagent at a constant flow-rate of 0.36 mL min^{-1}. A sample loop of 20 µL was used unless stated otherwise. Two columns were used, namely a 250 x 4.0 mm i.d. IonPac SCS-1 (Dionex, Sunnyvale, USA) packed with 4.5 µm poly(butadiene-maleic acid)-coated silica particles, and a 200 x 4 mm i.d. column packed with 5 µm IDAS (JPP Chromatography Ltd, Brentor, Devon, UK).

2.4 HPCIC with Fluorescence Detection

2.4.1 Reagents

All reagents were of an analytical grade. A NaCl-HNO_3 eluent (unless otherwise indicated) was made from stock 2 M and 1 M solutions respectively. All solutions were prepared from a Milli-Q Element purification system, (Millipore; North Ryde, NSW, Australia). A stock 1 M 2-(N-morpholino)ethanesulfonic acid (MES) (Sigma; Castle Hill, NSW, Australia) buffer was made and pH adjusted to 6.05 (unless otherwise stated) with concentrated NaOH. A stock 2 M NH_4OAc buffer was prepared from trace metal grade concentrated acetic acid (GFS Chemicals; Powell, Ohio, USA) and ammonia solution (isopiestic distilled concentrated NH_4OH) and pH

adjusted to 6.8. A stock 3 mM lumogallion (Pfaltz and Bauer, Waterbury, CT, USA) solution was prepared and refrigerated in dark conditions for up to 2 months. Working lumogallion buffers were prepared daily as were aluminium standards.

2.4.2 Apparatus

A Metrohm 844 UV/Vis Compact IC was used for all analyses. The system delivered the eluent at 0.3 mL min-1 and was set up with a post-column reactor, consisting of a 2m PTFE reaction coil (1/16" x 0.02"). This reactor was immersed in a silicon oil bath for heating above room temperature. Peristaltic pump tubing delivered the PCR reagent at a constant flow-rate of 0.36 mL min-1. A 20 μL sample loop was used unless specified.

A column heater set to 71 °C housed a 200 x 4 mm i.d. column packed with 5 μm IDAS (JPP Chromatography Ltd, Brentor, Devon, UK). Detection was carried out using a Varian Prostar 363 fluorescence detector fitted with a xenon lamp. The excitation and emission wavelengths were set to 500 and 550 nm respectively. The detector and Compact IC were connected through a Metrohm 830 IC Interface.

2.5 Samples

2.5.1 Paper mill process water sample

A sample of paper mill process water was obtained from the Boyer Mill, Hobart (Norske Skög). The sample was filtered (0.45 μm) before analysis. Sample acidification, when required, was achieved using twice distilled HCl.

2.5.2 Seawater samples

Surface seawater for use specifically during this project was collected aboard the Research Vessel Nathaniel B Palmer (USA) in the Ross Sea, Antarctica, by means of a towed fish. Samples were collected at a depth of approximately 7 m under trace metal clean conditions. The seawater was filtered (0.25 μm) and acidified to pH 2 using trace metal clean HCl. All handling of seawater samples was carried out under laminar flow, trace metal clean conditions.

Chapter Three -

FIA with Fluorescence Detection

3.1 Introduction

FIA coupled with fluorescence detection of the aluminium-lumogallion complex is by far the most widely employed technique for the ship-board determination of aluminium in seawater. The reasons for this have been discussed in detail already but in summary they include; transportability, sensitivity and relative ease of use. Considering that the objective of this project was to set up a method for the determination of aluminium in seawater for the purpose of analysis during a research voyage of the Ross Sea, Antarctica, it was logical to choose an established method which was likely to be suitable. Although the FIA method proposed by Resing and Measures [57] has been adapted by many groups since its publication in 1994, it was envisaged that further improvements would be possible given thorough investigation. The objective of this part of the project was, thus, to firstly establish a FIA system with fluorescence detection for shipboard determination of aluminium, and secondly, to improve the system through optimisation of the lumogallion chemistry and other adjustments.

3.2 Background to Statistical Optimisation

3.2.1 Overview

A preliminary step of this project was the development of an FIA system incorporating detection of aluminium by fluorescence detection of the aluminium-lumogallion complex. In order to thoroughly establish optimal conditions of the chemistry for this particular system, chemometrics were employed to ensure the process was carried out efficiently and effectively. Chemometrics is a chemical discipline that uses mathematical and statistical methods to design or select optimal measurements and experiments, and to maximise chemical information by analysing chemical data. This approach was considered most suitable given the substantial amount of prior research into the technique. Consequently, a brief introduction into the statistical approach of this work will be given in the following section.

3.2.2 Multivariate full factorial experimental design

Several variables contribute to the efficiency of the reaction between aluminium and lumogallion. Of these, pH, lumogallion concentration and reaction time were considered to be the most influential based on literature findings [57, 121]. To ensure maximum fluorescence was obtained from the aluminium-lumogallion reaction in the FIA system, and therefore, that the lowest limit of detection reached, optimum levels of these experimental parameters were required. A univariate approach of testing one factor at a time can lead to problems due to interactions between parameters. In order

to avoid such problems a multivariate method, in which multiple variables are tested simultaneously, is best.

The outcome (y) of an experiment is dependent on experimental conditions and may be approximated by a polynomial function based on the experimental variables. The most simple of these models contains only linear terms and for experiments with three variables is written as:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + \text{residual}$$

where residual is the difference between the calculated and experimental results. The next level of polynomial model contains terms that describe interaction effects between the variables:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3 + \text{residual}$$

Quadratic terms must be included within the polynomial model in order for an optimum to be determined. Including such terms allows for non-linear interactions to be explored:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3 + \text{residual}$$

A factorial design allows the researcher to investigate all possible combinations of the factor levels. The number of experiments required to achieve this is given by:

$$N = m^k$$

where N is the number of trials, k is the number of factors, and m is the number of levels. Therefore, if the number of levels is three then it is a 3^k factorial experiment, and the number of trials required for three factors is $3^3 = 27$.

The levels of factors are represented by plus (+) for high level, zero (0) for intermediate, and negative (-) for low level. A three factor, three level experiment can thus be presented in a design matrix as in Table 3.1.

In order to examine how interaction effects are treated, a 2^3 (three factor, two level) experiment for the optimisation of the lumogallion chemistry will be shown for simplicity. In this example the response variable, y, will be fluorescence intensity measured in fluorescence units (FU). Signs for the interaction coefficients must be calculated before determination of these values can be accomplished. This is achieved by multiplying the signs for the corresponding main variables (Table 3.2).

Table 3.1. Design matrix of a three factor, three level experiment.

Exp. #	Main Variables		
	x_1	x_2	x_3
1	-	-	-
2	0	-	-
3	+	-	-
4	-	0	-
5	0	0	-
6	+	0	-
7	-	+	-
8	0	+	-
9	+	+	-
10	-	-	0
11	0	-	0
12	+	-	0
13	-	0	0
14	0	0	0
15	+	0	0
16	-	+	0
17	0	+	0
18	+	+	0
19	-	-	+
20	0	-	+
21	+	-	+
22	-	0	+
23	0	0	+
24	+	0	+
25	-	+	+
26	0	+	+
27	+	+	+

Table 3.2. Interaction variables of a 2^3 experiment.

Exp. #	I	Main Variables			Interaction variables				(y)
		x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	$x_1x_2x_3$	(F.U.)
1	+	-	-	-	+	+	+	-	151
2	+	+	-	-	-	-	+	+	152
3	+	-	+	-	-	+	-	+	155
4	+	+	+	-	+	-	-	-	150
5	+	-	-	+	+	-	-	+	157
6	+	+	-	+	-	+	-	-	158
7	+	-	+	+	-	-	+	-	162
8	+	+	+	+	+	+	+	+	159

where x_1 is reaction pH, x_2 is reaction time and x_3 is lumogallion concentration.

Supposing that this study fits the experimental results to the following model:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3 + \text{residual}$$

To calculate the mean value (b_0), main and interaction effects, the signs in the corresponding columns of Table 3.2 are used to either add or subtract the value of the response, y:

$$b_0 = \frac{151+152+155+150+157+158+162+159}{8} = 156$$

For both the main and interaction effects the denominator becomes four since this is the number of comparisons made [143]:

$$b_1 = \frac{-151+152-155+150-157+158-162+159}{4} = -1.5$$

$$b_2 = \frac{-151-152+155+150-157-158+162+159}{4} = 2.0$$

$$b_3 = \frac{-151-152-155-150+157+158+162+159}{4} = 7.0$$

$$b_{12} = \frac{151-152-155+150+157-158-162+159}{4} = -2.5$$

$$b_{13} = \frac{151-152+155-150-157+158-162+159}{4} = 0.5$$

$$b_{23} = \frac{151+152-155-150-157-158+162+159}{4} = 1.0$$

$$b_{123} = \frac{-151+152+155-150+157-158-162+159}{4} = 0.5$$

The estimated effects can then be substituted back into the initial polynomial model to illustrate the influence of the experimental variables and their interactions:

$$y = 156 + -1.5x_1 + 2.0x_2 + 7.0x_3 + -2.5x_1x_2 + 0.5x_1x_3 + 1.0x_2x_3 + 0.5x_1x_2x_3 + \text{residual}$$

It can thus be seen from this function that variable x_3 (lumogallion concentration) has the largest influence on the fluorescence intensity and with an increase of one unit of the concentration an increase of 7 fluorescence units is possible.

3.2.3 Neural networks

Artificial Neural Networks (ANN) are information-processing systems with their theory based on the biological nervous system. Neural networks take a different approach to problem solving than that of conventional computers, since they do not use an algorithmic approach. Whereas, conventional computers require specific instructions, which restricts their problem-solving capability to problems that are already understood, neural networks 'learn by example' and can be used to extract patterns and detect trends which can then be used to provide projections and answers to unknown situations based on the examples provided. Neural networks are, thus, entirely model-free estimators.

ANNs consist of numerous simple process units (neurons) that can be modified in order to estimate a function. The structure of an ANN is that of three types of layers:

a layer of inputs (x_1, x_2,...,x_n), a variable number of layers of hidden units, and an output layer ($f(z)$). These layers are linked by weighted connections (w_1, w_2,...,w_n) that can be strengthened or weakened [144]. A typical network with a single hidden layer is depicted in Figure 3.1.

The most commonly used ANN's are Multi-Layer Perceptron (MLP) networks - simply networks of the basic perceptron shown in Figure 3.1. The output of such a network is achieved firstly by applying a linear relationship based on the weighted inputs and subsequently transforming the result non-linearly. Typically, the logistic sigmoid ($1/(1+e^{-x})$), is the non-linear function applied in MLP's, and more specifically in back propagation – a form of network training. Training is defined as a search process for the optimised set of weight values, which can minimise the squared error between the simulation and experimental data of units in the output layer [145].

A possible use of ANN's is the prediction of optimal experimental conditions for a particular system. The ANN is generated from a base set of experiment data. This input/output training data is fundamental in neural network technology, because it provides the necessary information for discovery of the optimal operating point through learning and therefore an informed selection of the ranges of all conditions must be made. Once a suitable ANN architecture is chosen, optimum levels of the investigated conditions can be predicted using the trained network.

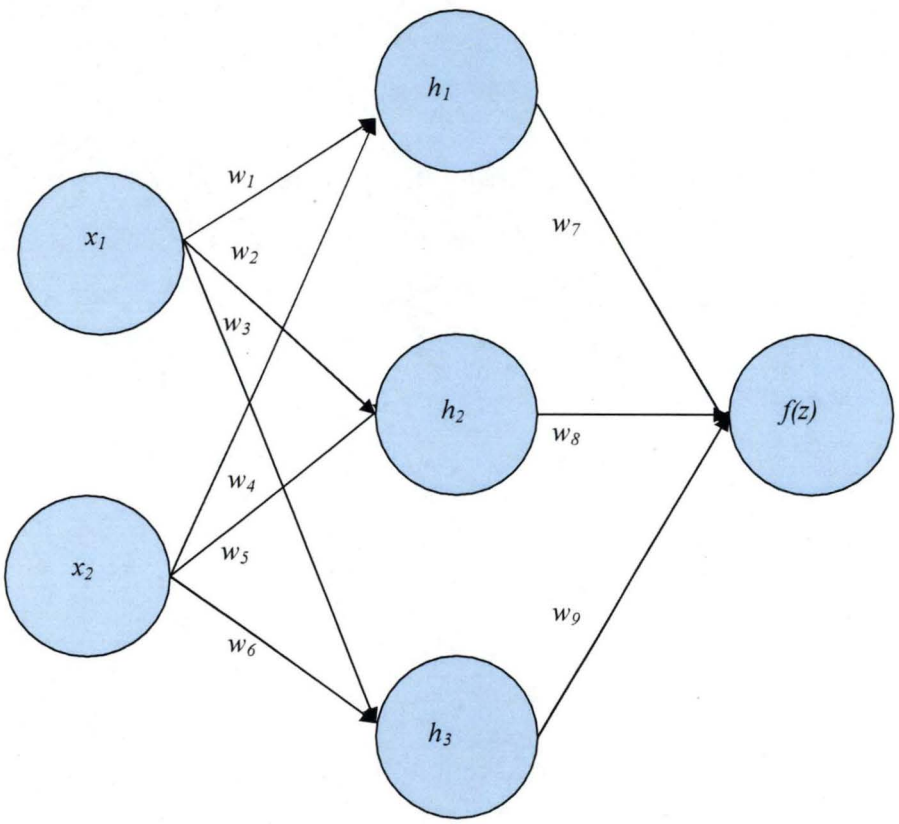

Figure 3.1. Layout of an artificial neural network with a single hidden layer (h_1, h_2, h_3)

3.3 Optimisation of Lumogallion Chemistry

3.3.1 Choice of experimental variables

As stated previously, the extensive amount of research carried out on the chemistry of aluminium with lumogallion, allows for a clear indication of the most influential variables to be derived from the literature. On this basis, it was decided that, of the possible variables, only lumogallion concentration, reaction time and buffer pH would benefit from further investigation into optimum operating levels for this specific FIA system. These variables have been chosen because they were shown to affect the efficiency of the reaction between aluminium and lumogallion significantly. This decision allowed for a full factorial multivariate experimental approach to optimisation to be implemented. As discussed previously, multivariate experimental design avoids issues of interaction between variables and allows for a detailed optimisation study to be undertaken without the drawback of an excessive number of experiments being required. No such systematic chemometric optimisation of the lumogallion chemistry has been conducted previously.

3.3.2. Preliminary investigation into lumogallion concentration

Whilst ideal ranges for both buffer pH and reaction time could be gained straight from the literature, the ideal lumogallion concentration was not as apparent. This is because most research groups report this as a concentration in the effluent. Given that every manifold differs slightly with regard to flow-rates/pump tubing etc., an initial investigation into applicable lumogallion concentrations specific to the present

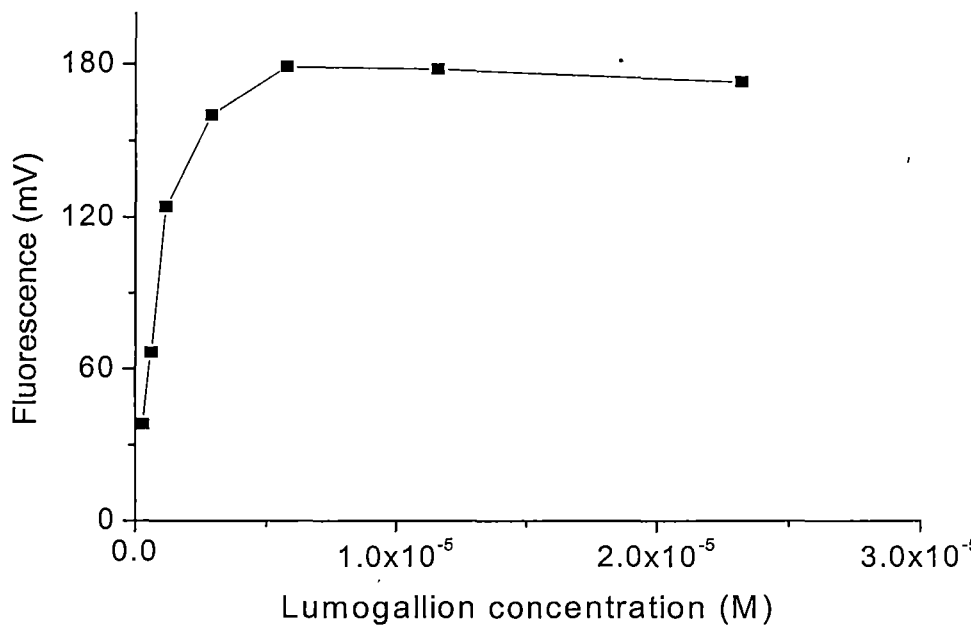

Figure 3.2. Influence of lumogallion concentration on fluorescence. Experimental conditions: 50 nM acidified aluminium standard; 2.75 min reaction time; 2 M ammonium acetate buffer (pH 6.0); reaction coil temperature 50 °C.

system was carried out. The results of this preliminary univariate study are given in Figure 3.2. It can be seen from the results that no apparent quenching effects were observed at the lumogallion concentrations tested and that a maximum fluorescence was reached at a concentration of 5.8 μM. This concentration can be converted to a lumogallion:aluminium ratio of ~ 26:1. Subsequently, the concentrations chosen for investigation during the multivariate optimisation experiments were based on this ratio.

3.3.3 Results of optimisation experiments

Following the multivariate experimental design, 27 experiments were carried out in order to determine an optimal level for each of the three variables investigated. Table 2.1 shows the levels at which each variable was tested and Table 3.1 the conditions of each of the 27 experiments. A summary of the results obtained is given in Table 3.3. From the 27 experiments, maximum response was reached at a pH of 5.5, reaction time of 2 min and a lumogallion concentration of 23.6 μM.

Table 3.3. Results of optimisation experiments.

Experiment #	Fluorescence (arbitrary units)
1	3.60
2	51.4
3	161
4	6.43
5	79.0
6	163
7	9.30
8	92.5
9	146
10	8.13
11	106
12	158
13	14.8
14	146
15	150
16	18.9
17	147
18	135
19	12.8
20	154
21	157
22	21.3
23	170
24	150
25	27.8
26	163
27	139

3.3.4 Analysis of optimisation results:

Statistical modeling based on general linear regression

3.3.4.1 Model selection

Equation 1 was used as the starting point for four separate statistical models.

$$Fl = \beta_0 + \beta_1 pH + \beta_2 T + \beta_3 L + \beta_4 pH.T + \beta_5 pH.L + \beta_6 T.L + \beta_7 pH^2 + \beta_8 T^2 + \beta_9 L^2$$

Equation 1

where:

Fl = fluorescence in arbitrary units

pH = buffer pH

T = reaction time in minutes

L = lumogallion concentration in M

The regression coefficients (β_i), F-ratios, residuals (e_i) and R^2 of each model were determined using the general linear model estimating option within SYSTAT® 10.2.05. These parameters were used to evaluate and select the final statistical model, where F-Ratio is the ratio between treatment mean square and error mean square, R^2 is the coefficient of determination [146].

A summary of four of models deemed most suitable is given in Table 3.4. The first model included the calculation of all of the regression coefficients (β_0 through β_9).

The "tolerance" option within SYSTAT®10.2.05 was set at 1.0 e^{-11} to give warning if any of the regressors was directly or highly correlated to the independent variable (fluorescence). The second model excluded the constant term (β_0). In order for the test of fit statistics to be calculated properly the "mixture model" option of SYSTAT®10.2.05 was used. The third model was one in which regression terms of low significance ($\alpha < 0.05$) were removed from the model and the remaining regression coefficients were recalculated ("stepwise" option). This was repeated until all of the remaining regression terms had a high degree of significance ($\alpha > 0.05$). This model also contained the constant term (β_0). The fourth model was one in which regression terms of low significance ($\alpha < 0.05$) and the constant term (β_0) were removed from the model and the remaining regression coefficients were recalculated. This was repeated until all of the remaining regression terms had a high degree of significance ($\alpha > 0.05$). Other models were also generated using various options of SYSTAT®10.2.05 but they will not be discussed here since they were considered to be highly unsuitable.

