CHAPTER I

INTRODUCTION

1.1 Overview and Background of This Research

There is currently much activity on the development of analytical methods for elemental speciation [1]. This is being applied in diverse fields such as medicine, agriculture, industry and the environment. While most work concentrates on determining the chemical forms of elements in samples there is also considerable interest in size-based speciation. This involves determining the distribution of elements across the size range of the sample. In the field of environmental science this is important as particle size greatly influences the transport and fate of the elements associated with particles in air, soil and aquatic environments.

Field-flow fractionation (FFF) is a technique for size separation and characterization of particles and macromolecules [2]. FFF instrumentation is similar to that used in liquid chromatography (LC). FFF takes place in a thin rectangular open channel and the separation is carried out by applying an external driving force perpendicular to the laminar flow of the carrier liquid [2]. Various driving forces can be employed in FFF (e.g. centrifugal, flow, thermal gradients, electrical and gravitational). This work used gravitational FFF (GrFFF) due to the simple experimental set up and low cost [3]. GrFFF can be set up by replacing the column in a standard high performance liquid chromatography (HPLC) system with the GrFFF channel. GrFFF is capable of separating particles in the diameter range of 2-50 μm and has been employed for biological samples such as red blood cells, parasites, wine yeast, and wheat starch [4-7].

The application of GrFFF for industrial particles has involved coal, silica and clay minerals[8-12].

Flow injection analysis (FIA) has been demonstrated to provide several advantages, including simple instrumentation and economic aspects [13-17]. FIA has been combined with other analytical techniques but not yet with FFF. This research then attempted to hyphenate FFF with FIA for size-based element distribution.

Natural aquatic and soil particles are thought to possess a coating of hydrous iron oxide and natural organic matter [18]. These coatings influence the uptake of contaminants such as trace metals, nutrients and biocides [19,20]. Thus the size distribution of readily released iron is of some importance in assessing the potential fate of some pollutants.

Combination of FFF with various analytical techniques has gained considerable interest recently [2]. This has led to size-based element distributions [21] and adsorption distributions [18,22]. This research involves the work on the hyphenated method involving gravitational steric/hyperlayer FFF and flow injection (FI) with chemiluminescence (CL) detection (GrFFF-FIA-CL). Trace amounts of iron catalyse the luminol oxidation, which emits light. In addition GrFFF coupling with off-line and on-line electrothermal AAS (ETAAS) for iron analysis was also performed.

1.2 Field-Flow Fractionation

1.2.1 Synopsis of Field-Flow Fractionation

Field-flow fractionation is an elution based separation method analogous in many ways to liquid chromatography. The FFF concept was proposed in 1966 by the late J. Calvin Giddings from the University of Utah and the principles were outlined in a journal article in 1973 [23,24]. FFF has been extensively used for separating and sizing particles and macromolecules [25-29] and has been applied to characterize a wide variety of samples including: polymers [30], soils [31], river suspended matter [32], bacteria [33], starch [34], proteins [35], blood [36] and milk [37].

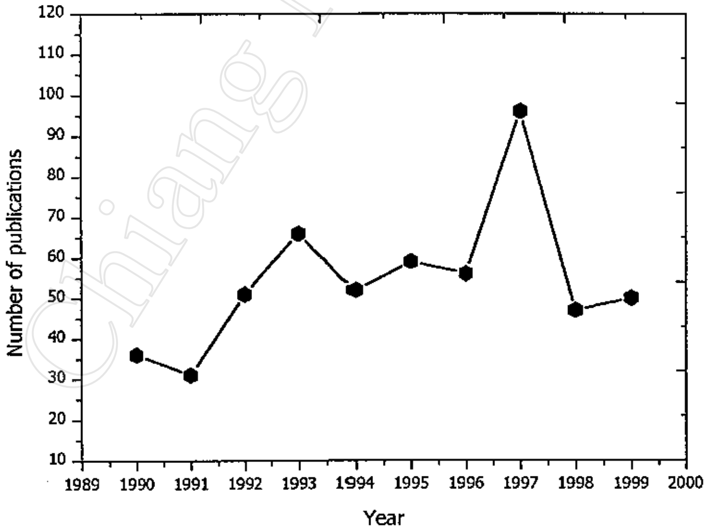


Figure 1.1 Publications on FFF in the last decade (1990-1999)