Data for the four fluorescence models, including their regression coefficients (β_i), F-ratios and R_2 values, are listed in Table 3.5. Of the four models shown model 3 and 4 were considered to have the strongest fit statistics based on their F ratio. Model 3 was considered the most suitable model, given that it was not based solely on one variable.

Table 3.4. Statistical methods used to model fluorescence response.

Model	Application	Description
1	All regression coefficients with constant	β_0 through β_9
2	All regression coefficients without constant	β_1 through β_9
3	Stepwise with constant	$\alpha \geq 0.05$ β_1 through β_9 with β_0
4	Stepwise without constant	$\alpha \geq 0.05$ β_1 through β_9

Table 3.5. Summary of model regressors and fit statistics

Coefficient	Model 1	Model 2	Model 3	Model 4
β_0	-1671		-1511	
β_1	539.6	-39.79	517.1	-32.74
β_2	71.60	-6.712		
β_3	9.086	2.194	1.663	
β_4	-7.248	1.998		
β_5	-0.579	0.205		
β_6	-0.255	0.030		
β_7	-40.77	8.961	-40.77	8.94
β_8	-6.124	-0.520		
β_9	-0.122	0.060		
F ratio	20	25	67	135
R^2	0.915	0.926	0.897	0.912

3.3.4.2 Model Testing

In order to ensure that the selected model was accurate it was tested in three ways. Firstly, the residuals (e_i) were plotted against experimental fluorescence, the modelled fluorescence and against each of the regressors in order to ensure that patterns did not exist thus, satisfying the modelling conditions. Secondly, the modelled fluorescence data were plotted against the actual fluorescence data. Lastly, the data from four experiments, not used in the generation of the statistical model, was used to test the predictability (as determined by R^2).

The residuals (e_i) were plotted against experimental fluorescence, the modelled fluorescence and against each of the regressors using the Scatter Plot Matrix (SPLOM) option of SYSTAT®10.2.05. It can be seen from the plot (Figure 3.3) that there is no clear pattern except in the case of β_1 and β_7 which may be expected given they are both derived from the variable pH.

The plot of experimental *versus* modelled fluorescence (Figure 3.4) gives an R^2 value of 0.9673. Despite this being an indicator of good linear relationship it can be seen from the plot that the modelled and experimental results differ significantly, particularly in the mid-fluorescence range. It can be deduced from this plot that the model is not accurate in predicting fluorescence over a wide range.

Fluorescence data from four experiments not used for the generation of the general linear regression model were plotted against modelled data for the same conditions

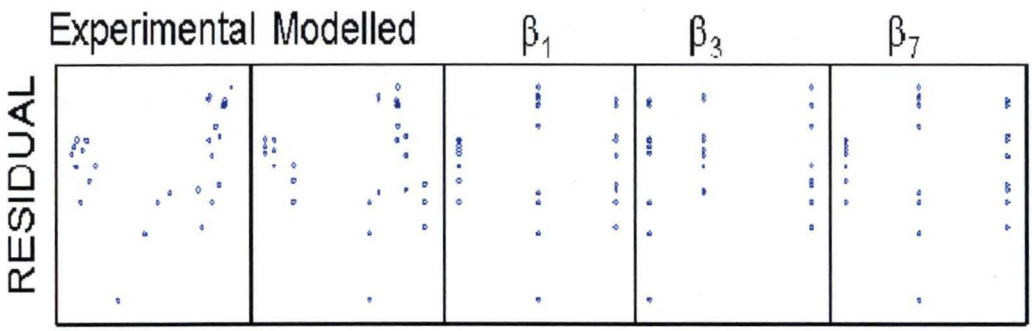

Figure 3.3. SPLOM of Model 3.

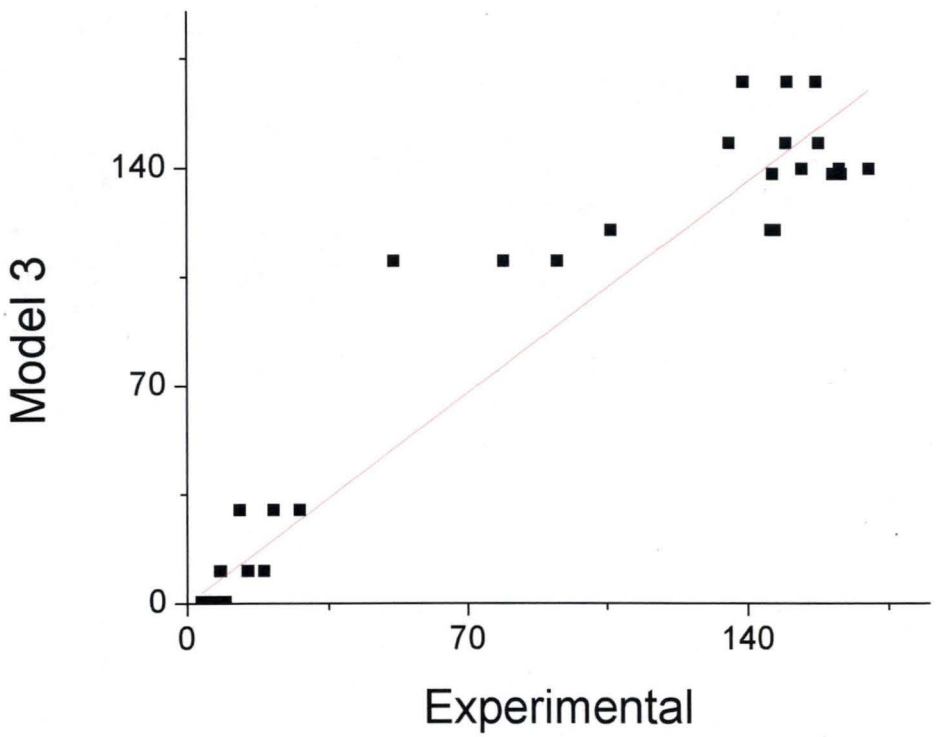

Figure 3.4. Agreement between data generated by Model 3 and experimental results.

gave a R^2 value of 0.9398 (Figure 3.5). Again, this result suggests that the model can predict fluorescence intensity fairly well, but, the four test experiments indicated limitations in the capability of the model in the mid-fluorescence range. It should be noted that these test data points were chosen prior to analysis of the optimisation results in order to avoid bias.

Whilst the model appeared to handle the four test data points well it was still a concern as to whether the model would hold true over a wide fluorescence range. Given this concern and the somewhat conflicting results as to whether the model was a suitable predictor of fluorescence, further analysis of the optimisation experiments was sought by means of ANN's.

3.3.5 Analysis of optimisation results:

Artificial neural networks

3.3.5.1 Model generation

Due to the obvious complexity of the aluminium-lumogallion chemistry, and uncertainty associated with the generated general linear regression model, results of the optimisation experiments were analysed using artificial neural networks. The Trajan Neural Network Simulator 6.0, Intelligent Problem Solver (IPS) was employed to generate multiple ANN's. Through this approach, ANN's of different types and complexity (hidden units) were generated with different modes of training

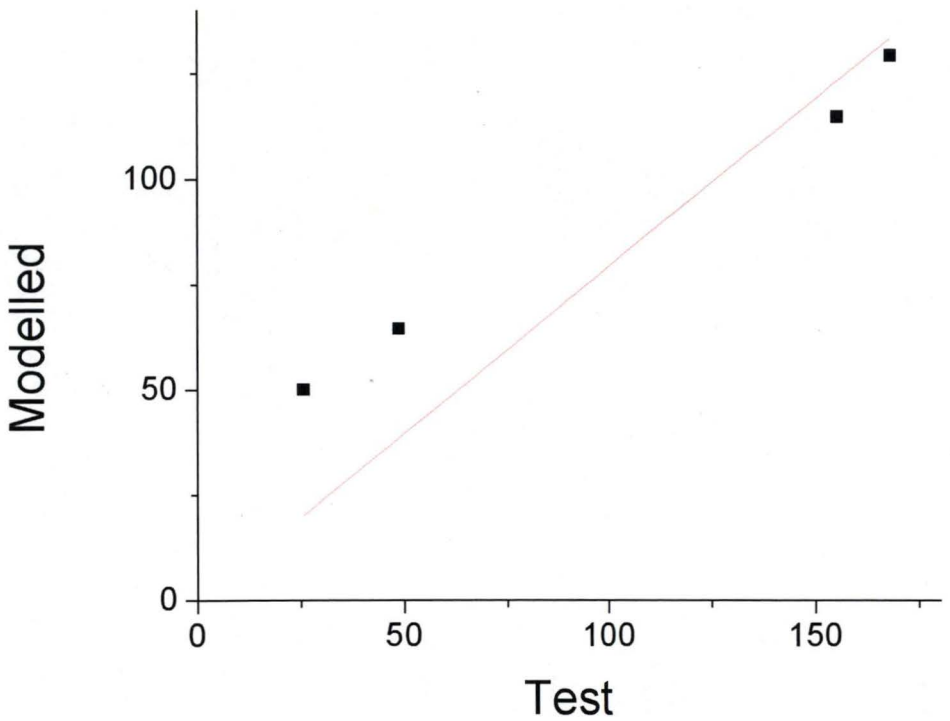

Figure 3.5. Fit of Model 3 to test data (refer to text for details).

also utilised. The 'best' ANN could be chosen by comparison of the performance parameters.

Of the ANN's generated from the aluminium-lumogallion optimisation data, a multilayer perceptron (MLP), with three inputs (concentration, pH and time) and two hidden layers was determined to be the most suitable. This ANN had ten nodes in the first hidden layer and three in the second and was chosen based on both favourable selection performance and good generalisation (as illustrated by training and selection errors).

3.3.5.2 ANN performance

The fit of the experimental results versus the Trajan predicted values, is shown in Figure 3.6. The highest residual was -15.8, however the average was -0.56 indicating the high capability of the ANN. It is important to note the ability of the neural network to predict accurately over the entire fluorescence range tested. In particular, the neural network was much more able to handle the mid-fluorescence range than the general linear regression model.

3.3.5.3 Optimisation of variables

On examination of the response curves of each variable it was noted that reaction time affected fluorescence the least. In fact a gain of only ~ 40 fluorescence units was achieved by changing the reaction time from 1-3 min and most of this gain was

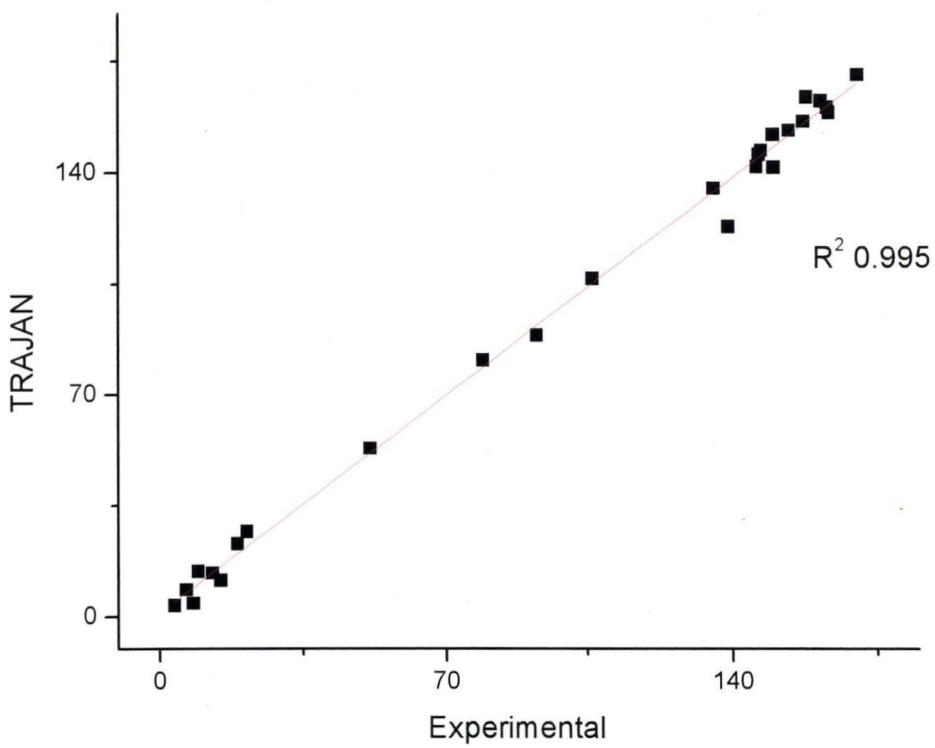

Figure 3.6. Agreement between Trajan ANN generated data and experimental results.

achieved by 2 min. Subsequently, 2 min was assumed to be an adequate time for the reaction and further optimisation of this parameter was not deemed necessary.

A response surface depicting the two most influential variables, as generated by the Trajan programme, is given in Figure 3.7. An area of maximum fluorescence can be seen clearly, as indicated by the red area. This maximum equates to a pH of 5.75 and a lumogallion concentration of 1.8×10^{-5} M. This pH optimum was in fairly good agreement with findings by Resing and Measures [57], who reported an optimal response between pH 5 and 5.5. In comparison, the SYSTAT model generated an optimal pH of 6.3, which is considerably higher than that reported elsewhere.

Given the apparent superior ability of the Trajan ANN to predict fluorescence over the entire range tested, and not just at the extremes, the optimal levels of pH and lumogallion concentration generated by this technique were chosen as the operating conditions for subsequent investigations utilising the FIA system.

3.4 Performance of the FIA System

The linear response of the FIA system was investigated between 5 and 200 nM. An additional measurement at 2.8 nM was made, with this value being the limit of quantification (LOQ) for the system as calculated by 10 times signal to noise. The result of this calibration plot is given in Figure 3.8.

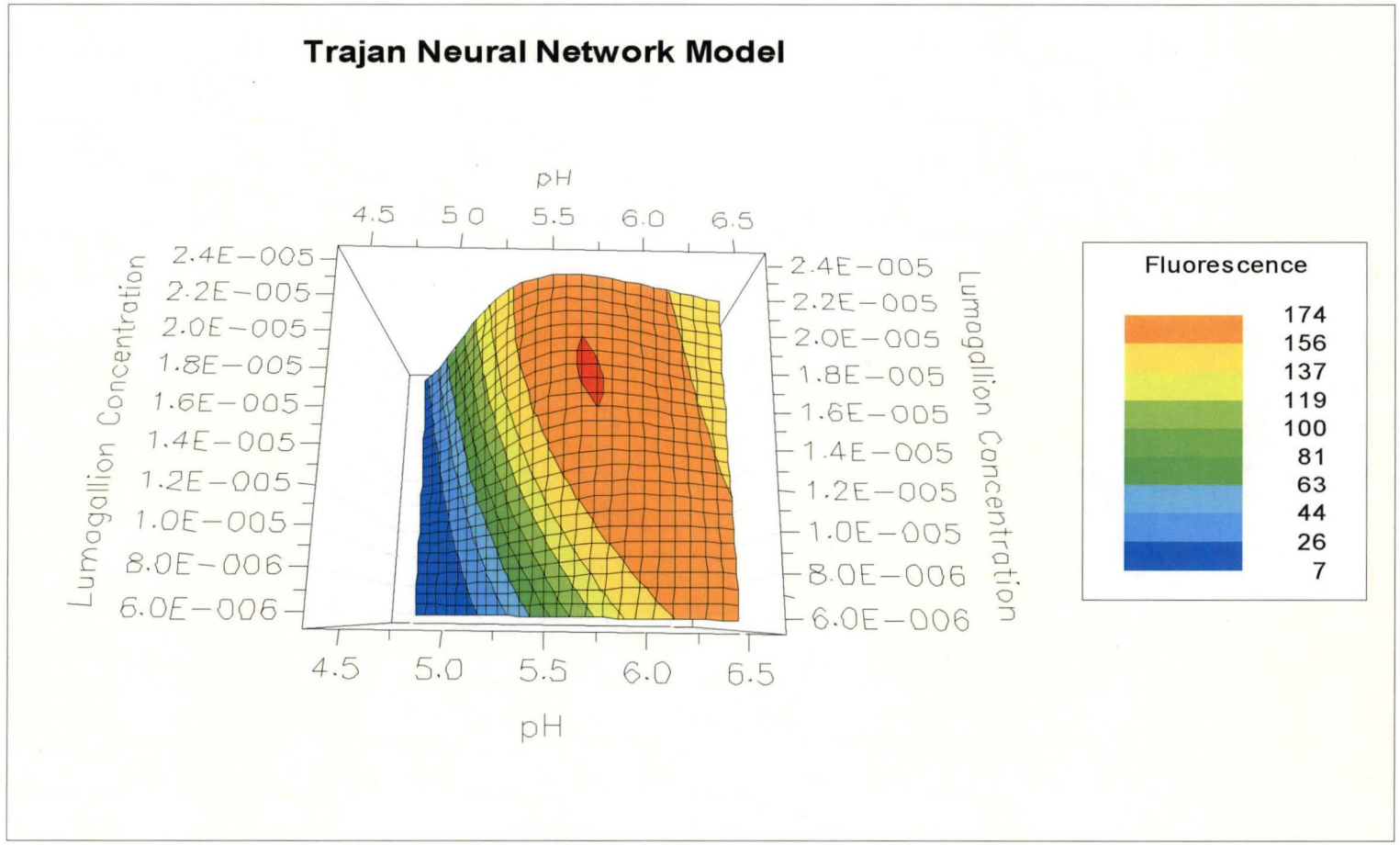

Figure 3.7. Response surface of the two most influential variables to aluminium-lumogallion complex formation.

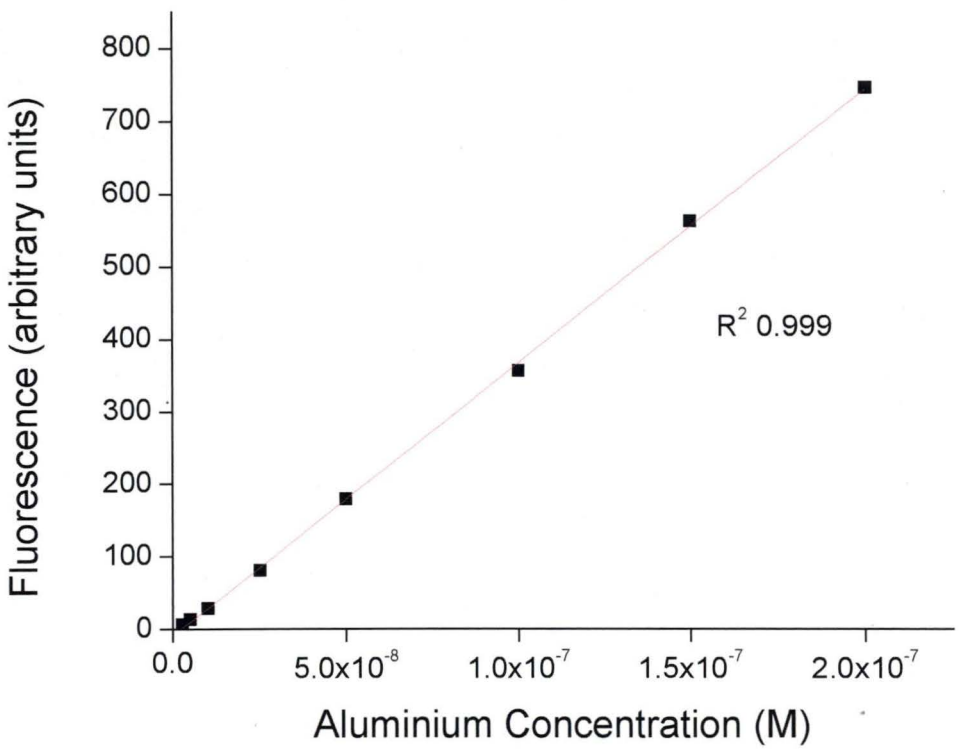

Figure 3.8. Linear response of FIA system coupled with fluorescence detection.

Despite a high correlation coefficient being obtained, the performance of the system specifically at lower concentrations was further analysed by means of a Cassidy Test [147] (Figure 3.9). This test illustrates whether the method is valid at lower concentrations by determining whether the calibration curve is in fact linear in this concentration range.

It can be seen that the Cassidy plot only becomes horizontal at a concentrations 50 nM or higher, as indicated by the control lines representing an error of 5%. Thus, it can be said that the true linearity of the system for the concentration range investigated lies between 50 and 200 nM. It must be noted at this stage that the linear range may well have been able to be extended to lower concentrations if more extreme clean techniques were employed during standard preparation. At a concentration range this low, contamination issues become more evident and may be, in part, responsible for the skew in results below 50 nM. Additionally, the Milli-Q system used to prepare the standards was later found to produce water which contained a detectable amount of aluminium, which would have again increased fluorescence. This contribution would have been particularly evident in the lowest concentration standards (see section 6.2.4 for further discussion on aluminium content of Milli-Q water).

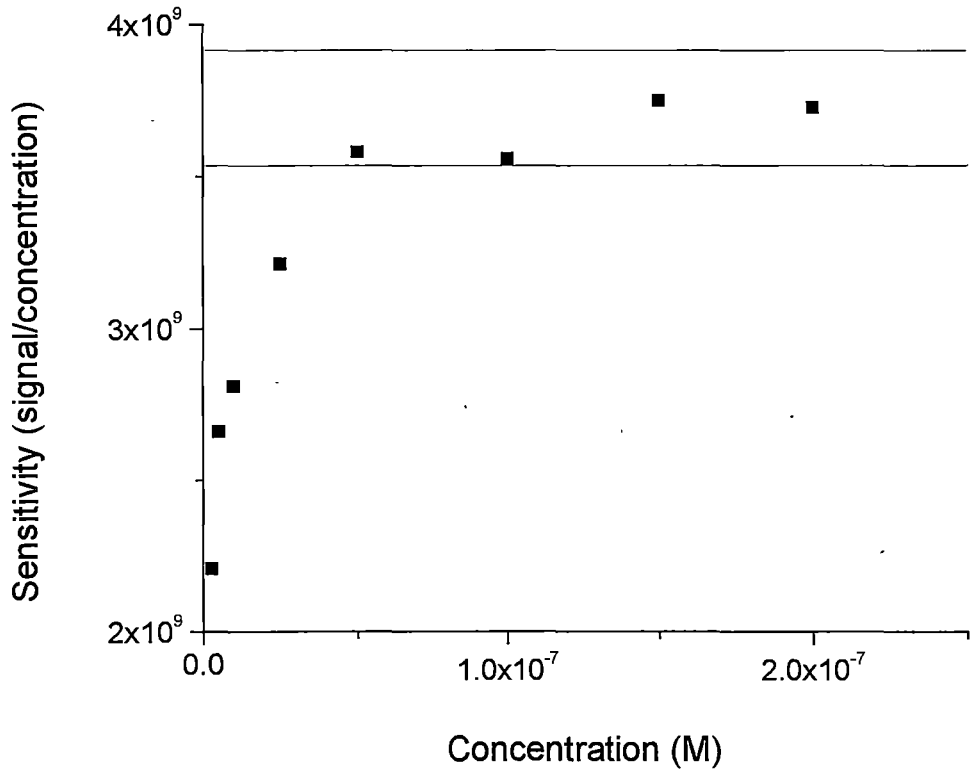

Figure 3.9. Cassidy plot of FIA system over nanomolar range of aluminium.

3.5 Preconcentration Using 8-Hydroxyquinoline Functionalised Resin

Given that the expected concentration of aluminium in seawater samples from the Antarctic region is considered to be in the low- to sub-nanomolar region, it was apparent that the FIA system was not capable of analysing such samples directly. Consequently, an initial preconcentration step needed to be added to the existing FIA manifold.

3.5.1 Synthesis of 8-hydroxyquinoline functionalised resin

As previously discussed in Section 1.5, resins with immobilsed 8-hydroxyquinoline (R8-HQ) is used most commonly for the purpose of preconcentration of aluminium in FIA. Not only does R8-HQ ensure enrichment of the sample concentration but it also serves to eliminate potentially interfering species (both the sea-salt matrix and other possible interferents). As was also mentioned, there are several possible methods for the synthesis of R8-HQ. Because the modified method of Landing [123] is an involved and prolonged procedure, the shorter simpler Dierssen method was chosen [124].

The Dierssen method cross-links 8-hydroxyquinoline directly to the resin by reacting epoxy-activated TSK-Gel AF-Epoxy-650 M resin (polystyrene divinylbenzene) with 5-amino-8-hydroxyquinoline in a single step. The synthetic steps involved in preparing the functionalised resin are illustrated in Figure 3.10.

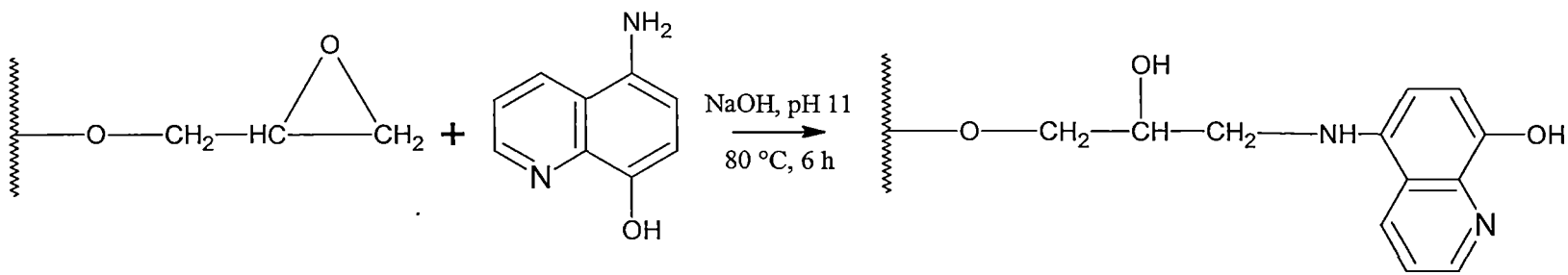

Figure 3.10. Synthesis of 8-HQ functionalised resin according to Dierssen method [124].

This technique was applied successfully to the concentration of trace metals from stored, acidified seawater samples [124]. However, this seawater was from an anoxic marine lake in the Palau Islands, and broader and extensive use of the synthesis had not been undertaken at the time of the method's utilisation in this project. Consequently, investigations of the performance of the resulting resin and a comparison with 8-HQ functionalised resin prepared by the modified Landing method were made.

3.5.2 Functionalised resin capacity

The equilibrium capacity for copper has been used as a measure of the chelating capacity of various resins, however, the dynamic exchange 'breakthrough' capacity is a more useful measurement for column operation in flow analysis [124]. Columns of the same shape and size, and packed under the same conditions, containing both types of 8-HQ functionalised resin (Dierssen and Landing) were each flushed with acid and Milli-Q water and preconditioned with a sodium acetate buffer (pH 5.4). A 20 ppm Cu^{2+} standard, buffered at pH 5.4, was then passed through each column and the breakthrough recorded by a UV-Vis detector. Copper was chosen for capacity investigations due to being easily detected by UV-Vis.

Whilst the 8-HQ functionalised resin synthesised by the modified Landing method resulted in a capacity equivalent to 69.6 μg of Cu^{2+}, the same column packed with the Dierssen resin had a capacity of only 39.1 μg. These results suggest that the efficiency of the synthetic process of Landing is greater than that of Dierssen. Despite

the fact that the latter resin had obviously not been functionalised completely with 8-HQ, or at least not to the same extent, it was used for subsequent investigations. This choice was supported by the fact that the resin would still act to preconcentrate effectively trace level amounts of aluminium and also that a larger supply was more readily available. If a higher preconcentration factor was found to be required in order to achieve the required sensitivity, then use of the Landing resin would be considered.

3.5.3 FIA with preconcentration using 8-HQ functionalised resin

3.5.3.1 Carrier

Whilst previous optimisation experiments had employed a pure Milli-Q water carrier, an acidic carrier was required for use of the FIA system incorporating an 8-HQ column. A low pH was necessary in order for the aluminium preconcentrated on the column to be stripped from the functionalised resin. Initially, a carrier acidified to pH 2.5 with HCl was trialled, but due to issues of incomplete elution of the loaded aluminium, this was lowered to pH 1. Results (not detailed here) indicated that the ammonium acetate buffer was able to maintain an optimal pH in terms of the lumogallion chemistry, with no decrease in fluorescence observed for a 50 nM aluminium standard (sample loop) using an acidic carrier in comparison to a Milli-Q water carrier.

3.5.3.2 Milli-Q water standards

Aluminium uptake onto 8-HQ has been studied previously. Whilst De and co-workers [148] reported extraction of aluminium to be quantitative between pH 4.5-11, Resing and Measures found the pH range of optimal uptake of aluminium on 8-HQ columns to be much narrower, between 5.3-5.7 [57]. It is believed that only over this pH range are kinetics of aluminium sorption suitably rapid.

Initial investigations of the performance of the 8-HQ column were carried out using aluminium standards prepared in Milli-Q water. A 5 nM standard, at pH 5.5 (adjusted with ammonium acetate buffer), was loaded for 30 s and subsequently injected into the manifold. The column appeared to be effectively preconcentrating the aluminium, to at least some extent, registered by an increase of almost twice the fluorescence compared to a non-preconcentrated 5 nM standard injection of the same volume. A drawback of the preconcentration procedure with R8-HQ was the appearance of a second peak in addition to the expected aluminium peak. This peak was not reproducible in terms of retention time or fluorescence intensity. Milli-Q water rinsing of the column before elution of the aluminium did not eliminate this extra peak, even with prolonged rinsing.

Subsequent investigation into elimination of this extra peak and also optimal conditions for column loading using Milli-Q water standards were hampered by excess back-pressure in the system manifold. This was, in part, rectified by modifications to reaction coil lengths and tubing diameters. However, the continually

irreproducible nature of the results obtained meant that no clear conclusions could be drawn from this series of experiments.

In trying to gain an understanding of the poor results obtained using Milli-Q water standards, published research revealed that aluminium is not well absorbed onto R8-HQ from deionised water solutions [149]. Addition of fluoride, as NaF, has been found to rectify this problem [149], but the disadvantage of this approach is an increase in blank fluorescence due to aluminium present in the NaF. Investigation into the possible improvement of results via this method was not pursued further given the unnecessary complications involved and the fact that seawater samples, rather than deionised water samples, were the focus of this project.

3.5.3.3 Online buffering

Considering that all seawater samples collected for the determination of aluminium are acidified (~ pH 1.8) for storage purposes, it was apparent that pH adjustment of any seawater sample would be required before loading onto the column. In view of the multiple potential sources of contamination for such samples, minimal sample manipulation is preferred. Due to this reason, an online buffering system was added to the manifold to ensure the correct pH change on loading, whilst minimising sample exposure to contamination. This system involved the addition of a buffering line to the peristaltic pump, which was then joined via a T-junction to the sample line. A sample pH of 5.4 was obtained using a 2 M ammonium acetate buffer (pH 5.75). By means of the manually-operated T-junction, either seawater or Milli-Q water flowed

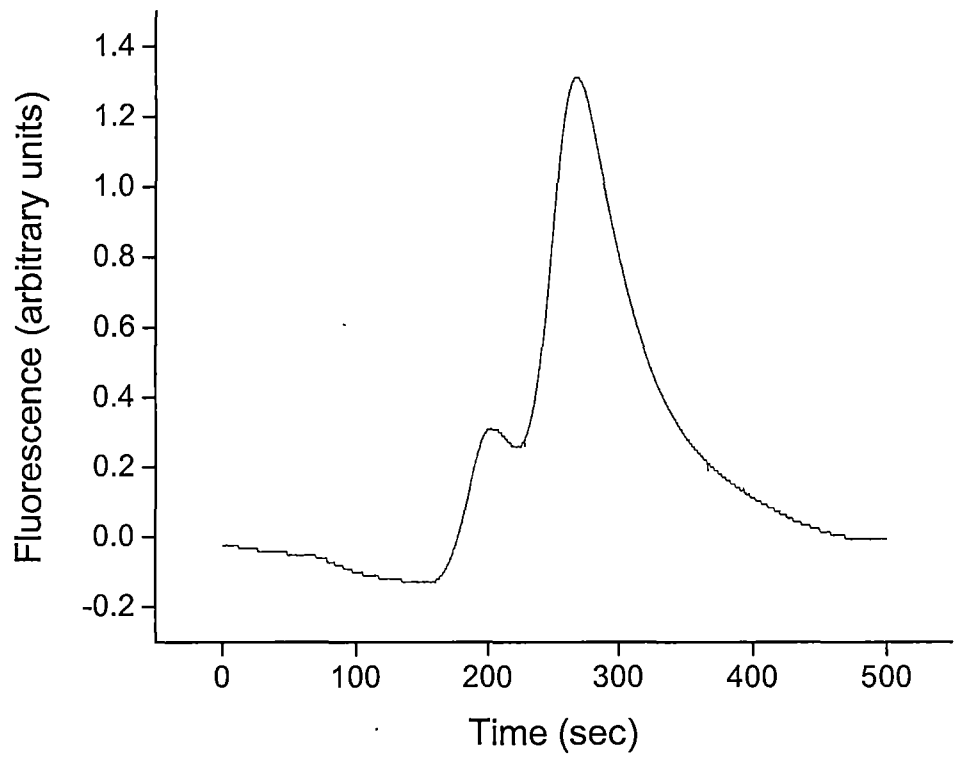

Figure 3.11. FIA injection of towed fish Antarctic surface seawater.

through this sample line as determined by whether sample loading or column rinsing was required. The injection valve was configured in such a way as to ensure a back-flush elution process.

3.5.3.4 Seawater samples

Despite the issues of irreproducibility encountered with preconcentration of the standards made in Milli-Q water, the column was trialled at sea aboard the R/V Nathanial B. Palmer (NBP). Initial findings were promising. Although a shouldered aluminium peak was observed, aluminium was detected and the system showed high precision (< 3%). An example of the output of a sample is shown in Figure 3.11. The minor dip before the peak is believed to be caused by the Milli-Q water rinse eluting prior to the aluminium. Given Milli-Q water has a lower aluminium concentration than most of the reagents responsible for the background fluorescence, it follows that a dip in fluorescence intensity below the baseline would occur.

Calibration, by way of standard addition to Antarctic surface seawater, showed good linearity between 0 and 10 nM additions (Figure 3.12). Peak area was calculated so as to include the entire split peak, based on the fact that both sections of the peak increased with spiking. From the standard addition curve a concentration of 3.1 nM could be determined for a bulk towed fish sample (surface seawater).

At this stage, work was continuing aboard the NBP within Antarctic waters. The method had not been validated and thorough investigation into the nature of the split

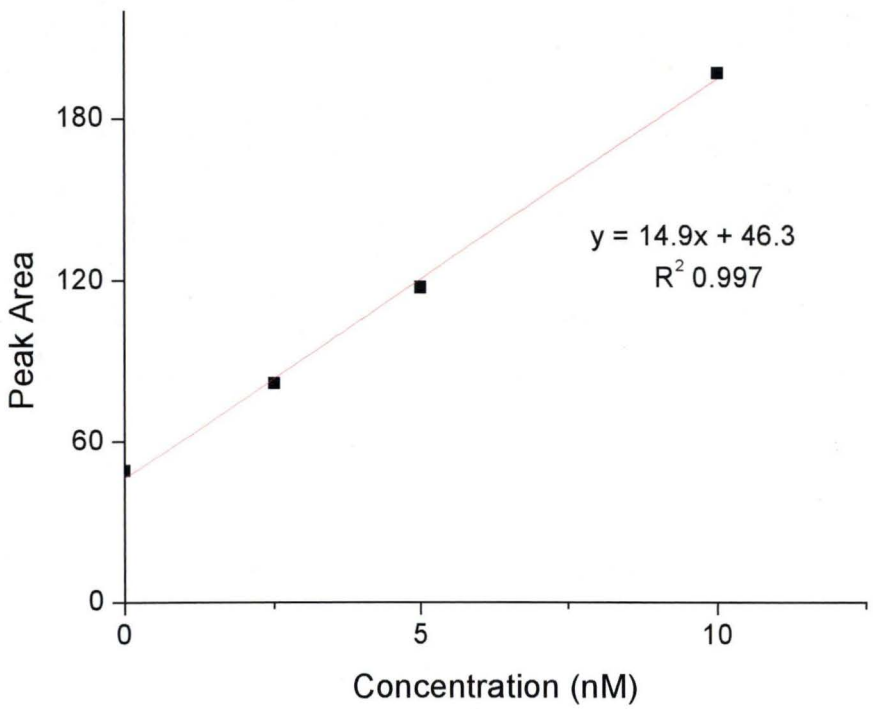

Figure 3.12. Standard addition calibration curve for Antarctic surface seawater using FIA.

peak was not undertaken onboard due to time constraints, sampling schedules and limited resources. Ideally, a fully validated system would have been used for real-time determination of aluminium concentrations throughout the voyage, but due to instrument inconsistencies encountered prior to departure (discussed in more detail in section 3.6) and the limited opportunity for ship time, the system was deployed mid-way through the developmental process.

3.6 Difficulties Encountered with FIA System Using Preconcentration on 8-HQ Functionalised Resin

Although the FIA system initially showed promising signs of being able to quantify aluminium in seawater, it was fraught with both recurring and random problems. The manifold seemed to be able to cope well with simple determinations of aluminium standards using sample loop injections (as per optimisation experiments) and was robust in day-to-day operation during this time, but faltered once the preconcentration system was added.

At first these matters were confined to elution inconsistencies and peak splitting, and it was believed that further and more thorough investigation into operating conditions of the column would be able to rectify them. However, the column itself was of major concern. The 8-HQ functionalised resin showed significant compression during consecutive runs leaving a large dead volume within the column. Additionally,

frequent breakages at connections arising from the resulting increase in back - pressure occurred. Despite subsequent trials with commercially available and more robust column housing, the problem of back pressure could not be resolved. This back-pressure was also believed to be one possible cause of a significantly oscillating baseline, which occurred intermittently. As well as oscillations, severe downward and upward spiking of the baseline were also observed at random intervals.

A possible explanation of apparent peak splitting has been made when fairly high levels of aluminium are present in the sample [149]. When the flow reverses at the valve, the first "plug" of liquid that reaches the detector is non-preconcentrated sample that was in the line between the valve and the resin in the column. With high analyte concentrations, that "plug" will show up as a visible peak, that will scale with (but of course is much smaller than) the main peak. However, due to the fact that a column rinse step was utilised it is believed that this non-preconcentrated "plug" should not be present.

Whilst onboard the NBP, a first incidence of sensitivity loss was encountered. The system had been functioning quite well for over two weeks and, on the particular day, for several hours. However, between consecutive runs of a seawater sample almost half the normal sensitivity was lost. Nothing was altered; and the xenon lamp of the detector, flow-rates and all other variables appeared to be unchanged. The sample was the same seawater sample that had been used for the past week and was not fouled or contaminated in any way. Numerous attempts were made to re-establish the system, including acid flushing all components, installation of a new Xe lamp,

replacement of the column with one containing fresh R8-HQ, new pump tubing and new reagents; however no improvement could be made. When the system did finally re-establish itself, the sensitivity lasted only one day until it once again halved, again for no apparent reason. After which, no adjustments could re-instigate normal function and the remainder of the cruise was spent attempting to trouble shoot this issue to no avail.

On return to the laboratory, the FIA manifold was re-established in its most simple form, with only a sample loop and no preconcentration column. This posed no problem and results were obtained with expected sensitivity and high precision. On addition of the 8-HQ column it quickly became apparent that back-pressure was once again an issue. This problem, however, was monitored closely and for the most part, operation of the manifold was uninterrupted.

It was at this stage that an attempt was made to investigate further the issue of the split aluminium peak. Considering that the Dierssen resin was shown to be inferior in terms of capacity to the Landing synthesised resin, in terms of capacity, it was thought that perhaps using the latter could improve the performance of the system with regard to preconcentration. Resins synthesised according to the modified Landing method from both the University of Tasmania (synthesised by Dr A. Bowie) and from Chris Measures' laboratory (University of Hawaii) were made available for testing.

Unfortunately at this point of the investigation, the system began to once more display the original problem of an oscillating baseline. All flows appeared normal and back-pressure was not elevated. A new pump was considered a logical option to try. Two alternate pumps were tested with neither making a difference. The peristaltic pump was therefore, not deemed to be the issue. Substitution of the preconcentration column with others of different shapes and sizes provided at best only a temporary improvement over a few days.