Figure 1.1 presents the number of publications on the FFF in the last decade (1990-1999). In the beginning of the decade (1990-1993) the number of publication increased to an almost constant rate of about 55 papers per year. A notable exception was the year of 1997, when the number increased dramatically to 96 due to the unfortunate passing of J. Calvin Giddings, the FFF inventor. Consequently his FFF colleagues published numerous articles in several memorial journal issues dedicated to his distinguished career.

FFF separations are based on a combination of physical principles involving the force induced on particles by an applied field or gradient and hydrodynamic laminar flow in a thin unpacked channel. Different fields and gradients can be employed in FFF such as centrifugal, electrical, magnetic, fluid crossflow, thermal gradient and acoustic standing waves. In addition back diffusion away from the accumulation wall in response to the concentration gradient may also be important. In the current experiments in this thesis the earth's gravitational field will be utilized and the particles will be too large for concentration diffusion, which causes particle transport by Brownian motion, to be significant.

1.2.2 The FFF Separation Principle

1.2.2.1 Instrument Components

Commercial FFF equipment is available (www.postnova.com) but like HPLC and GLC it is relatively expensive [38,39]. The architecture of an FFF instrument is similar to LC with the essential components being a pump, sample injection port, FFF separation channel and a detector. The difference between

FFF and LC is the mechanism of separation occurring in the channel. In most forms of LC differential migration of sample components is caused by chemical interaction with a stationary phase. Exceptions include size exclusion chromatography (SEC) and hydrodynamic chromatography (HDC). In this respect FFF is more similar to SEC and HDC, as selective migration in the channel is purely due to physical effects.

1.2.2.2 The FFF Channel

FFF separation process takes place within the channel. The conventional FFF channel is a thin, paralleled-wall duct with triangular inlet and outlet end pieces. The channel is constructed by tightly sandwiching a thin film cut as a spacer between solid blocks (see Section 2.4.1.) Typical dimensions are 20-50 cm x 1-2 cm x 0.01-0.05 cm.

1.2.2.3 Field Relaxation of the Sample

After injection of a sample suspension at one end of the channel the flow is usually turned off while the remains field on. This allows the field to force the sample particles across the channel to the accumulation wall in a process referred to as sample relaxation (see Figure 1.2). It may take only a few seconds or much longer (10–60 minutes) depending on the field strength and its interaction with the particles through the appropriate properties.

1.2.2.4 Sample Relaxation Time

The relaxation time must be sufficient for the smallest particles in the sample starting at the top wall to settle to the bottom wall (i.e. a distance equivalent to the channel thickness. By transposing the Stokes settling velocity equation for a gravitational field we obtain:

$$t_{relax} = Uw = \frac{18\eta w}{g\Delta\rho d^2} \tag{1.1}$$

where U = settling velocity,

w = channel thickness,

η = viscosity of fluid,

g = gravitational acceleration constant,

$\Delta\rho$ = density difference between the particle and the fluid,

d = equivalent spherical particle diameter.

1.2.2.5 Laminar flow in the FFF Channel

Following the relaxation period the carrier flow is initiated. Due to the thin channel, non-turbulent laminar flow results and the velocity profile is parabolic across the channel, which is shown in Figure 1.2(c). The fluid has maximum velocity at the center of the channel and is essentially zero at each wall. The fluid velocity profile is given by

$$v_{(x)} = 6 < v > \left(\frac{x}{w} - \frac{x^2}{w^2} \right) \tag{1.2}$$

Where $v_{(x)}$ is the velocity at distance x from the lower accumulation wall and $<v>$ is the mean velocity of the fluid.