To try and overcome the consistent problem of high back-pressure and uneven mixing of reagents at T-junctions, flow-rates of several reagents were varied. It was hoped that by altering these flows, pressures at specific junctions within the manifold would balance and the flows would become more consistent, perhaps in turn improving the baseline. The results were disappointing, with no combination of changes tested making any difference to either back-pressure or the baseline.

Although the system baseline remained unstable, the alternate resins (as discussed above) were introduced into the system. This served only to further confuse issues, with large negative peaks resulting. These negative peaks were, on average, ten times that of the dip detected prior to the elution of the aluminium peak depicted in Figure 3.11 with no positive peak following. What this result, and the others preceding it, seemed to suggest was that the sorption of Al to the 8-HQ functionalised resin was a more complicated process than was initially envisaged. Furthermore, the inclusion of a preconcentration cartridge in the flow-analysis manifold used here

caused the performance capabilities of the system to be exceeded. This was shown in the high back pressure, and the irregular flow-rates and detector baseline.

3.7 Conclusions

It had become apparent by this stage that the FIA manifold was not robust and that despite exhaustive efforts to correct problems, all attempts were proving futile and further issues were constantly emerging. In terms of achieving the goals of the project further use of the FIA system was considered to be neither reliable nor productive. In order to make progress with regard to accurate quantification of aluminium in seawater, investigation into a new analytical technique was determined to be the best option to take. In the following chapters, the development of a high performance chelation ion chromatography (HPCIC) system for the determination of aluminium in seawater is presented.

Chapter Four -

HPCIC Separation of Aluminium

4.1 Introduction

The chemistry of aluminium in water is dominated by its predisposition to undergo hydrolysis, with the extent and type of hydroxy species formed being highly dependent on pH. The hydrolysis product of interest in the pH range of this study is the divalent hydroxy species ($Al(OH)^{2+}$), which forms at a pH of around 2.5 (Al 68.5 μM) [150]. The species distribution profile of the hydrolysis products can be altered by the addition of an electrolyte, such as potassium chloride. Chloride can suppress the degree of hydrolysis due to the formation of its own complexes with aluminium, in particular $Al(Cl)^{2+}$, which is somewhat stable (log K –1.0) [151] and exists at low pH (< 4). The other common aluminium species in water include different complexes with carboxylic acids, polyphenols, fluoride and phosphate. The separation and identification of all species of aluminium is an extremely difficult task, so researchers usually determine only the labile soluble forms of aluminium after their conversion to a form suitable for detection as a single species. Such conversion of aluminium species can be performed by either addition of a suitable complexing reagent to the sample or by preconcentration on a chelating resin, followed by elution with an inorganic acid.

The purpose of this section of the project was to develop and test the suitability of a high performance chelation ion chromatography system for the determination of aluminium in complex samples. The initial developmental work focussed on the optimisation of separation conditions in order to achieve peaks of good shape and reproducibility. Photometric detection was coupled firstly to the HPCIC system based on the fact that a simple method of detection enabled uncomplicated optimisation of separation conditions and assessment of column performance. Although it was acknowledged that photometric detection would provide insufficient sensitivity for the direct detection of aluminium in the seawater of interest (Antarctic), a detailed investigation into the optimal operating conditions was a logical approach before any improvement to detection was attempted.

4.2 Optimisation of Separation Conditions

4.2.1 Overview

Aluminium forms stable complexes with O,O-coordinating ligands, so carboxylic-type ion-exchangers can be used for separation of this cation by HPCIC. Two silica-based stationary phases with either a poly(butadienemaleic acid) copolymer (PBDMA) surface layer or iminodiacetic acid (IDAS) functionalities (see Figure 4.1) were evaluated in terms of the peak profile of eluted aluminium. The peak of aluminium obtained with PBDMA was very broad and tailed, so the IDAS column was used for further experiments. Eluents containing KCl and HNO_3 were examined,

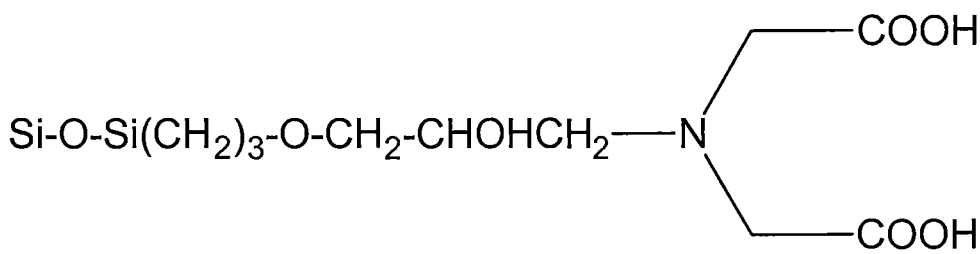

Figure 4.1. Structure of iminodiacetic acid functionalised silica.

using 0.3 mM Tiron in 1 M ammonium acetate as the post-column reaction (PCR) reagent. Experiments were carried out without column heating, unless specified otherwise.

4.2.2 Eluent pH and ionic strength

The retention of aluminium on IDAS should depend on both acidity and ionic strength of the KCl-HNO_3 eluent. The acidity of the eluent can affect separation in three ways. First, changing the acidity of the eluent affects the dissociation of the carboxyl moiety on the iminodiacetic acid functional group as can be appreciated by noting the applicable pK_a values of 2.59 (H_2L) and 1.85 (H_3L) [151] An increase in acidity reduces the number of negatively charged carboxyl groups through protonation which will, in turn, decrease electrostatic interactions. Second, conditional stability constants of the corresponding complexes between aluminium and IDA groups will also decrease. Both of these effects will result in a reduction in retention. Finally, a positive effect of increased acidity on retention is the reduction of hydrolysis of aluminium, which may also affect the separation efficiency.

The ionic strength of the eluent governs the extent of electrostatic interactions with the ionised IDA groups. At high ionic strength these interactions are suppressed and chelation becomes the dominant separation mechanism [112]. However, since too high an ionic strength can lead to a decrease in column efficiency due to increased viscosity, a balance between separation and column efficiency must be made.

Ionic strengths in the range of 0.1–0.75 M KCl were investigated, with the effects on retention time and column efficiency being illustrated in Figure 4.2. Although a decrease of approximately 35 s in retention time resulted from increasing the ionic strength from 0.1 to 0.5 M, an increase in column efficiency of more than two-fold was accomplished. It should be noted that at concentrations higher than 0.5 M KCl, a decrease in column efficiency was observed due to viscosity effects. In view of this, 0.5 M KCl was chosen as the optimal eluent concentration. In order to exhaust possible improvements through changes to ionic strength, different salts were examined. These included potassium sulfate, ammonium sulfate and ammonium chloride. K_2SO_4 and $(NH_4)_2SO_4$ were dismissed as alternatives based on resulting poor column efficiencies and whilst NH_4Cl compared well with the KCl in terms of retention time and column efficiency, KCl was ultimately chosen based on its superior peak heights.

In summary, the optimal eluent conditions were determined to be 0.5 M KCl and 30 mM HNO_3, which gave satisfactory peak shape, peak height and separation efficiency.

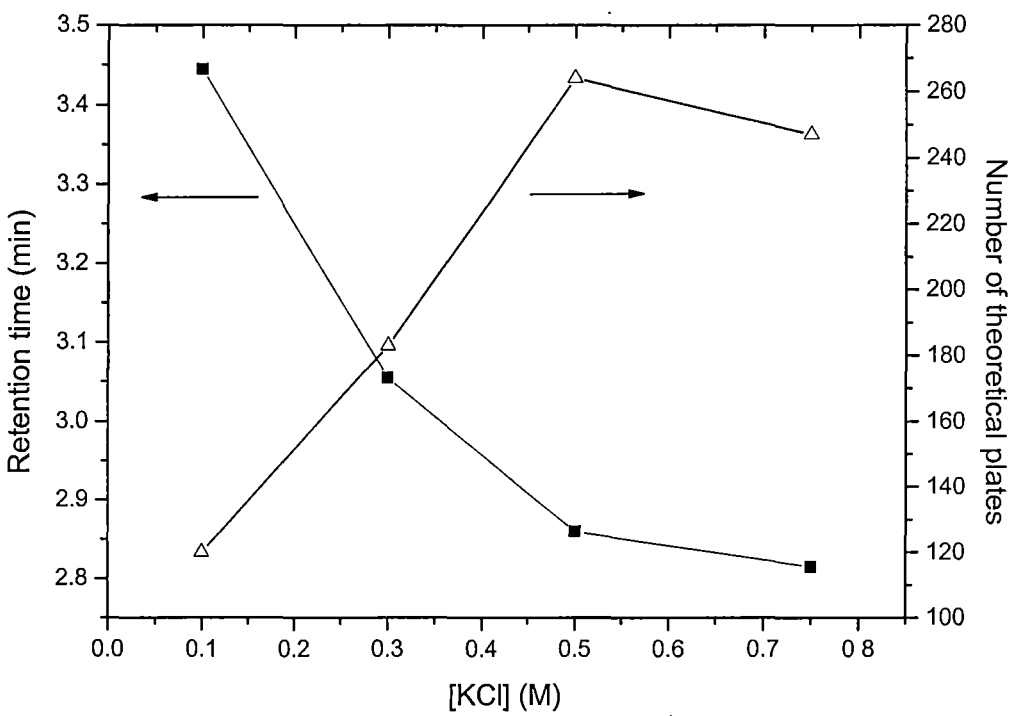

Figure 4.2. Dependence of retention time and column performance on concentration of KCl in the eluent. Experimental conditions: 15 cm IDA-silica column, 30 mM HNO_3, eluent delivery 0.8 mL/min.

4.2.3 Column temperature

Temperature exerts considerable influence on separation in HPCIC. The thermodynamic effects of column temperature on retention can be described by the van't Hoff equation and these have been previously explained in detail by Nesterenko and co-workers [112]. The impact of temperature change on retention is heavily reliant on the enthalpy of a system. For chromatographic systems in which chelation is the dominant mechanism, the enthalpy of reaction may be either exothermic or endothermic, so an increase in temperature may increase or decrease retention times. Additionally, the heats of sorption (ΔH) for chelating systems are generally significant in magnitude, so observable changes in retention in response to temperature change can be expected for HPCIC systems.

Response of the system at temperatures in the range of 24–75 °C was studied, with the latter temperature being the maximum possible for the Metrohm column heater. Figure 4.3 shows the dependence of retention of aluminium on column temperature. An increase in retention time of over 2.5 min was obtained by increasing the temperature from 24 to 75°C. This result agrees well with previous findings for 15 rare earth elements on IDA-silica [152]. The sorption enthalpy of aluminium with IDA was estimated to be 20.2 ± 1.3 kJ $(\text{mol K})^{-1}$ from the slope of the plot in Figure 4.3 and for such endothermic complexation reactions, higher temperatures shift the equilibrium in favour of complex formation and therefore increased retention results.

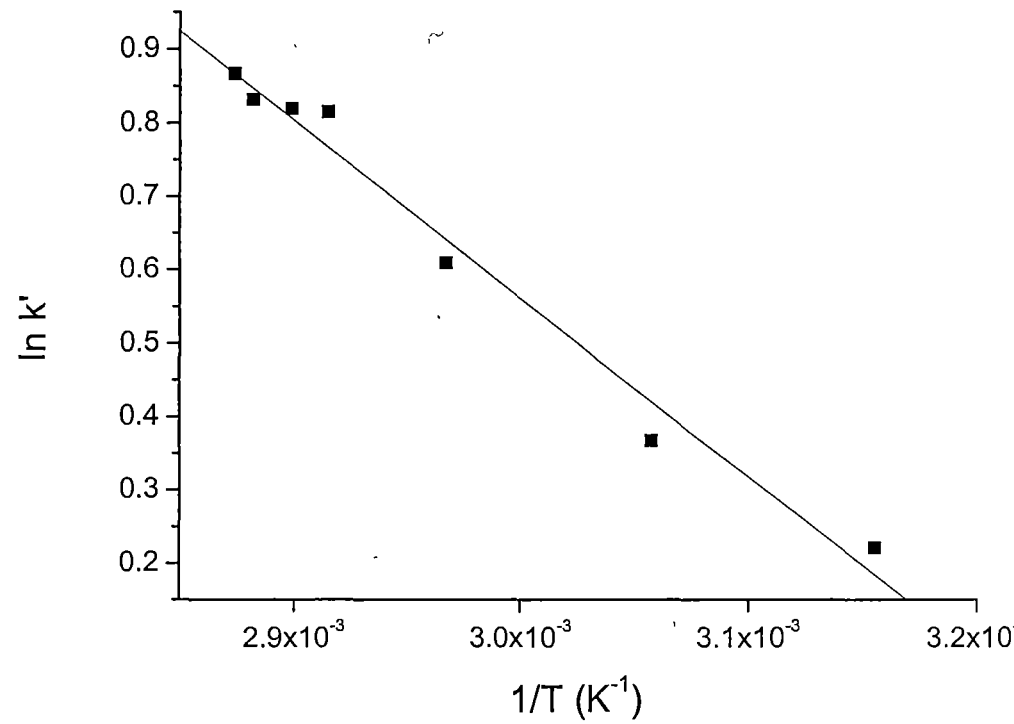

Figure 4.3. Dependence of retention of aluminium on column temperature. Experimental conditions: 15 cm IDA-silica column, 0.5 M KCl–30 mM HNO_3 eluent delivered at 0.8 mL/min.

Temperature also influences separation efficiency and it has been demonstrated that efficient HPCIC or IC separations of aluminium are possible only at column temperatures above 60 °C [106, 114, 115]. The reason for this is the very slow interaction kinetics of the aluminium cation with chelating groups and the slow dissociation rates of complexed aluminium species normally present in real samples. Figure 4.4 shows column efficiency for an IDA-silica column and illustrates that between 64 and 70 °C there is a sharp increase in efficiency, followed by a rapid decrease above 70 °C. To the author's knowledge, this type of dramatic response of column efficiency to column temperature has not been reported before and is believed to be specific to this particular chromatographic system.

A possible explanation is the influence of localised temperature-induced viscosity changes on the shape of the sample band. This is believed to result from the specific performance characteristics of the column heater and also the use of low thermal conductivity PEEK for the column and connecting tubing. Differences between the temperature of column components and that of the entering eluent impose viscosity effects. This in turn influences the shape of the chromatographic band and consequent column efficiency.

For example, it is believed that at low temperatures, the column heater provides insufficient heat to equilibrate the entire volume of the column and so the column housing is at a lower temperature than the eluent. This means that the eluent in immediate contact with the column walls is cooled, causing viscosity differences within the plug of eluent and tailing of peaks (Figure 4.4 (i)). At a temperature of

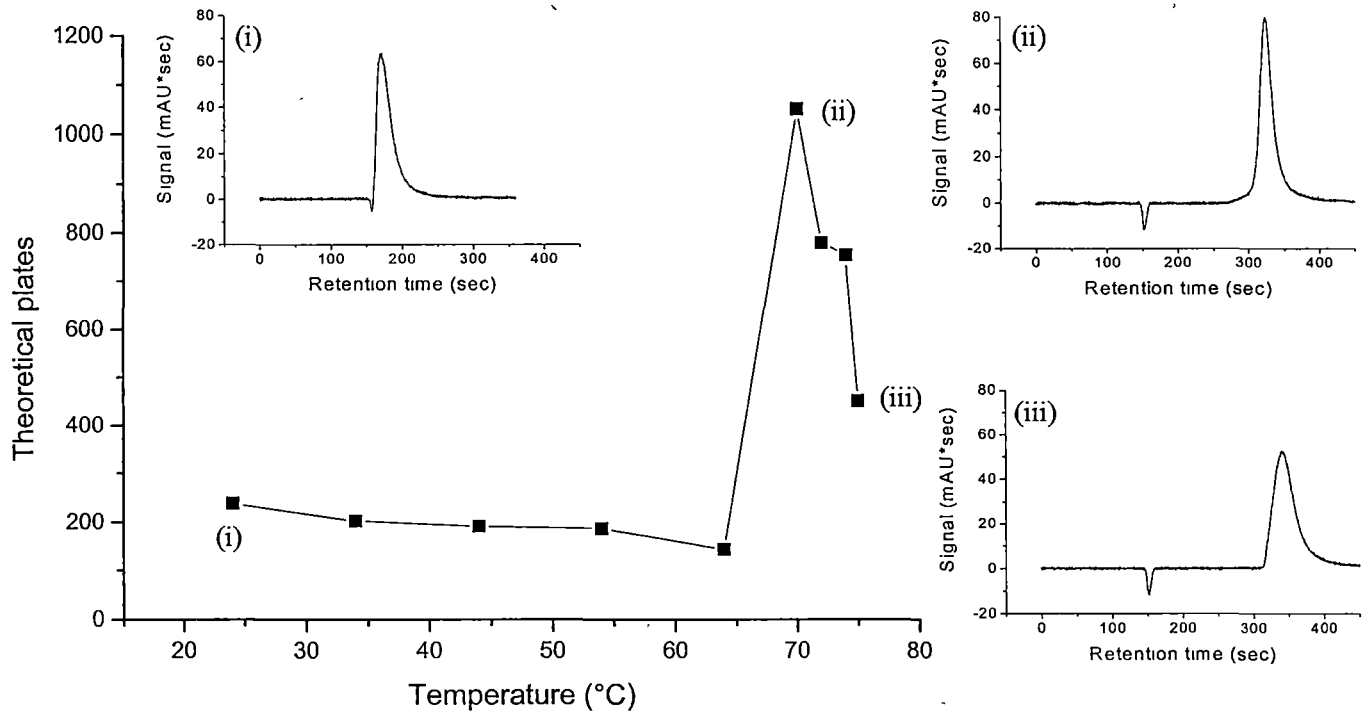

Figure 4.4. Dependence of column efficiency on column temperature. Experimental conditions: 15 cm IDA-silica column, 0.5 M KCl–30 mM HNO_3 eluent, delivered at 0.8 mL/min.

(i) Peak corresponding to 24°C. (ii) Peak corresponding to 70°C. (iii) Peak corresponding to 75°C.

70 °C, it is likely that the column heater provides sufficient energy for the entire column volume to be equilibrated to this temperature and so peak efficiency reaches a maximum (Figure 4.4 (ii)). At temperatures above 70 °C, a decrease in column efficiency is again observed. Figure 4.4 (iii) illustrates the typical peak shape at 75 °C and it can be seen that there is a definite increase in peak fronting. This is thought to be caused by the column reaching a higher temperature than the entering eluent. It is the author's belief that had the column heater been capable of heating to higher temperatures, further and more distinct fronting would have been observed.

This series of experiments was repeated using a water bath for heating the column and eluent and the strong dependence of efficiency on temperature shown in Figure 4.4 was not observed. However, the highest efficiency achieved in this series of experiments was only 409 theoretical plates per column and for this reason the Metrohm column heater was preferred.

4.2.4 Final adjustments to separation conditions

In an attempt to ensure the separation system was well prepared to handle samples with a complex matrix an additional 5 cm length of IDAS column was added to the existing 15 cm previously used. This served to increase the retention time by 2 min and also increase column efficiency slightly.

Up until this stage all experiments had been carried out with an eluent flow-rate of 0.8 mL/min. Adjustment of this rate was an obvious further approach to increasing the

retention of aluminium, ensuring adequate separation from potential interfering analytes in samples with a complex matrix. Flow-rates of between 0.2-1.2 mL/min were investigated with an expected increase in retention time observed a lower flow-rates. Additionally, higher column efficiency, as measured by the number of theoretical plates, also resulted at lower flow-rates. Of the flow-rates investigated, 0.3 mL/min was chosen based on a suitable retention time of ~15 min and the highest column efficiency (>3500 plates).

Because of the gains in retention time achieved through the modifications described above, maximum performance of the IDAS column was sought through further adjustment of the eluent composition. Acidity was increased to 40 mM to promote column efficiency and the expected subsequent decrease in retention time was negated by a simultaneous decrease in ionic strength to 0.1 M. However, despite the retention time remaining fairly constant, a significant reduction in column efficiency resulted. This was believed to be an effect of ionic strength limiting the extent of chelation to the point where it was no longer the dominant separation mechanism. Consequently, the ionic strength was increased to 0.25 M at the same acidity. The result was an overall reduction in retention but a significant increase in efficiency (>22%).

Optimised conditions for the separation of aluminium on IDAS were thus determined to be a 0.25 M KCl-40 mM HNO_3 eluent delivered at 0.3 mL/min for a 20 cm column housed at 71 °C.

4.3 Photometric Detection of Aluminium

4.3.1 Overview

Photometric detection utilising different post-column reagents was comprehensively investigated once optimisation of separation conditions was complete in order to gain an initial understanding of the potential of the column for the analysis of complex samples. An extensive study of this area allowed for realisation of both the potential and limitations of this type of detection and was deemed significant in order to illustrate the applicability of the system to a variety of samples with different requirements e.g. limits of detection and applications.

4.3.2 Optimisation of post-column reaction detection

4.3.2.1 Post-column reaction detection

Post-column reaction (PCR) spectrophotometric detection is very common in HPCIC and IC [112], with Tiron and pyrocatechol violet (PCV) being the reagents used most commonly for the determination of aluminium. The sensitivity of systems employing PCR for the detection of aluminium has been improved continually, with Jones *et al.* [153] reporting a detection limit of 37 nM (0.1 mL injection volume) using fluorescence detection of the 8-hydroxyquinoline-5-sulfonate–aluminium complex. An objective of the present study was to develop a system utilising a post-column reagent capable of such a detection limit but using only photometric detection. Tiron was chosen initially as the post-column reagent, based on its widespread use for the detection of aluminium.

4.3.2.2 Tiron

Initial working conditions for the Tiron post-column reagent, such as buffer type and reagent concentration, were chosen based on literature findings [104-106, 154] (Dionex Application Note AN 69, 1991). None of the reported methods, however, involved the inclusion of a surfactant to the reagent mixture. It has been shown that the addition of a surfactant to post-column reagents can often intensify the signal through interaction with micelles and in this study it was found that an addition of 0.5% w/v of Triton X-100 to the PCR reagent resulted in a small improvement to both peak height and efficiency. A calibration plot of the optimised system showed good linearity between 7.4 and 370 μM (see Table 4.1 for regression data).

4.3.2.3 Alternate PCR reagents

Alternate PCR reagents were examined in order to lower the limit of detection (LOD) of the system. The results for the optimised Tiron system were compared to those obtained for PCV, Chrome Azurol S (CAS) and Eriochrome Cyanine R (ECR), with this being the first reported use of the latter reagent for PCR determination of aluminium in a HPCIC system. Column and eluent conditions were maintained as outlined above and concise optimisation of buffer and surfactant conditions and detection wavelength for each reagent was undertaken.

(i) Pyrocatechol violet

For PVC, both imidazole and hexamine were investigated as possible buffers. Hexamine appears to be the buffer of choice in many literature articles. However, since the extent of complexation of aluminium by PCV increases with pH (to ~ pH 6) [155], imidazole was initially trialed. This choice was based on imidazole's higher pK_a and expected increased capability to maintain an optimum pH on mixing with an acidic eluent stream. Various wavelengths were tested for the PCV- imidazole system with 585 nm determined to be the most suitable. The sensitivity of the system was enhanced by the addition of Triton X-100, with a LOD of 1.0 μM achieved. Replacement of imidazole by the same concentration of hexamine resulted in complete loss of the aluminium peak. Increasing the hexamine concentration to 1.4 M served to rectify this problem, however, the peak shape was distorted and subsequently efficiency was poor.

(ii) Eriochrome Cyanine R

Both imidazole and hexamine were also tested for the PCR reagent ECR. Both buffers were investigated at a concentration of 0.2 M and it was found that hexamine performed much better in terms of both sensitivity and peak shape. Consequently, hexamine was chosen as the buffer for all subsequent experiments involving ECR. The pH of the hexamine was also optimised with a range of between pH 6-7.5 examined. A pH of 6.1 was found to be the most suitable.

Two factors; ECR and surfactant concentration, appeared to influence the extent of the baseline noise significantly in the ECR system. Initial experiments were carried out using

0.5 mM ECR but it was found that by reducing this concentration by half the baseline noise could be reduced ten-fold without significant loss to sensitivity. Also, the addition of the cationic surfactant cetyltrimethylammonium bromide (CTAB) seemed to have a stabilising effect on the baseline. The concentration at which this stabilising effect was active was limited to 1 mM, with higher and lower additions resulting in a dramatic increase in baseline noise. An alternate surfactant, cetylpyridinium chloride, also cationic, was trialed however no significant improvement to either peak shape or sensitivity was achieved.

Final adjustment of the ECR system was carried out through optimisation of the wavelength at which absorbance was measured. A range of 520-600 nm was investigated with maximum absorbance reached between 570 and 590 nm. Subsequent experiments were carried out at 580 nm.

(iii) Chrome Azurol S

CAS was also examined briefly in terms of performance as a post-column reagent for the detection of aluminium. Operating conditions were based on the work of two groups [156, 157], with minor adjustments made to the wavelength at which absorbance was measured. However, since a highly noisy baseline for the CAS system was observed and because it was considered that the limit of sensitivity using photometric detection had been exhausted through extensive optimisation of the ECR system, further investigation into CAS was not carried out.

4.3.2.4 Comparison of PCR reagents

Whilst peak shape was similar between Tiron, PCV and ECR, their sensitivities for the detection of aluminium differed considerably (Table 4.1). It can be seen that ECR performed well with regard to both sensitivity and column efficiency, and optimal performance was observed using 0.25 mM ECR with 1 mM CTAB, in a 0.2 M hexamine solution buffered at pH 6.1 and with detection performed at 580 nm. When a 100 μL sample loop was used, the LOD (3σ) was 100 nM. Linear calibration was observed over the range of 3.7 – 370 μM (see Table 4.1).

4.4 Conclusions

Following extensive optimisation of separation conditions, including column temperature and eluent composition, it was apparent that innovative use of IDAS could achieve successfully and effectively the separation and preconcentration of aluminium. Optimised conditions for the separation of aluminium on IDAS were determined to be a 0.25 M KCl-40 mM HNO_3 eluent delivered at 0.3 mL/min for a 20 cm column housed at 71 °C. Additionally, a unique dependence of column efficiency on temperature, believed to be specific for this system, was shown to exist.

High performance chelation IC systems employing post-column reaction were developed successfully and optimised for the determination of aluminium. Of the PCR reagents investigated, ECR, which was used for the first time for PCR aluminium detection in a

flow system, gave the best results, with a LOD of 100 nM obtained for a 100 μL sample loop. The applicability of this newly developed HPCIC system to the determination of aluminium in a complex sample will be presented in chapter six.

Table 4.1. Comparison of conditions and performance of different post-column reagents.

Reagent	λ (nm)	Reagent mixture composition	LOD (μM)[a]	Linearity[b] range and regression data
Tiron	310	0.3 mM Tiron in 1 M ammonium acetate, (pH 6.7) with 0.5% w/v Triton® X-100	6.7	7.4 – 370 μM S = 9.8(±0.1)c + 3.3(±13)
PCV	585	0.1 mM PCV in 0.2 M imidazole, (pH 6.9) with 0.5% w/v Triton® X-100	1.0	
ECR	580	0.25 mM ECR in 0.2 M hexamine, (pH 6.1) with 1 mM CTAB	0.6	3.7 – 370 μM S = 166(±5)c + 930(±900)
CAS	590	0.26 mM CAS in 50 mM MES (pH 6.0) with 2% w/v Triton® X-100	5.0	

[a] For a sample loop of 20 μL using standards prepared in deionised water.

[b] where S = signal (mAU s); c = concentration (μM)

Chapter Five -

Fluorescence Detection of Aluminium

5.1 Introduction

The sensitivity of photometric detection was never considered to be adequate for the determination of aluminium in the Antarctic surface seawater samples collected in the Ross Sea, nor seawater samples from many other oceanic regions. Whilst the high performance chelation IC system developed in chapter four was capable of detecting aluminium in the low μM range, it was estimated that an increase in sensitivity of approximately 100 times would be required for seawater analysis. Consequently, investigation into the coupling of a highly sensitive detector to the HPCIC system was always required.

Fluorescence detection was an obvious choice given the low detection limits achievable and the range of applicable fluorescent reagents available for the determination of aluminium. The possible reagents have been discussed in detail previously in section 1.3.2.4 but include, amongst others, lumogallion, 8-hydroxyquinoline and morin.

For this study, lumogallion was considered the most suitable fluorescent reagent for a variety of reasons including: previous experience throughout investigations using FIA, extensive and successful use in the literature, and most importantly, the low detection

limits previously reported for the detection of the aluminium-lumogallion complex. Considering that coupling a fluorescence detector to the newly developed HPCIC system had not yet been investigated and because of the differences in operation between it and FIA, a comprehensive study into optimal conditions of the lumogallion-based post-column reaction specific for this system was deemed necessary. The approach taken was to reinvestigate and optimise experimental variables that had been shown previously to have a high impact on the efficiency of the fluorescence reaction. However, additional parameters to those studied in the optimisation experiments for FIA, such as buffer type and reaction temperature, were also included given that differences in response were considered likely between FIA and HPCIC.

5.2 Separation Conditions

Optimum operating conditions for the separation of aluminium using IDAS have been detailed previously in chapter four. The only modification to these conditions was the use of NaCl rather than KCl in the eluent. The reason for this was the availability of high grade chemical reagent in order to ensure low background fluorescence. In summary, these conditions were a 0.25 M NaCl-40 mM HNO_3 eluent delivered at 0.3 mL/min with separation on a 200 x 4 mm i.d. column packed with 5 μm IDAS housed at 71 °C.

5.3 Background Fluorescence

A significant dip in fluorescence away from the baseline before the elution of aluminium was observed in preliminary experiments. This dip was up to one fifth the size of the peak of a 3.7 μM aluminium standard. It was decided that the probable cause was the effect of the sample plug on the high background fluorescence due to the reagents used to prepare the eluent, in particular the chloride salt. Initially, KCl was used for the preparation of the eluent and despite choosing an analytical grade KCl, the level of aluminium contamination was obviously high. A possible solution to this was the addition of a trap column positioned before the separation column. The column was packed with Eichrom Diphonix® resin (particle size 100-200 mesh). This resin has diphosphonic and sulfonic acid groups bonded to a polystyrene/divinylbenzene matrix. It is capable of extraction of a range of metals from both neutral and highly acidic solutions. The column, measuring 250 x 4 mm i.d., effectively removed the majority of the aluminium from the eluent, reducing the dip by a factor of 25. In addition, trace metal grade sodium chloride and nitric acid were used in the eluent for subsequent experiments.

5.4 Optimisation of Lumogallion-Based PCR

5.4.1 Buffer

Previous work by Howard and co-workers [121] reported the optimum pH of the aluminium-lumogallion reaction to be between 4 and 5.5. Resing and Measures later

found the maximum response to be in a much narrower range between pH 5 and 5.5 [57]. Based on this fact, MES was chosen as the buffer for initial experiments given its pK_a of 6.27 at 25 °C and subsequent useful buffering range [120]. Although initial chromatograms of a 37 μM aluminium standard, using a 40 mM MES solution at pH 6.2, were promising in terms of sensitivity and efficiency, the pH of the effluent was found to be only 2.9. Increasing the MES concentration to 120 mM served to improve this situation, but also resulted in an increase in baseline noise and reduction in both sensitivity and efficiency. Consequently, it was decided to continue investigations using ammonium acetate, a buffer used extensively for the aluminium-lumogallion reaction.

Firstly, the effect of varying the concentration of the ammonium acetate buffer on sensitivity was studied. This was carried out by diluting a stock 3 M buffer (pH 6.7) to 0.25, 0.5 and 1 M. The results indicated that a concentration of 0.25 M gave the best result in terms of peak area and also for achieving an effluent pH closest to optimum for the lumogallion reaction. It was shown that peak area of a 3.7 μM aluminium standard increased almost 1 ½ times through the use of 0.25 M compared with 1 M ammonium acetate and over 8 fold compared with 40 mM MES.

Seawater samples intended for quantification of aluminium required acidification to between pH 1.8-2. Consequently, the buffer utilised in the lumogallion reaction needed to be able to maintain an optimum pH even on mixing with this acidified sample. The 0.25 M ammonium acetate buffer was shown to have insufficient buffering capacity when mixed with an acidified sample. Not only did the retention time decrease but a loss

in sensitivity also resulted. Given that a decrease in sensitivity was also observed previously with an increase in buffer concentration for ammonium acetate, the only alternative was to increase the pH of the buffer. This was attempted but it appeared that even increasing the buffer pH to 8 resulted in little improvement. This meant that, short of sacrificing sensitivity for buffering capacity, ammonium acetate was not the best choice for the analysis of acidified seawater.

The choice of buffers capable of maintaining a pH of approximately 5.5 is fairly limited. This fact led to the decision to reinvestigate MES. For comparative proposes, a 0.25 M solution of MES (pH adjusted to 6.05 with NaOH) was used initially. The result was the attainment of sensitivity equivalent to that of ammonium acetate, but with the added advantage of no loss in sensitivity between acidified and non-acidified samples. Similarly, changes in retention times were negligible. The remaining issue with the use of MES was the increase in baseline noise and subsequent increase in detection limits. This problem was overcome by pre-cleaning the buffer using a column packed with Eichrom Diphonix® resin. The resulting baseline noise reduced approximately three times and the corresponding background fluorescence was almost seven times less.

It was thus determined that a pre-cleaned buffer of 0.25 M MES adjusted to a pH of 6.05 with NaOH, was the optimum buffer choice for the determination of aluminium in acidified seawater samples.

5.4.2 Temperature of PCR

The response of the reaction between aluminium and lumogallion to temperature has been investigated in both batch techniques and flow systems. In the batch method, an optimal temperature of 80 °C is generally accepted [120, 121], whereas FIA methods tend to use 50 °C. The latter is based on investigations carried out by Resing and Measures, who concluded that most of the temperature-based reaction rate gain had been achieved by this temperature [57]. Independent investigation into the effect of temperature on the rate of reaction was undertaken in this study due to the fact a different buffer was used. It was found that the highest response, in terms of peak area, was obtained at temperatures between 65 and 75 °C (Figure 5.1). Based on this response, 70 °C was chosen as the temperature at which to operate the post-column reactor for all subsequent analyses.

5.4.3 Lumogallion concentration and reaction coil length

The extent of chemical reaction needs not be complete for an analytical technique to be valid. However, it is desirable to obtain as high a reaction yield as possible in order to ensure the technique has good precision. For the reaction between aluminium and lumogallion, the concentration of post-column reagent may be changed, along with temperature and reaction time, in order to control the extent of reaction. Three concentrations of lumogallion (0.03, 0.04 and 0.05 mM) were tested in order to exhaust possible improvements to the system via this approach. The concentrations chosen were based on those used in flow systems. It was found that at concentrations higher than

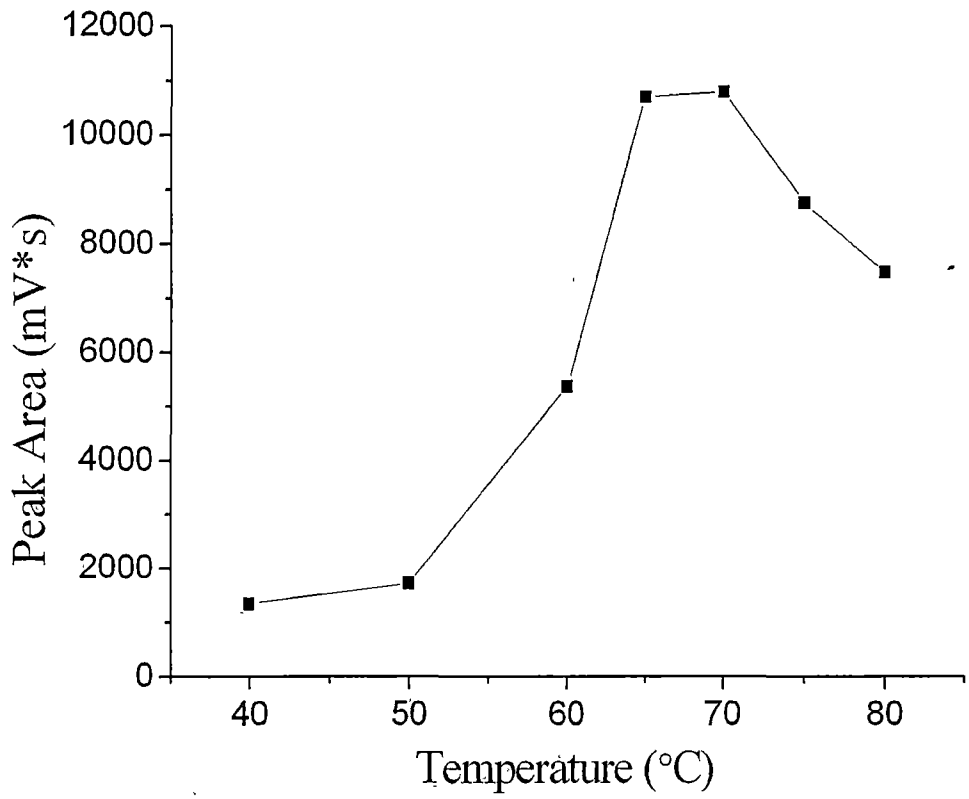

Figure 5.1. Dependence of fluorescence on temperature.

0.03 mM, no significant improvements were achieved.

FIA manifolds based on that of Resing and Measures [57] typically use a reaction coil length of 8-10 m. This is considerably longer than the 2 m used in investigations thus far with HPCIC. The effect of increasing the length of the post-column reaction coil from 2 m to 4 m was therefore studied. The result, however, was a slight reduction in fluorescence. Further, the effect of adding a cooling coil after the initial PCR coil was also tested. Again, a reduction in fluorescence was recorded even when the total length of both coils was kept at 2 m.

A MES buffer containing 0.03 mM lumogallion together with a 2m reaction coil were thus used in all subsequent analyses.

5.4.4 Effect of surfactant addition

Howard and co-workers reported an increase in the fluorescence intensity of the aluminium-lumogallion complex of as much as 5-fold through the addition of a non-ionic surfactant [121]. Further investigation has been carried out by Resing and Measures [57], which showed that Brij-35 enhanced fluorescence to a greater extent than other surfactants, such as Triton X-100 and cetyltrimethylammonium bromide (CTAB). In order to ensure the lowest limit of detection was achieved for this system, an investigation into the effect of surfactants was also carried out.

The results differed substantially from those discussed earlier. It was found that although the addition of Brij-35 enhanced fluorescence marginally, a simultaneous increase in baseline noise negated any improvement achieved. Interestingly, when CTAB was tested, the aluminium peak disappeared altogether. This was considered to be an effect of the surfactant adhering to the tubing walls and effectively stripping the aluminium from the reagent stream. The system required flushing with methanol in order to resume normal operation. Consequently, further investigation into the possible use of surfactants was abandoned, with the decision to explore other approaches to lowering the detection limit being deemed more favourable.

5.5 Effect of Injection Volume

A more attractive approach for achieving a low LOD was increasing the sample loop volume. All previous experiments had been carried out using a volume of 20 μL. The response of the system to higher volumes was investigated and the results for a 37 nM standard are depicted in Figure 5.2. It can be seen that for volumes between 20 and 500 μL, the system follows a linear response, as expected. It was also noteworthy that no reduction in column efficiency was experienced at higher volumes. The highest efficiency was achieved for a 500 μL sample loop, which was unexpected considering that band broadening is generally associated with increased sample size and is often responsible for an observed reduction in performance of the chromatographic column.