For the region very close to the channel wall the second term can be neglected (i.e. $(x/w)^2 << (x/w)$), thus for $x<<w$ equation (1.2) becomes:

$$v_x \approx \frac{6<v>x}{w} \qquad (1.3)$$

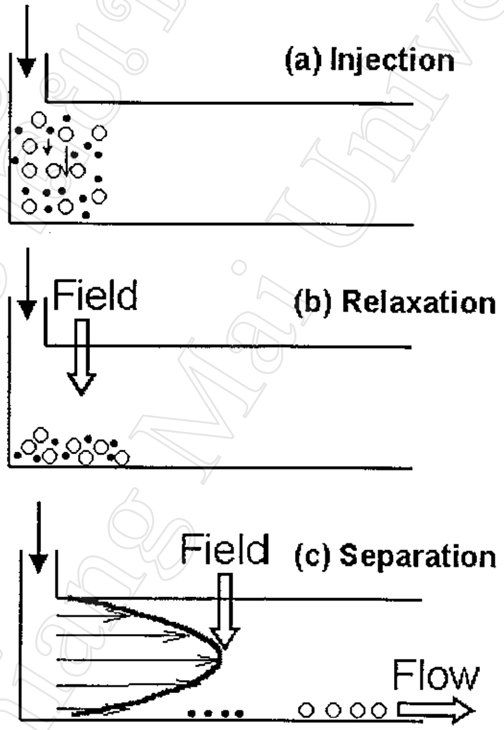


Figure 1.2 Cross-section of separation channel showing the steps involved in the separation mechanism

1.2.2.6 Steric FFF Separations

After relaxation the particles are pushed close to the accumulation wall as shown in Figure 1.2(b). For particles in the micron size-range back diffusion effects can be ignored. A simplified model for the separation of particles according to size is illustrated in Figure 1.3(a). As an approximation the particles can be imagined to be pushed along by the fluid velocity vector acting at its center (i.e. at a distance d/2 above the accumulation wall). Thus from equation (1.2) and (1.4) this velocity will be

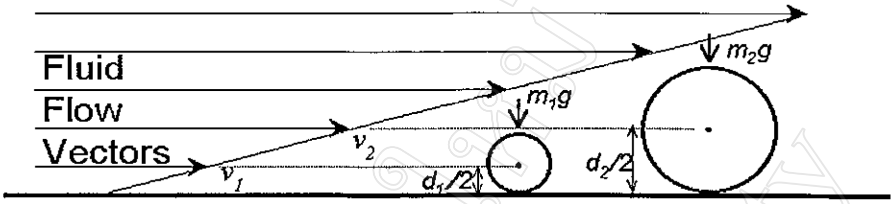
$$v_r = 6 < v > \left(\frac{d}{2w} - \frac{d^2}{4w^2} \right) \tag{1.4}$$

$$v_r \approx \frac{3 < v > d}{w} \tag{1.5}$$

This indicates that the retention volume or time required for a given component to elute should decrease as the particle size increases (Figure 1.3).

Unfortunately this simple model is not accurate due to a number of additional effects not taken into account. These perturbing influences include wall attractive and repulsive forces, near wall viscous drag and hydrodynamic lift forces [40-42].

(a) Steric GrFFF



(b) Steric/ hyperlayer GrFFF

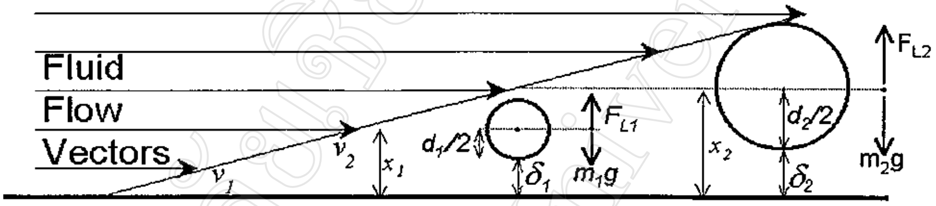


Figure 1.3 Two different modes of separation mechanism in GrFFF

This can be accounted empirically by including a correction factor γ yielding

$$v_r = \frac{3 < v > \gamma d}{w} \tag{1.6}$$

Processes pushing the particles away from the wall result in faster elution and $\gamma > 1$, whereas attractive wall forces and additional drag slows the particle migration along the channel and will result in $\gamma < 1$.