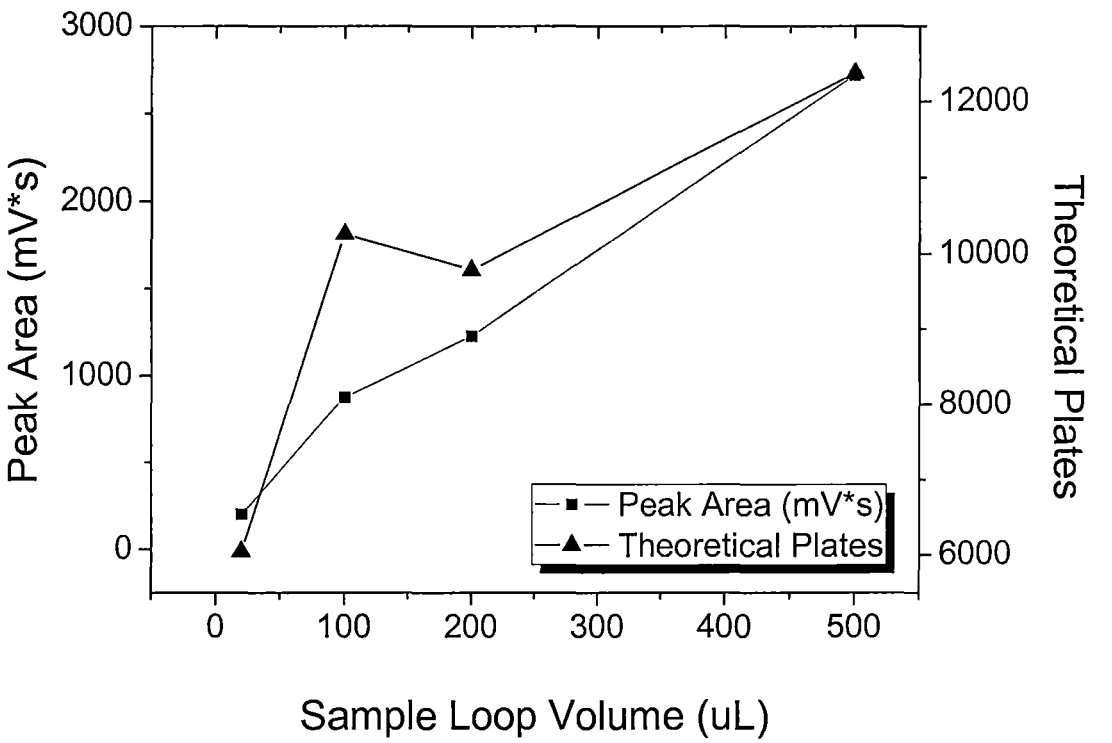

Figure 5.2. Effect of increasing sample volume of a 37 nM aluminium standard on column performance and fluorescence response.

Another unexpected result of increasing the sample volume was an increase in retention time (see Figure 5.3). Generally, a decrease in retention time would be expected due to competition from other analytes for chelation sites; more so for samples containing multiple analytes. This was shown not to be the case when using IDAS for standards or seawater (see Chapter 6) and may be explained in terms of the formation of negatively charged aluminium complexes and the high ionic strength of the eluent. Ionic strength has been reported to affect the retention of ions in chelation IC studies [113, 158]. The increase in retention is actually considered favourable as it allows for additional stabilisation of the baseline between the minor dip in fluorescence and elution of the aluminium.

The linear response and sensitivity of the HPCIC system with fluorescence detection was carried out by standard addition to a seawater sample with a low aluminium content and will be discussed in the following chapter.

5.6 Conclusions

A fluorescent detection unit was coupled successfully to the existing HPCIC system developed in chapter four in order to provide greater sensitivity. Post-column reaction with the fluorescent reagent lumogallion was utilised and a thorough investigation into optimal operating conditions of the PCR was undertaken specifically for this HPCIC system.

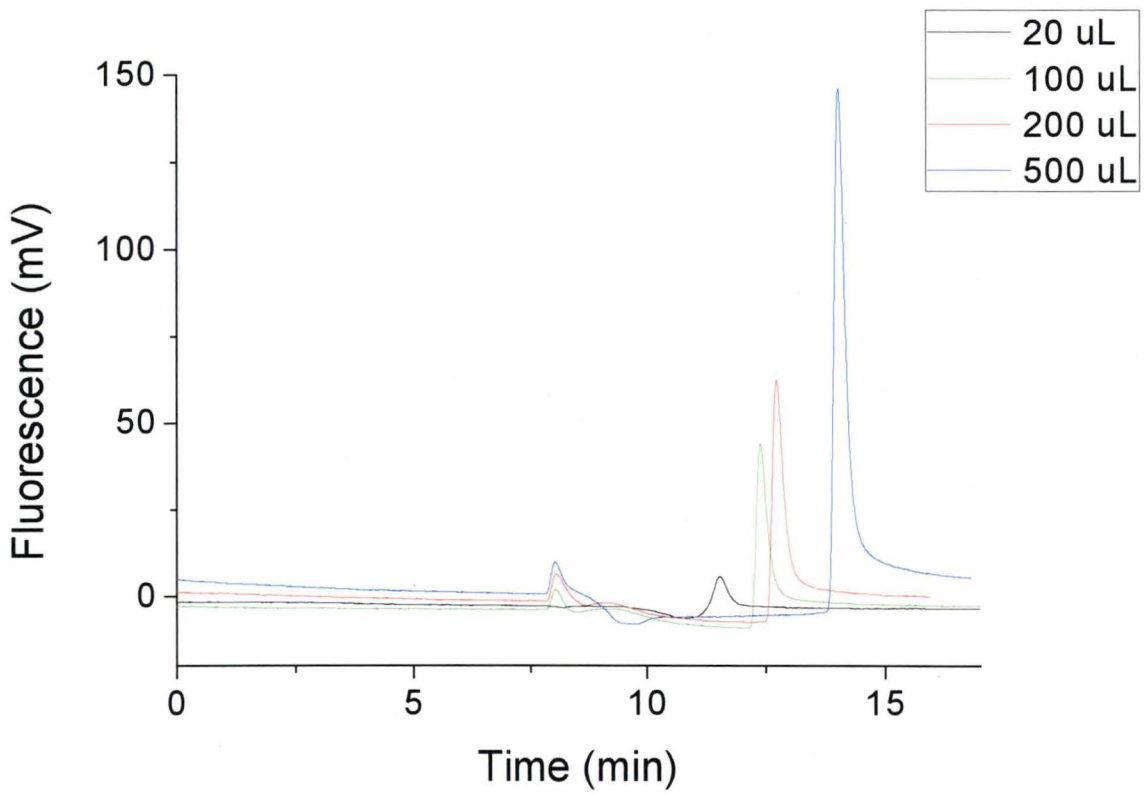

Figure 5.3. Response of HPCIC system to different injection volumes of a 37 nM aluminium standard.

A 0.25 M MES buffer adjusted to a pH of 6.05 with NaOH to which the lumogallion was added, was chosen based on good peak shape and high sensitivity, in addition to its ability to effectively maintain an adequate pH on mixing with acidified (pH 1.8-2) samples. The extent to which the aluminium-lumogallion reaction proceeded was maximised by optimising the reaction coil temperature, lumogallion concentration and coil length. The optimal temperature of the reaction coil was found to be 70°C and a lumogallion concentration of 0.03 mM along with a 2 m reaction coil was observed to provide the highest sensitivity.

The sensitivity of the HPCIC system coupled with fluorescence detection was able to be further improved through increased sample injection volume. A maximum volume of 500 μL was tested, with both retention time and column performance highest at this volume in comparison to 20, 100 and 200 μL. No improvement to sensitivity could be made through the addition of surfactants.

Chapter Six

Application of HPCIC to Real Samples

6.1 Introduction

The difficult nature of seawater as a matrix has been discussed previously. Based on this knowledge it was decided that a sample with a less complex matrix would firstly be investigated in order to gauge the capability of the HPCIC system. Paper mill process water was deemed to be an appropriate sample because whilst such a sample has a relatively complex matrix the potential problems of excessively high salt content, together with an extremely low aluminium concentration, are avoided. The concentration of aluminium in this sample was known to be in the μM range and so photometric detection was deemed adequate in terms of sensitivity. The initial primary focus of this section of work was to observe how the IDAS column coped with a more complex matrix than the Milli-Q water used thus far rather than the issue of sensitivity.

It was envisaged that if no problems were encountered with the IDAS column for injections of the paper mill process water then seawater injections could be investigated subsequently. In light of the low LOD required for the Antarctic seawater samples of interest, fluorescence detection would be used for this section of work.

6.2 Analysis of Paper Mill Process Water

A sample of paper mill process water was obtained from the Boyer Mill. This water is originally used to transport fibre onto the paper machine, at which stage it contains fibre from a variety of sources (softwood mechanical pulp, hardwood mechanical pulp and recycled fibre), clay and polymeric additives (used to aid in the retention and drainage of fibre on the paper machine). The process water is recirculated many times and the main source of any aluminium is the clay added in the papermaking process, or carry-over as $Al(OH)_3$ flocculant from the water treatment plant.

The mill process water was analysed using the optimised ECR photometric system (using a 20 μL sample loop). Initial chromatograms of the sample showed two major peaks at 11.2 min (peak A) and 13.5 min (peak B) (Figure 6.1 (a)). The latter was determined to correspond to free aluminium, as confirmed from spiking experiments. Increased acidification of the sample decreased the retention time for peak B but not for peak A, such that at pH 1.5 coelution of the two peaks occurred (Figure 6.1 (b)). Identification of the initial peak was attempted by injection of standards of multiple metals that were considered likely, given the composition of paper mill process water, including iron, copper, zinc and magnesium. Despite injecting an extensive number of possibilities no peaks were observed and the peak remained unidentified. ICP-MS was thus employed in the hope of finally identifying this peak. ICP-MS analysis of the collected fraction of the effluent corresponding to peak A in Figure 6.1 (b) was carried out by Dr Ashley Townsend (Central Science Laboratory, University of Tasmania). Results showed that this peak was not a result of the elution of another metal but was also due to aluminium.

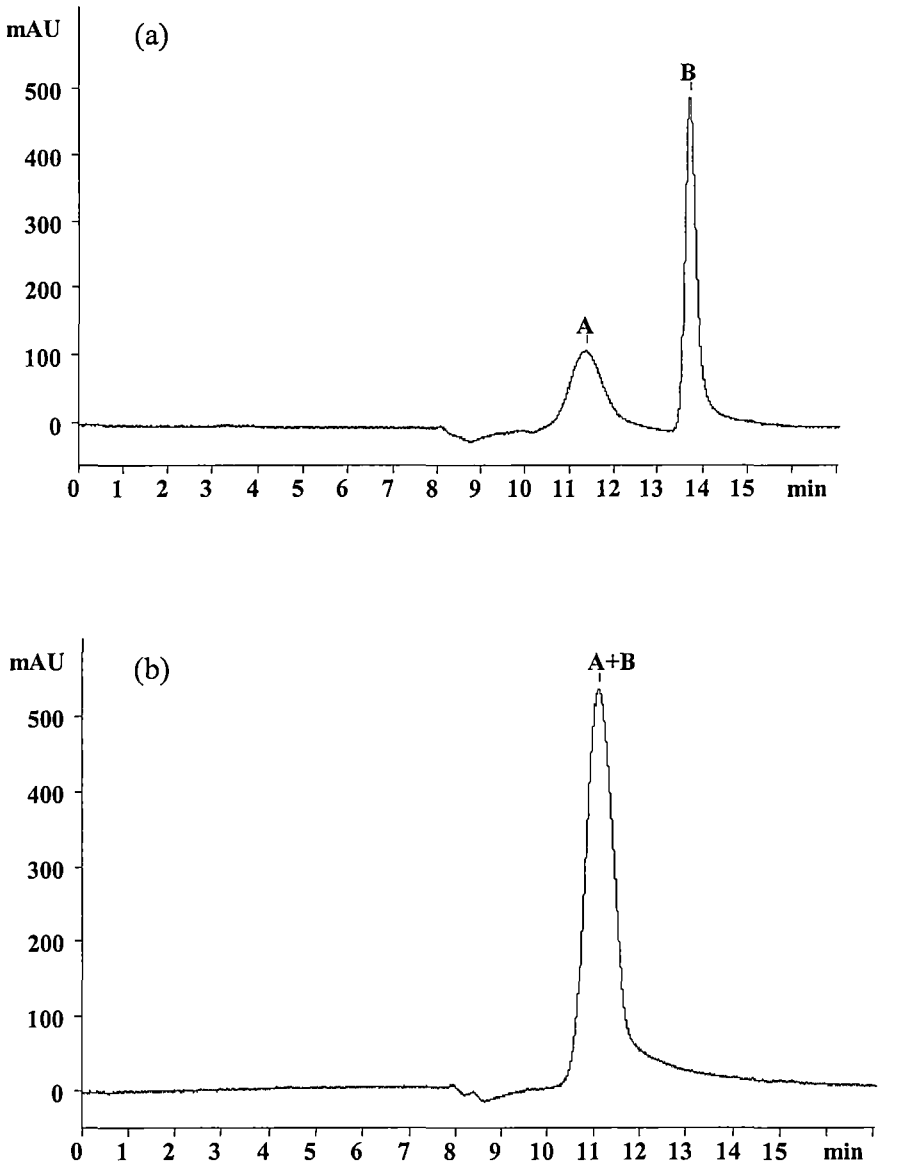

Figure 6.1. Chromatogram of paper mill process water using optimised ECR-system (20 µL sample loop). Experimental conditions: 20 cm IDA-silica column at 71°C, 0.25 M KCl–40 mM HNO_3 delivered at 0.3 mL/min.

(a) Sample filtered and at natural pH (pH 4.8).

(b) Sample filtered and acidified to pH 1 with HCl.

This species of aluminium appeared to be neutral, as its retention did not depend significantly on the pH of the sample (see Figure 6.2), being stable under strongly acidic conditions. In addition, this species was evidently kinetically inert in view of the fact that it could be eluted as a discrete peak on the IDAS column, which has strongly complexing functional groups and is expected to behave similarly to Chelex 100 when used for resin titration speciation [111, 159]. However, this species can still react with ECR to produce a coloured, detectable complex. The exact identity of this early eluting species is not clear, but the aluminium must be bound very strongly by ligands in the sample in order to account for this shorter retention time. The existence of such stable complexes of aluminium has been reported elsewhere [159-161], but again, the exact identity of the complexes has not been determined. It has been noted that the contribution of these strong ligands to the complexation of aluminium seems to be more important at low pH and when their concentration is in excess of aluminium [159].

Using IC with conductivity detection (analyses carried out by Dr. Eadaoin Tyrrell, University of Tasmania), chromatograms of both the sample and eleven common anions were compared in an attempt to identify possible ligands responsible for the formation of the highly stable aluminium complex. Despite expected anions, such as carbonate and sulfate, being identified no other anion detected was deemed likely to be able to form such a stable complex with aluminium. In a sample of this nature it is believed that humic or fulvic acids may potentially form complexes of such high stability with aluminium, however this is merely speculative.

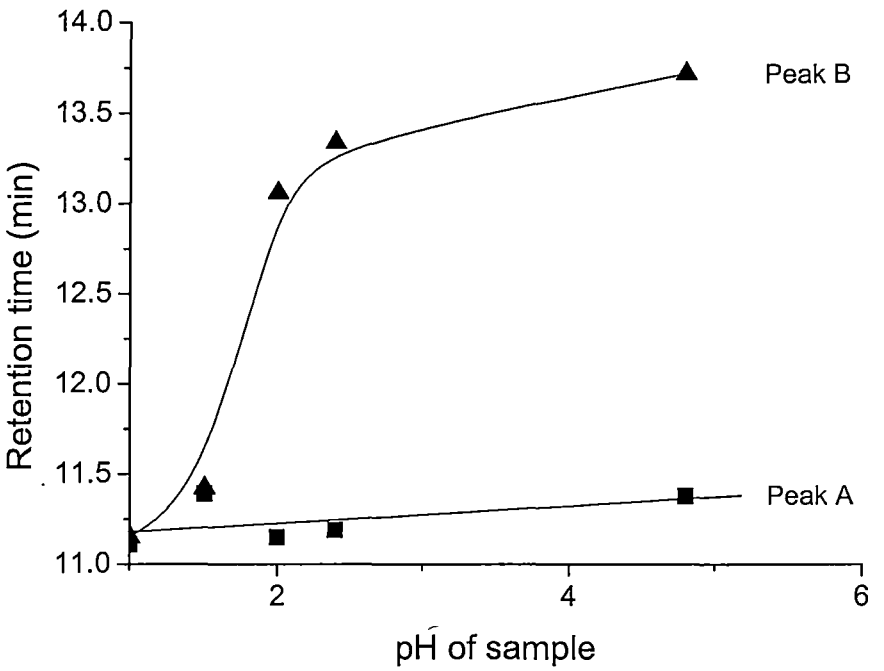

Figure 6.2. The effect on retention of peak A and B (inert and labile Al species) with increasing sample pH.

The concentration of the labile aluminium species in the process water (i.e. peak B) was determined to be 44.8 ± 1.8 μM (n = 3, p = 0.95). Standard addition of 18.5 μM of Al(III) to the sample gave a recovery of 99.3%. ICP-MS showed total dissolved aluminium in the unacidified sample to be 69.3 ± 1.1 μM (n = 5, p = 0.95). This value verifies the findings by the HPCIC method since the difference between the amounts measured by both techniques would have been due to the inert species of aluminium. The chromatographic peak area ratio for species B and A from the analysed sample is about 4.04, so the concentration of species A can be estimated from the PCR calibration plot (see Table 4.1) as 10.4 μM. In this case, the sum of concentrations of both species will give 44.8 + 10.4 = 55.2 μM. This is significantly less than the total concentration 69.3 μM of aluminium in the sample, as determined by ICP-MS, and it indirectly confirms the chemical inertness of specie A under conditions of PCR with ECR.

6.3 Analysis of Seawater Samples

6.3.1 Overview

At this stage, the optimised HPCIC system coupled with fluorescence detection had been shown to be applicable to the determination of aluminium in acidified standards prepared in Milli-Q water. Previous work with photometric detection showed that IDAS could be applied successfully to the analysis of samples with a complex matrix but it had not yet been used for the analysis of aluminium in seawater. Seawater is difficult to analyse not only in terms of the high salt content, but also due to the number of other potentially

interfering ions, such as iron and magnesium. However, preliminary chromatograms of seawater using the HPCIC system with fluorescence detection showed no co-elution of different elements and only one additional peak (at ~8 min) other than aluminium. Based on previous findings this peak is likely to be due to iron and/or a mixture other analytes e.g. sodium and calcium.

6.3.2 Calibration

Calibration of the system with both 200 and 500 μL sample loops was carried out by means of standard addition to an Antarctic seawater sample containing low levels of aluminium. The limit of detection was calculated from the standard deviation of low aluminium seawater and determined as the signal equivalent to three times this value (i.e. 3σ). LOD's of 1.2 nM and 0.39 nM were achieved using a 200 and 500 μL sample loops, respectively. Good linearity of the system was observed between 3.7 and 37 nM additions for the 200 μL sample loop (Figure 6.3) and 1.8 and 37 nM additions for a 500 μL injection volume (Figure 6.4).

6.3.3 Injection Volume

Chromatograms of different injection volumes of Antarctic seawater are given in Figure 6.5. As per the acidified Milli-Q water standards, an increase in retention time and response can be seen. A distinct difference can, however, be seen in the performance of the IDAS chromatographic column with increasing injection volume of seawater in comparison to acidified Milli-Q water standards as seen in Figure 5.2. A decrease in the

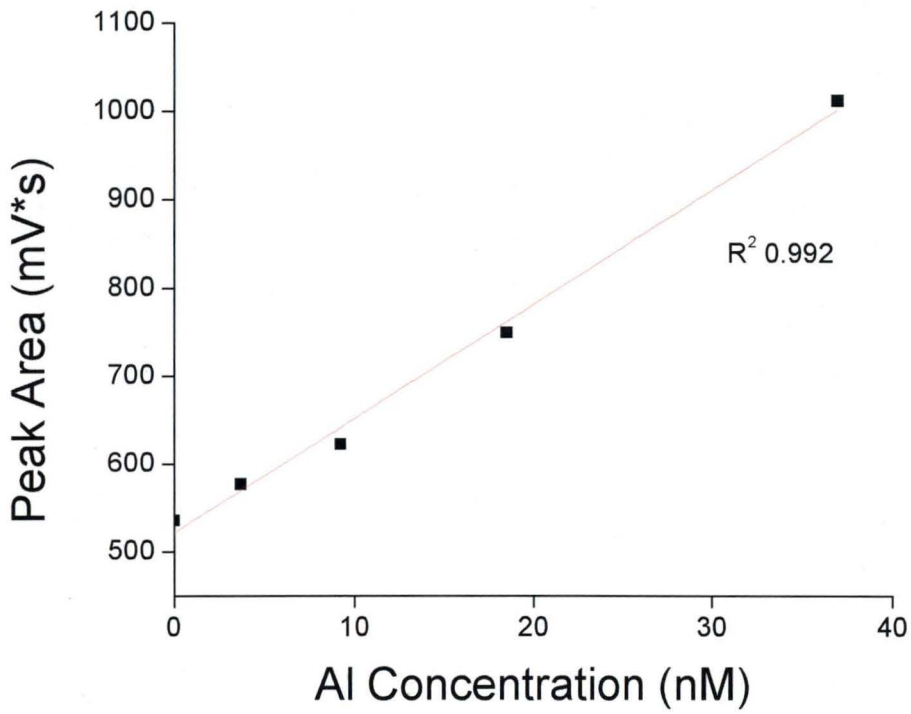

Figure 6.3. Calibration plot by standard addition using Antarctic surface seawater and a 200 μL injection volume.