1.2.2.7 Hydrodynamic Lift Forces

When particles in the close vicinity of the accumulation wall are pushed along the channel by fluid flow they experience an upward lift force due to the increased pressure in the liquid film between the particle and the wall. This hydrodynamic lift force has been discussed in more details by Williams, et al. (1992), Williams et al. (1994) [41,42].

The lift force (F_L) decreases as the distance between the particle and the wall increases [42]. At equilibrium the particles are elevated a distance δ above the wall, where the gravitational force (mg) is balanced by the lift force (F_L) as shown in Figure 1.3(b).

1.2.2.8 Steric/Hyperlayer Mode of FFF

As the channel flow increases the particles become more elevated as the magnitude of the lift force increases. As discussed above the particle velocity, and hence the retention time and volume, will depend on the distance of the particle centre above the wall, which for particles in these hyperlayers will be at position

$$x = \delta + \frac{d}{2} \tag{1.7}$$

thus the retention volume of a given component is not constant but decreases with increasing flow rate. Consequently equations (1.4) or (1.5) cannot be used for quantitative determination of particle diameter from the measured peak position and calibration with size standards of the same particle density is required at each flow rate.

1.2.2.9 Retention Ratio

Retention of a sample is usually expressed in terms of the dimensionless retention ratio

$$R = \frac{v_r}{< v >} = \frac{t^0}{t_r} = \frac{V^0}{V_r} \qquad (1.8)$$

where v_r = fluid velocity vector at the center of the particle

t^0 = void time

V^0 = void volume

t_r = retention time

V_r = retention volume

From equation (1.6)

$$R = \frac{3\gamma d}{w} \qquad (1.9)$$

Using the approximate expression for $v_{(x)}$ (equation (1.3)) the elevation of the particle center above the wall is

$$x = \frac{wR}{6} \qquad (1.10)$$

Thus the measured retention ratio can be used to estimate x from equation (1.10) and if the particle radius is known, δ is obtained through equation (1.7).

1.3 Detection Systems Used with Field-Flow Fractionation for Size-based Speciation

Size-based speciation can be performed by combining particle size separation with various analytical methods. Separation techniques for example, high performance liquid chromatography [43,44], size exclusion chromatography (SEC) [45], field-flow fractionation, as summarized in Table 1.1 and Table 1.2 and capillary electrophoresis (CE) [46] have been reported in combination with the elemental detection systems for size-based speciation.

Field-flow fractionation has been recognized for several decades as a feasible separation technique for hyphenation with analytical detection in order to provide elemental and particle size information. Sensitive detection systems are needed due to the low particle concentrations eluted from FFF. The following sections describe the reports of the detection systems, namely inductively coupled plasma mass spectrometry, electrothermal atomic absorption spectrometry, flow injection analysis and other methods which have been used for this purpose.

1.3.1 Inductively Coupled Plasma Mass Spectrometry

FFF can be combined with inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS is sensitive and has the advantage in term of being multi elemental analysis technique. FFF-ICP-MS was first introduced as a promising analytical technique by Beckett (1991) [32]. In practice, combining FFF and

ICP-MS is relatively simple since the flow required by FFF is compatible with many the ICP-MS nebulisers. Two FFF subtechniques, sedimentation FFF (SdFFF) and flow FFF (FlFFF), have been widely combined with ICP-MS. Tables 1.1 and 1.2 summarize the studies reported on sedimentation FFF and flow FFF in combination with ICP-MS, for elemental determining size distributions.