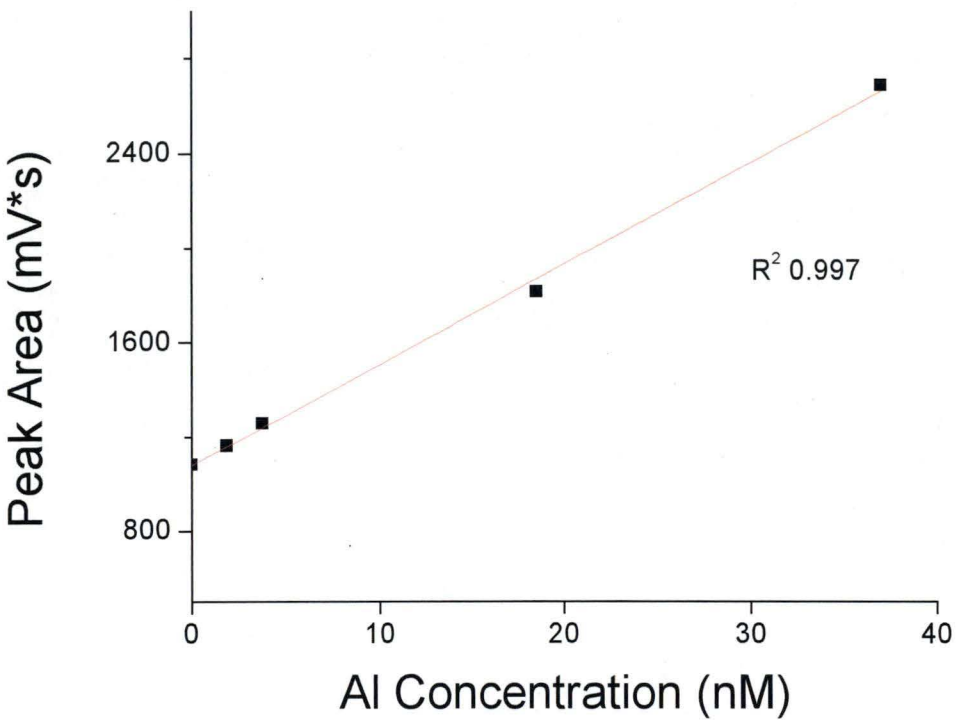

Figure 6.4. Calibration plot by standard addition using Antarctic surface seawater and a 500 μL injection volume.

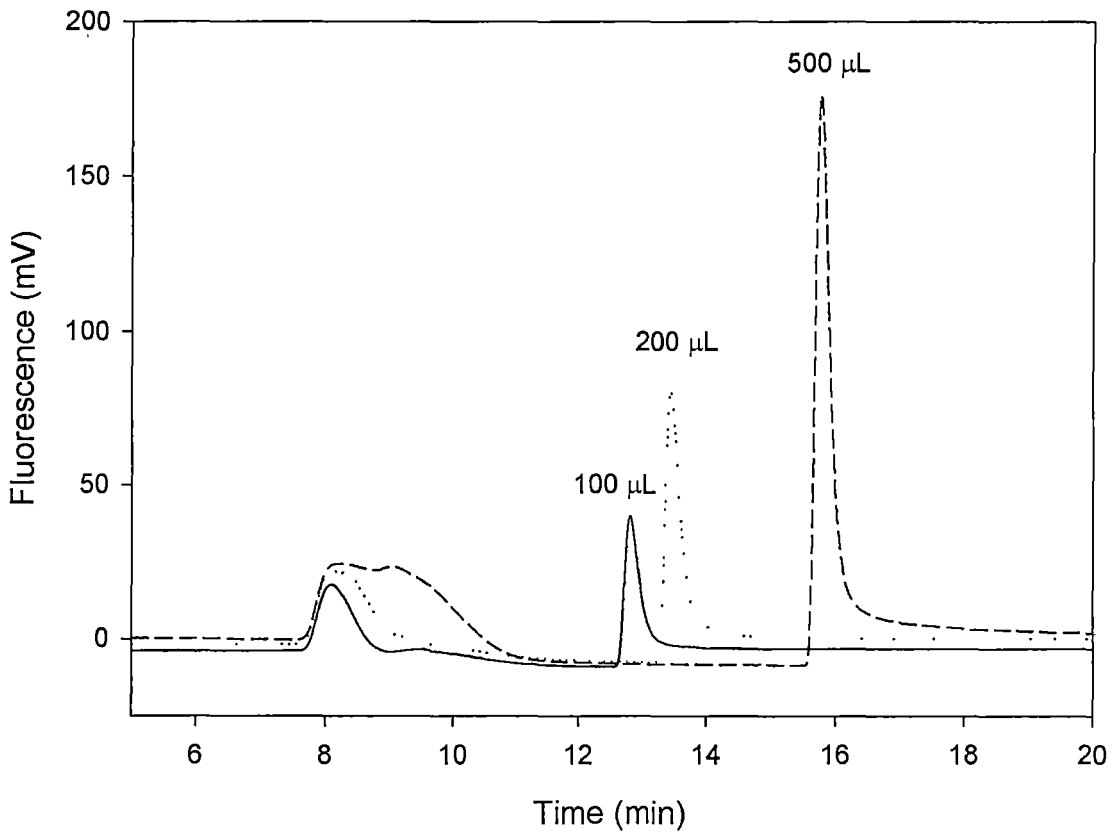

Figure 6.5. Chromatograms of increasing injection volumes of Antarctic surface seawater.

number of theoretical plates is observed at volumes higher than 100 μL (Figure 6.6). This decrease is likely to be a result of the high ionic strength of the sample, with not only the amount of aluminium increasing with larger injection volumes but also the numerous other common ions found in seawater. Regardless of the relative decrease in performance witnessed, the column still acts efficiently to produce peaks of good height and shape, even at the highest injection volume tested.

6.3.4 Quantification of Aluminium in Seawater Using HPCIC

Thus far, the HPCIC system coupled with fluorescence detection had shown good reproducibility (1% RSD for 500 μL injections) and linearity, however quantitative validation had not yet been carried out. Consequently, in order for the overall aim of the project to be realised, further testing in this area was required. Validating a system for the quantification of aluminium in seawater is difficult, owing to the fact that no certified reference material for aluminium in seawater exists. Direct comparison with techniques, such as ICP-MS, was also not possible without considerable sample manipulation, due to inadequate sensitivity. Therefore, the only available option was analysis of seawater considered within the FIA oceanographic community as equivalent to a certified aluminium reference sample [122]. This sample was collected in October, 2004, in the North Pacific, as part of the SAFe (Sampling and Analysis for Fe) iron intercomparison study cruise. Both open ocean surface water (S) and 1000 m (D2) were collected and had been stored at a pH of 1.7 in LDPE bottles since this time. Analysis by FIA (based on the Resing and Measures method [57] has shown the concentration of the S and D2 samples to be 1.7 and 1.0 nM respectively.

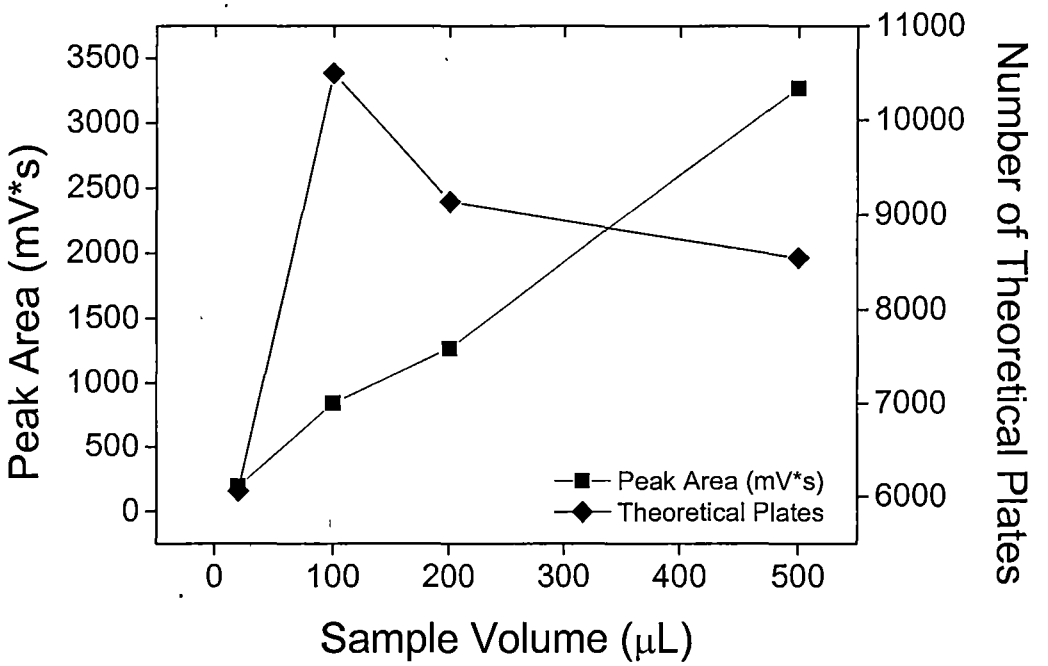

Figure 6.6. Effect of increasing injection volume on column performance and fluorescence response for Antarctic surface seawater.

As discussed previously, calibration of the HPCIC system was carried out by standard addition to low aluminium Antarctic seawater. Because there was concern that slight differences in the matrix of seawater from different regions could alter chromatographic behaviour, standard addition to the SAFe samples was carried out in order to determine the concentration of the samples. Analysis by HPCIC gave readings approximately seven times higher for both S and D2, than that obtained by FIA.

It was thought that perhaps the higher readings were a factor of some sort of background fluorescence. An injection of the eluent indicated no system blank. Likewise, whilst the eluent and PCR reagent contribute to constant background fluorescence, due to being continuously pumped throughout the IC system and detector, they could not be responsible for increased fluorescence on injection of a sample.

Given the complexity of seawater and the possible contribution of other analytes to fluorescence, an attempt was made to quantify the fluorescence of an "aluminium free" seawater sample. A 25 mL volume of Antarctic towed fish sample was left overnight to equilibrate with approximately 0.7 g of Diphonix resin (the same as used for the trap column). Given the extremely high affinity of the resin for aluminium, it was expected that all traces of aluminium would be extracted. The peak area of a normal towed fish sample and "cleaned" sample was compared. The resulting peak area of the "cleaned" sample was ~35% less than that of the normal sample. Consequently, by subtracting the fluorescence of the aluminium free seawater, the concentration of the Antarctic surface water was determined to be 14 nM rather than 44 nM.

It was, at this point, still expected that the Antarctic sample should be much lower in aluminium than the 14 nM obtained, based on FIA results of other oceanic regions. One possibility for the high content of aluminium in the Antarctic sample was the affinity of the Diphonix resin for other metals besides aluminium. If the Diphonix resin had extracted additional ions that would have otherwise contributed to background fluorescence then the "blank" reading may have been smaller than expected and the resulting concentrations still higher than true.

Because of the difficulty of extracting only aluminium from seawater, it was thought that Milli-Q water injections could help to identify the source of any additional fluorescence. Water from three available Millipore systems; Milli-Q Academic, Milli-Q Gradient and Milli-Q Element were injected, the latter having been developed by the company specifically for trace metal work and specifying sub-ppt elemental contamination. Despite a clear reduction in the aluminium content of the water from the Element system compared with the Academic and Gradient systems, as could be appreciated from differences in peak area, it was evident that all water types still contained aluminium to a degree. It must be noted at this stage, that work was not carried out inside a class 100 clean room, with only sampling being undertaken under laminar flow. Consequently, aluminium fluorescence from the Element system water could have been, in part, due to contamination.

The existence, and indeed source, of any additional fluorescence was still unclear at the conclusion of this series of experiments. This was because it remained impossible to

completely eliminate the contribution to fluorescence from aluminium itself. Additionally, a Milli-Q water injection containing no aluminium would only have served to eliminate the instrument itself as a source of additional fluorescence and not the seawater matrix.

In order to assess the possible contribution of the seawater matrix to fluorescence, a differential refractometer was used to trace an injection of seawater. It was hoped that if potentially interfering components of seawater were present at the same retention time as aluminium then a change in the refractive index would be observed. It was considered that perhaps the seawater matrix was not eluted fully at ~8 minutes as previously thought, and that this plug of matrix eluted gradually, overlapping with the retention time of aluminium. This could, in turn, falsely increase fluorescence and the subsequent quantification of aluminium. However, detection by refractometry confirmed that the plug of seawater matrix was completely eluted before aluminium, disproving this theory.

Continuing the line of thought that there must be fluorescence additional to that caused solely by the reaction between aluminium and lumogallion, co-elution of two fluorescing compounds was considered a possibility. If this was the case, then further changes to separation conditions were needed in order to separate these species. The first attempt to separate the two species was made by the addition of an extra 10 cm of IDAS-packed column of the same dimensions to that used previously, giving a total length of 30 cm. As would be expected, the retention time of aluminium was increased, however still only one peak (besides that of iron/matrix) was observed.

At this stage, it was unclear as to whether the potentially co-eluting species were different elements or two distinct aluminium species. It was, however, more likely to be the latter, considering the performance and specific nature of ion chromatography and the results of the previous experiments. Changes to eluent composition were made in the hope of causing variation to the retention of at least one of the aluminium species, so as to differentiate it from the other. The first approach was to observe the effect of increasing ionic strength of the eluent. As was discussed in section 4.2.2, changes to ionic strength affect separation by suppressing electrostatic interactions and ensuring that chelation is the dominant separation mechanism. The ionic strength was initially increased to 0.4 M NaCl with acidity maintained at 40 mM HNO_3. Despite a decrease in fluorescence being observed for a seawater injection - a consequence of increased viscosity, the emergence of two peaks also became evident. These peaks, whilst only partially separated had a retention time only negligibly different to that of a normal injection of aluminium in seawater.

Consequently, a further increase of ionic strength to 0.5 M NaCl was made. Only a slight decrease in fluorescence resulted but the separation between the two peaks became more evident. A final ionic strength adjustment to 0.75 M NaCl was made and again, the fluorescence and peak shape were significantly diminished. At the same time, further distinction between the two peaks also resulted. For comparative purposes, the same eluent composition (0.75 M NaCl-40 mM HNO_3) was also used for an injection of an acidified 37 nM aluminium standard. The same magnitude of peak separation was achieved as per the seawater; however, the two peaks were more obvious, given the

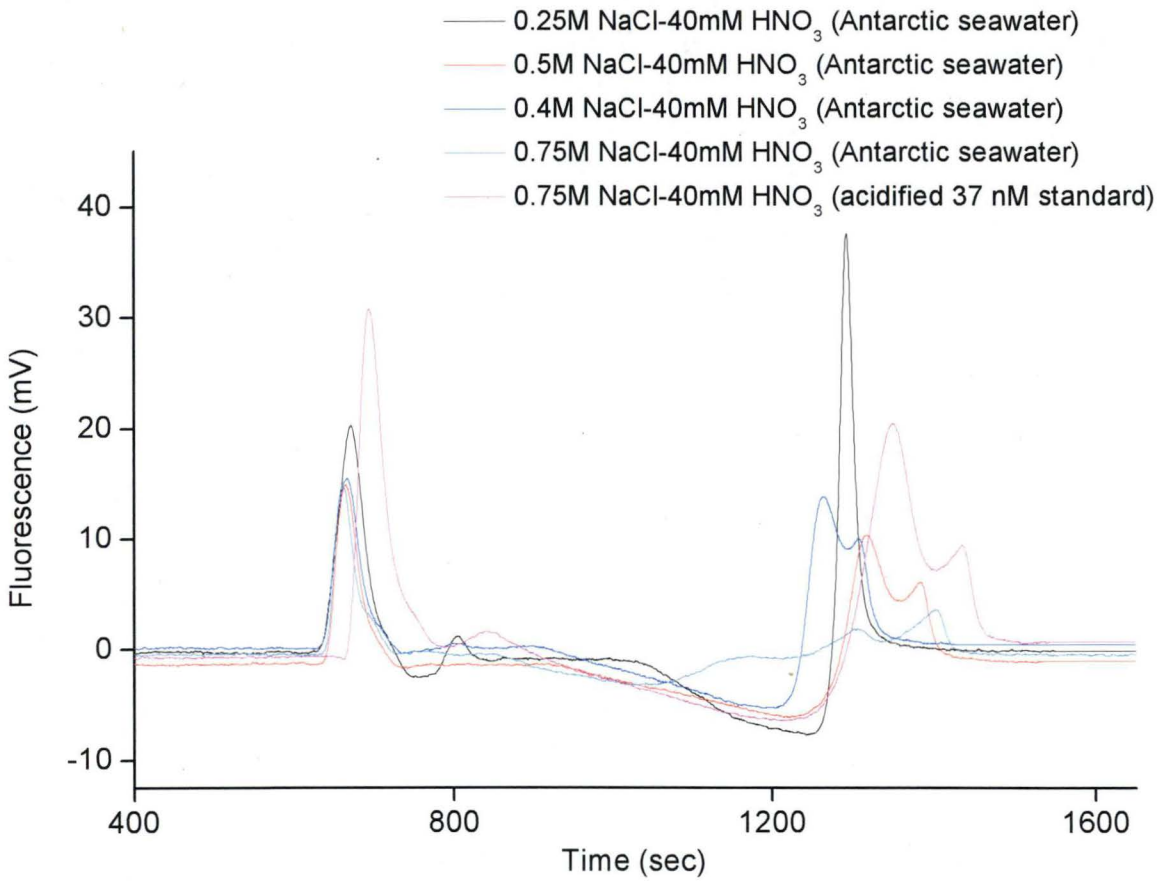

Figure 6.7. Effect of changes to ionic strength of eluent on the separation of two co-eluting species (believed to be both aluminium). 30 cm IDAS column.

higher concentration of aluminium. The chromatograms of the resultant injections are illustrated in Figure 6.7. Despite fairly good separation of the two species at high ionic strength, the consequent reduction in sensitivity and column performance meant that employing such a high NaCl concentration was not a feasible option. Instead, further separation of the two peaks was sought by means of changes to the acidity of the eluent. An ionic strength of 0.5 M was maintained and the acidity of the eluent decreased from 40 to 25 mM so as to cause an increased retention time. The resulting chromatograms are given in Figure 6.8.

It can be seen that although an increase in retention time resulted, as expected, both species appeared to be affected equally. Decreasing acidity did not aid in separating the co-eluting species; in fact, it served to negate the effect of high ionic strength, such that complete co-elution was once again observed at approximately 29 min. The reduction and consequent elimination in separation of the two species may be explained in several ways. Firstly, by decreasing acidity, the number of negatively charged carboxyl groups will increase, which results in an increase in electrostatic interactions - an opposite effect of increased ionic strength. Additionally, conditional stability constants of aluminium complexes with IDAS will be increased by a reduction in acidity. Since the acidity was almost halved from 40 to 25 mM, it may be that the stability constants for both aluminium species were maximised at such a pH resulting in identical retention.

From this series of experiments it was apparent that two ions were indeed being co-eluted and subsequently quantified by the HPCIC system. Due to the improbability that two

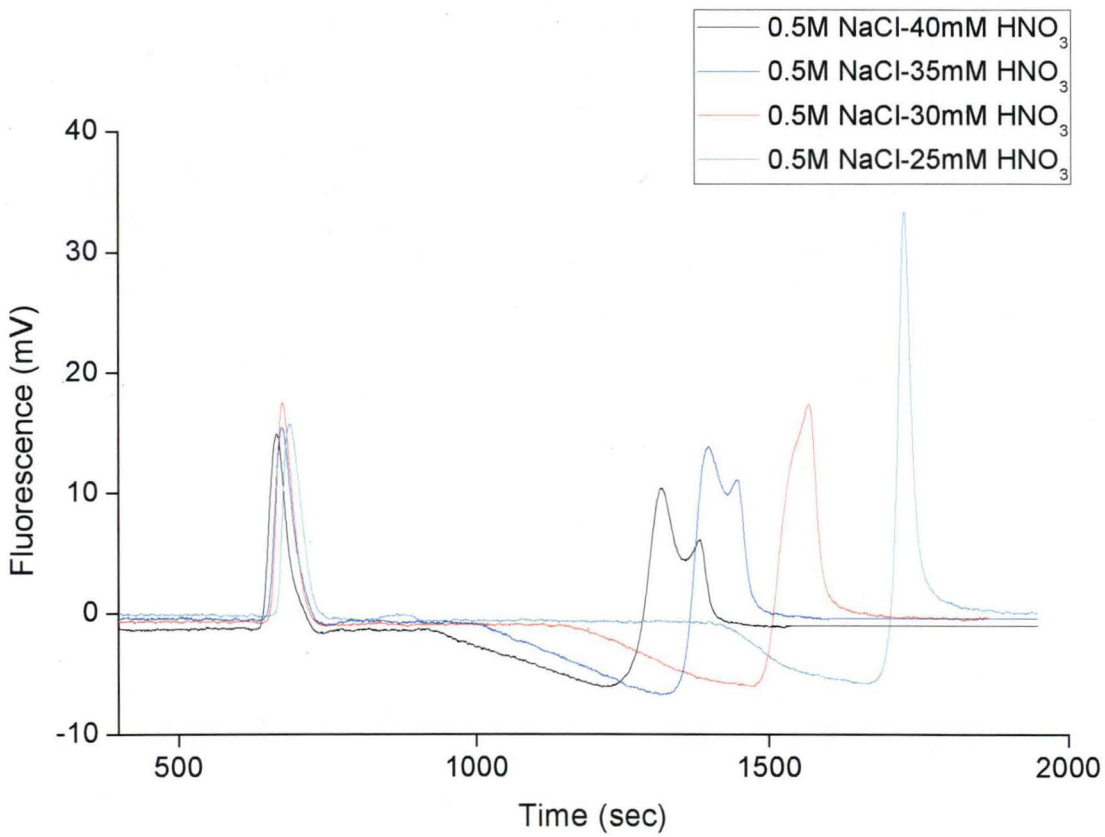

Figure 6.8. Effect of changing acidity of eluent on the separation of two co-eluting species (believed to be both aluminium), 30 cm IDAS column.

different elements would co-elute because of the uniqueness of conditional stability constants during complex formation of individual elements, it was considered that the two species were aluminium.

6.3.5 Aluminium speciation in acidified seawater

The ability of the IDAS column to differentiate between two aluminium species has been discussed previously in detail in section 6.2. Analysis of the paper mill process water showed that the technique could identify both inert and labile aluminium species. This work also highlighted the effect of sample acidification on the retention of these species. Whilst the retention of the inert species remained relatively unchanged with decreasing sample pH, the retention of the labile species was reduced, until, at a sample pH of approximately 1.5, co-elution of both species occurred.

It is believed that the effect of acidification on aluminium speciation also occurs in seawater. This means that at a higher sample pH, two peaks of aluminium would be expected to elute separately. However, at a low sample pH, such as that which HPCIC analyses were carried out, no such separation would take place and quantification would be of both the inert and labile aluminium species. Whilst both peaks obtained for the paper mill process water sample could be confirmed as aluminium using mass spectrometry, the same could not be carried out for the Antarctic seawater sample, once again given the sensitivity limitations of the technique.

The FIA method based on Resing and Measures work and the newly developed HPCIC system operate very differently. A large amount of sample manipulation is undertaken before seawater is analysed by FIA. This typically includes: sample acidification on collection (~ pH 2), sample buffering before preconcentration (~ pH 5.5), extraction via a solid phase such as 8-HQ functionalised resin (R8-HQ) and elution using an acidic carrier. In comparison, the HPCIC system directly analyses the seawater at the pH to which the sample was originally acidified, for storage purposes. There is no pH change. It is believed that this difference between the two techniques is extremely significant and could help explain the variation in concentration of aluminium in the Pacific Ocean samples. Additionally, it raises the question as to what exactly is being measured by both systems, in particular questioning the term "total dissolved aluminium".

In order to explain the differences in results of FIA and HPCIC, two suggestions are put forth. Firstly, it is the author's belief that the HPCIC system measures a true concentration of total dissolved aluminium in seawater, when the sample is acidified to pH 1.8 (as per the Antarctic samples). This means that both the labile and inert species are quantified. On the other hand, it is theorised that at a pH of ~5.5, FIA only accounts for one of these species, most likely the labile content. Considering the obvious inert nature of the other aluminium species, it is feasible that the preconcentration phases employed by FIA do not extract both species, or at least not to entirety. This would mean that the total aluminium content of the seawater is, in fact, not being accounted for by FIA. This partial or non-existent extraction of one species may, or may not be pH dependent but could be reliant on the type of solid phase employed.

In order to help substantiate this theory of two species of aluminium in seawater, an ambient pH seawater sample was obtained and an injection made using the HPCIC system. This sample was collected in February 2007 from the subAntarctic Southern Ocean, south of Tasmania and had been stored unacidified since this time in an acid-washed LDPE container. It was hoped that two distinct aluminium peaks would be observed, however, only one peak was again detected. The single peak from the unacidified sample eluted less than 30 seconds later than the acidified sample. The similar elution time suggested that either co-elution of the species was occurring even at a higher sample pH (unlikely), or, only one species was present because the length of storage time of the sample before analysis (almost two years) had affected speciation. It has been shown that water samples for trace metal analysis require acidification for storage purposes in order to prevent speciation change and loss via other means, such as adsorption. It is thus feasible to assume that because the sample was stored unacidifed for such a length of time, the labile fraction had been lost or possibly converted. Due to the inert nature of the other species, it remained in solution and was consequently the only species detected.

In conclusion, due to the age of the unacidified sample, it was difficult to determine whether the single peak observed was due to one species resulting from storage, or whether co-elution of both species was still occurring. Therefore, this experiment neither substantiated nor disproved the original theory. However, the separation of the two co-eluting peaks in the Antarctic sample through modifications to the eluent composition still strongly supported the existence of two aluminium species in seawater.

An alternative explanation for the difference in results for the Pacific Ocean samples obtained by FIA and HPCIC could be the effect of pH change on the preconcentration process. The pH change to 5.5 prior to preconcentration in FIA, is achieved using an ammonium acetate buffer and is required in order for aluminium to be retained optimally on R8-HQ. However, this pH change may inadvertently mean that only certain species are extracted. This assumption is supported by the complex solution chemistry of aluminium at different pH values. Figure 6.9 illustrates a species phase diagram for aluminium in a solution containing chloride (e.g. seawater) between a pH of 1-12. It can be seen that at a pH higher than 4, the speciation of aluminium becomes quite complicated with the free Al^{3+} ion no longer being the dominant species. In fact, at a pH > 4.5 the insoluble hydroxide $Al(OH)_3$, becomes the dominant species. Upadhyay and coworkers [162] also describe this dependence of aluminium speciation on pH and specifically mention the solubility minimum of aluminium in the pH range 5.5-6.5. Although this paper focuses primarily on river water a similar pH range could be expected for seawater. Since only a small fraction of labile aluminium would exist in this pH range it may be that FIA measures only this portion of aluminium, ignoring the contribution of more inert aluminium complexes.

The hydrolysis of aluminium at the pH of seawater has been discussed previously (section 1.2.1) and it is expected that insoluble hydroxides would exist. The affect this hydrolysis has on the quantification of aluminium has not, however, been discussed in detail nor has the difference in concentration of aluminium been measured between samples at different pH values.

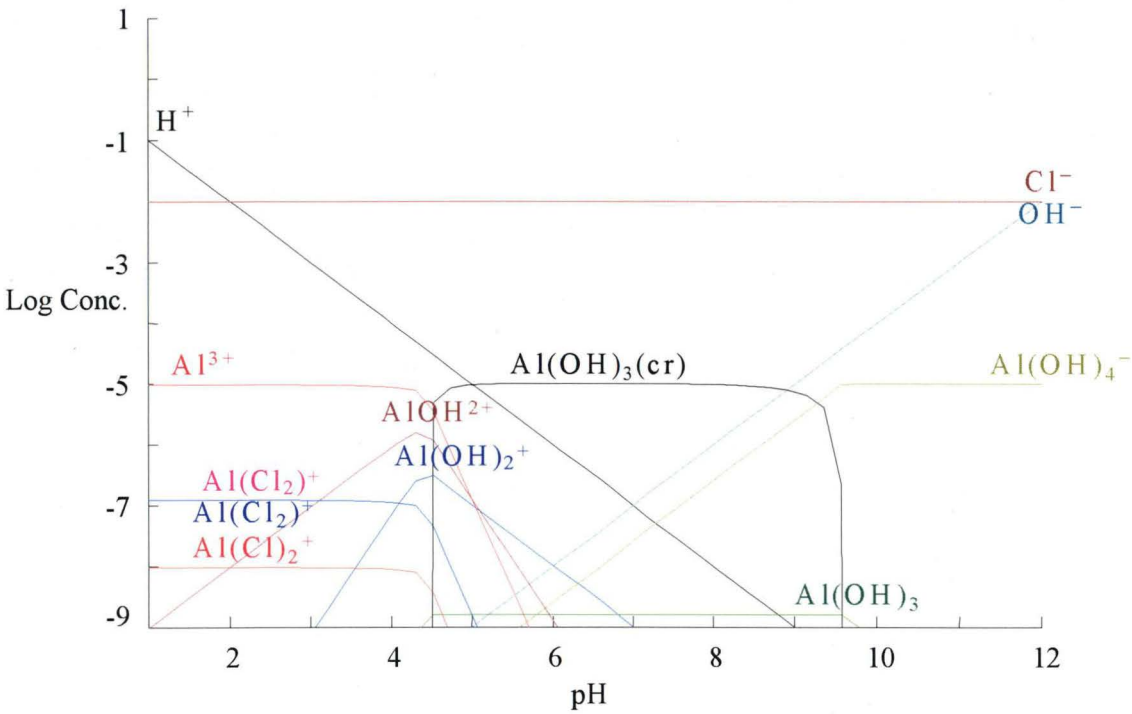

Figure. 6.9. Species phase diagram of aluminium between pH 1-12 in a solution containing chloride.

It is not the author's intent to infer that FIA incorrectly quantifies aluminium in seawater, only that the definition and identity of what is being measured should perhaps be questioned. It is obvious that pH has a significant effect on the speciation of aluminium and, in turn, those species that are quantified by particular techniques. Possible future investigations that could help in furthering understanding into the effect of pH on speciation and the differences between the two techniques are given in the following chapter. It should also be noted that the MADCOW model (used for the calculation of dust deposition estimates) has been developed specifically using dissolved aluminium concentrations obtained by FIA and therefore may still give realistic predictions. However, if differences in the aluminium content of surface seawater are indeed due to the nature of the technique employed, this model may need to be re-evaluated.

6.4 Conclusions

It has been shown that the IDAS column can handle effectively samples with a complex matrix. Peaks of aluminium from such samples compare well to standards prepared in Milli-Q water in terms of shape and chromatographic efficiency. The use of photometric detection, employing ECR as the post-column reagent, illustrated the ability of the HPCIC system to determine the concentration of the labile fraction of dissolved aluminium in paper mill process water. In addition, this work also highlighted the presence of both labile and chemically inert species of aluminium in such samples, the ability of the system to differentiate between these species, and the potential to quantify

each separately. The dependence of the retention of these species on sample pH was also demonstrated.

The applicability of the HPCIC system coupled with fluorescence detection to the determination of aluminium in seawater was also assessed. Again, peaks of good efficiency were observed, however a slight decrease in column performance was noted at injection volumes higher than 100 μL. LOD's of 1.2 nM and 0.39 nM in seawater were achieved using 200 and 500 μL sample loops, respectively. Good linearity of the system was observed between 3.7 and 37 nM additions for the 200 μL sample loop and 1.8 and 37 nM additions for a 500 μL injection volume.

Verification of the system's ability to accurately determine aluminium was attempted by comparing results obtained for two Pacific seawater samples (regarded as a "certified" reference material for aluminium in seawater by oceanographers) with those acquired using FIA. Analysis by HPCIC gave readings approximately seven times higher for both samples, in comparison to those obtained by FIA. This led to an investigation into the discrepancy between the two techniques. Findings suggested that HPCIC determined a true total dissolved aluminium content whilst FIA may not account for both labile and inert species, or at least not entirely, as a result of the mechanisms of solid phase extraction and/or the effect of sample pH on speciation.

It is appreciated that further work needs to be carried out in order to fully substantiate these theories and to validate fully the ability of the HPCIC system to accurately determine aluminium in seawater.

Chapter Seven

Conclusions and Future Work

7.1 Project Summary

The overall aim of this project was to establish a system capable of determining extremely low levels of aluminium in seawater, specifically from the Antarctic region. The most logical approach was to develop an FIA system based on that of Resing and Measures, which is used extensively within the oceanographic community. Significant effort was spent optimising conditions for the aluminium-lumogallion reaction including, reaction pH, time and lumogallion concentration. Both conventional statistics and artificial neural networks (ANN) were used to calculate these optima. However, it was found that an ANN gave the most accurate predictions and subsequently the most reliable estimates of optimal values.

The FIA system showed good reproducibility for injections of standards prepared in Milli-Q water. However, the addition of an 8-HQ preconcentration column resulted in the development of numerous problems, including excessive back-pressure, baseline instability and irreproducibility. Despite exhaustive efforts, these issues could not be alleviated to any degree of acceptability and so the decision was made to seek an alternative, more reliable technique for the purpose of this project.

This led to the development of a HPCIC system capable of detecting aluminium and subsequently the assessment of its suitability for determining aluminium in samples with complex matrices, most importantly, seawater. Given the innovative use of IDAS for the separation and preconcentration of aluminium, optimal conditions of separation were examined. Investigations were made into eluent composition, column temperature and eluent flow-rate. Photometric detection was utilised for these studies in order to concentrate on separation conditions without additional complications from the detection technique. Once separation conditions were determined, a detailed study into several different post-column reagents was undertaken in order to maximise the achievable sensitivity of photometric detection. Of the reagents examined (tiron, pyrocatechol violet, Eriochrome Cyanine R and Chrome Azurol S), ECR gave the lowest limit of detection, namely 100 nM for a 100 μL injection volume. Working conditions for this post-column reaction were 0.25 mM ECR with 1 mM CTAB, in a 0.2 M hexamine solution buffered at pH 6.1 and with detection performed at 580 nm. Linear calibration was observed over the range of 3.7 – 370 μM.

This fully optimised photometric HPCIC system utilising ECR was employed for the first injection of a sample with a complex matrix. Due to the combination of high ionic strength and extremely low concentration of aluminium in seawater, paper mill process water was considered an appropriate sample of intermediate complexity in order to gauge how the IDAS column performed with a complex sample. Work on this sample illustrated the ability of IDAS to not only elute aluminium free from interferences, but also to differentiate between inert and labile aluminium species. The concentration of the labile

aluminium species in the process water was determined to be 44.8 ± 1.8 μM. ICP-MS showed total dissolved aluminium in the unacidified sample to be 69.3 ± 1.1 μM. This value verifies the findings by the HPCIC method since the difference between the amounts measured by both techniques would have been due to the inert species of aluminium.

Following this work, a fluorescence detector was coupled to the existing HPCIC system. This was done in order to develop a detection method capable of the low limit of detection required for analysis of Antarctic seawater. Lumogallion was chosen as the fluorescent reagent and given the uniqueness of HPCIC as a technique, optimisation of parameters such as buffer type, reaction pH and temperature, was undertaken. A LOD of 0.39 nM was achieved for a 500 μL injection of seawater using 0.03 mM lumogallion in a 0.25 M MES buffer adjusted to pH 6.05 and a 2m reaction coil held at 70°C.

A significant amount of time was spent validating the HPCIC system coupled with fluorescence detection for the quantification of aluminium. This task was made difficult due to the fact that no certified reference material existed for aluminium in seawater. In the absence of such a standard, comparisons were made with results obtained by FIA for two samples of Pacific Ocean seawater. This comparison revealed that values obtained by HPCIC were approximately 7 times higher than those achieved by FIA. Multiple investigations were undertaken in an attempt to identify the reason behind this discrepancy. At the conclusion of this project the most probable explanation is that HPCIC accounts for both the labile and inert aluminium species at a sample pH of ~2.

This assumption is supported by the fact that the aluminium peak could actually be separated into two distinct peaks through changes to eluent composition. In comparison, FIA, which extracts aluminium at a sample pH of 5.5, most likely fails to quantify both species either due to variations in speciation at this pH or perhaps because the solid phase employed for preconcentration is unable to extract effectively the inert aluminium species.

7.2 Suggested Future Work

The potential of the developed HPCIC system with fluorescence detection for the determination of aluminium in seawater is evident. The only limitation in its application to oceanographic studies is the discrepancy between results obtained in comparison to the widely accepted FIA method. Differences in the species quantified by each technique, has been given as a possible explanation, however it is the author's opinion that this theory is best tested in an environment where fresh seawater is readily available and comparisons with an established and robust FIA system can be made. Ideally, this would occur shipboard during an oceanographic cruise. Considering time limitations of the project, this was not a feasible option for this particular study, nonetheless important investigations for the completion of this work are suggested below.

Firstly, it is necessary to test freshly collected seawater using HPCIC, considering the fact that the storage time of the ambient pH seawater tested during this project caused concern over the validity of the findings,. This would determine conclusively whether

two species are indeed present at the natural pH or not. It is, however, a possibility that only one species will exist at this pH given the extent of hydrolysis that occurs naturally in seawater.

Ideally, a direct comparison between HPCIC and FIA analyses, of the same sample of seawater needs to be made. This would require any sample manipulation to be identical, which may mean that modifications need to be made to both techniques in order for precisely the same aluminium species to be quantified. Given the restrictive pH range of extraction using R8-HQ, this would most likely mean changes to the solid phase used for preconcentration in FIA. Possible analysis into the differences in concentration obtained by both methods for samples at varying pH values is also suggested. This work is vitally important in order to address the issue of what forms of aluminium are being quantified by specific techniques and subsequently what concentration is required to ensure calculations of dust deposition are made with acceptable accuracy.

An alternative or concurrent investigation may be the analysis of a sample for which there is a certified reference value of aluminium content, not necessarily seawater (e.g. freshwaters). This would not only serve to help validate the HPCIC system but also expand the range of applications to which the technique can be used.

7.3 Conclusions

This project has investigated in detail two techniques for the purpose of the determination of ultra-trace aluminium in seawater. It has been shown that FIA, currently the most

widely employed method for this purpose, can suffer from severe shortcomings in terms of robustness, particularly in association with the preconcentration column. In addition, the question has been raised as to what exactly is being measured by this technique. Whilst the term 'total dissolved aluminium' is commonly used, it has been suggested through comparison with results obtained by HPCIC, that this may not be what is actually measured. It is theorised that FIA may only account for certain species in seawater and not the entire aluminium content.

In response to the issues encountered with FIA, a high performance chelation ion chromatography system has been developed successfully for the determination of aluminium. Separation and/or preconcentration is achieved through the novel use of IDAS, with conditions of separation optimised fully for aluminium. The system may be coupled with either a photometric or fluorescence detector, and employs post-column reaction. HPCIC has illustrated the presence of two separate aluminium species and the technique can differentiate between the two. A strong dependence of retention (of at least one species) on sample pH has been shown. The HPCIC system has been shown to be applicable to the determination of labile aluminium in paper mill process water. As yet, the system is not validated for the quantification of aluminium in seawater, but it exhibits excellent chromatographic performance and linear response for seawater injections.

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