Table 1.1 Sedimentation FFF with ICP-MS applications

Sample	*Size range*	*Element*	*Reference*
Colloidal particles	0.05-2 μm	Si, Al, Mg, Rb, Fe	Murphy, et al. (1993) [47]
Suspended river colloid and soil colloid	0.08-0.5 μm	Fe, Al, Mg	Berkel and Beckett (1996) [48]
Water-borne river particles	0.1-1 μm	Al, Fe, Mg	Contado, et al. (1997) [49]
Clay-rich surface sediment	0.1-0.6 μm	Al, Si, Fe, Mn, Cs, Cd, Cu, Pb, Zn	Hassellov, et al (1999) [50]
Soil colloid	0.05-1 μm	Fe, Al, Mg, Sr, Ti, Ba, Rb, Ce, Nd	Ranville, et al. (1999) [21]
Colloid fraction of contaminated	0.1-0.5 μm	Fe, Cu, Al, Mn, Pb	Chen, et al. (2001) [51]

Table 1.2 Flow FFF with ICP-MS applications

Sample	*Size range*	*Element*	*Reference*
Colloidal in natural water	0.1-0.5 μm	(28 elements, see reference)	Hassellov, et al. (1999)[52]
Proteins	0.08-0.5 μm	^{112}Cd, ^{114}Cd, ^{63}Cu, ^{63}Cu ^{64}Zn, ^{66}Zn, ^{127}I	Siripinyanond, et al. (1999) [53]
Soil and compost derived humic acid, colloidal organic matter in municipal waste	3790-3950 Da	Fe, Al, Mn, Cu, Zn	Amarasiriwardena, et al. (2001)[54]
Ethylene imine polymer	600000-1000000 MU.	Cu, Zn, Cd, Pb, Zr, Mo, Y, Te, Sn, As, Re, Tl	Al-Ammar, et al. (2001) [55]
Colloidal and particulate materials from river sediment core	1-15 nm	Cu, Fe, Mn, Pb, Sr, Ti, Zn	Siripinyanond, et al. (2002) [56]

1.3.2 Electrothermal Atomic Absorption Spectrometry

Electrothermal atomic absorption spectrometry (ETAAS) provides good sensitivity for elemental analysis. It was investigated as a possible detection system for FFF. ETAAS has the advantage of being capable of analysis slurries. There have some reports on the utilization ETAAS with FFF.

Contado et al. (1997) reported employing ETAAS coupled with SdFFF for size-based element speciation and compared the results with those obtained by ICP-MS. Colloid size particles from a reference standard sample of Kaolin and a natural river colloid were used. The major elements Al and Fe were analyzed in submicron size particles [49].

Chen and Beckett (2000) worked on coupling of SdFFF and ETAAS for characterizing soil and sediment colloids and a model iron oxide coated kaolin. The combined technique exhibits high-resolution particle separation capability and relatively high sensitivity for element analysis. The results obtained from ETAAS detection were compared with those obtained using ICP-MS and good agreement was found [19].

The worked report in this thesis employed ETAAS for iron analysis of silica model sample coated with goethite [57].

ETAAS has the advantage of good sensitivity however single element for the analysis for measurement. However, usually only a single element can be analyzed in each FFF runs. The multi element capability of ICP-MS and ICP-AES in a distinct advantage in this respect however, ETAAS is a cheaper and more common instrument than ICP-MS.

1.3.3 Flow Injection Analysis

The use of flow injection analysis (FIA) after FFF separation is of interest reported by Chantiwas, et al. (2001) [58]. Flow injection is quite a simple and cost effective analytical method. In this thesis of the combination of gravitational field-flow fractionation (GrFFF) and reverse flow injection analysis with chemiluminescence (FIA-CL) would then be attempted. It would also lead to size-based iron speciation. The FFF was used for separation of particle sizes, the rFIA-CL for iron determination, the CL analysis and is based on the catalytic reaction of alkali luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) and hydrogen peroxide [59-63].

Previous studies using flow injection analysis with chemiluminescence (FIA-CL) for iron determination are summarized in Table 1.3.

Table 1.3 Summary of iron determination by FIA-CL

Analysts	*CL-Conditions**	*Linear range (Detection limit)*	*Sample*	*Reference*
Fe(II), Fe(III) and Total Fe	5 mM Luminol, 0.5 M NH_4Cl	10^{-9}-10^{-6} M ($2x10^{-8}$M, Fe(III))	-	Sarantonis, et al. (1986) [64]
Fe(II), Fe(III)	Luminol, Ti(II), carbonate buffer	(10^{-9} M)	-	Alwarthan, et al. (1987) [65]
Fe(II)	Luminol, H_2O_2 with 8-quinolinol immobilized	(10^{-13} M)	water	Alwarthan, et al. (1990) [66]
Fe(II), Fe(III)	1 mM alkaline BSF, H_2O_2	(0.45 nM)	seawater	Virignia, et at (1991) [67]
Fe(II), total dissolved iron	Luminol-KIO_4, H_3BO_4-NaOH	$1.0x10^{-4}$-$5x10^{-6}$M ($3x10^{-6}$ M)	groundwater	Obata, et al. (1993) [63]
Fe(III)	Lumiol, 0.4 M NH_4OH, H_2O_2	(0.05 nM)	oceanic water	Zhang, et al. (1996) [68]
Fe(II), Fe(III)	Luminol-dissolved oxygen, EDTA	10^{-9}-10^{-5} M ($3.5x10^{-10}$M Fe(III)), ($2.7x10^{-10}$M, Fe(II))	-	Zhou, et al. (1997) [60]
Dissolved Fe (II+III)	Luminol, 8-quinolinol	(40 pM, 3σ)	seawater	Bowie, et at (1998) [61]
Fe(II), Total Fe	Luminol enhanced TTAB, 10^{-2} M H_2O_2, borate buffer	10^{-9}-10^{-6} M ($5x10^{-11}$M, 3 S/N)	natural hair and river water	Saitoh et al. (1998) [69]
Fe(II), Fe(II)	0.25 M immobilized Luminol	10^{-9}-10^{-6} M	water	Qin, et al. (1998) [62]
Fe(III)	Luminol-TETA, 8-quinolinol	0.25-2 nM	seawater	de Jong, et al. (1998) [70]
Fe(II+III)	Luminol and CTAB	(40 pM, 3σ)	seawater	Cannizzaro, et al. (2000) [71]

*Abbreviations:
Luminol: 5-amino-2,3-dihydrophthalazine-1,4-dione
BSF: Brilliant Sulfoflavin (4-amino-N-(p-tolyl)-naphthalimide-3-sulfonate)
EDTA: ethylenediamine
TTAB: tetradecyltrimethylammonium bromide
TETA: triethylene tretamine
CTAB: cetylmethylammonium bromide

1.4 Research Aims

This thesis aims to develop analytical systems combining FFF with FIA and with ETAAS, which should be simple and cost-effective for particle size separation and elemental determination. The overall objective of the research was to develop an effective method for size based Fe speciation in environmental and industrial particles.

The specifications were:

1. To develop a simple GrFFF system composed of easily available components.
2. To test performance of the GrFFF using model samples of silica gel and chromatographic silica particles.
3. To develop a rFIA system using a commercial flow through liquid scintillation counter as a chemiluminescence detector for iron, using the luminol-hydrogen peroxide reaction.
4. To combine GrFFF with rFIA to produce a hyphenated analytical system for particle separation and elemental determination.
5. To test GrFFF-rFIA-CL method for size-based speciation of iron using a model sample of goethite coated silica particles.

6. To develop the combination the GrFFF with ETAAS both off-line and on-line speciation for use in determining the size-based speciation of particles.
7. To apply the developed hyphenated systems to real clay mineral samples.