CHAPTER II

EXPERIMENTAL

This chapter presents information on chemicals and reagent preparation. Methods used for sample preparation and instrumentation are also given.

2.1 Chemicals and Reagents

Chemicals and reagents were obtained from various suppliers, as shown in Table 2.1.

Table 2.1 List of chemicals

Chemical	**Supplier**
Sodium hydroxide; $NaOH$	BHD (Poole, England)
3-aminophthalhydrazide(Luminol); $C_8H_7N_3O_2$	Sigma-Aldrich (Steinheim, Germany)
di-Sodium hydrogen phosphate; Na_2HPO_4	Carlo Erba (Milano, Italy)
Hydrogen peroxide (30% m/m); H_2O_2	Carlo Erba (Milano, Italy)
Iron Nitrate; $Fe(NO_3)_3 9{\cdot}H_2O$	BHD (Poole, England)
Nitric acid (70% w/w); HNO_3	J. T. Baker (Phillipsburg, USA)
Standard solutions; Iron, Manganese, Zinc, Copper, Lead (1000 mg L^{-1})	BDH SpectrosoL® (Poole, England)

Preparation of Reagents and Standard Solutions

All chemicals were analytical grade and were used without further purification. All solutions were prepared using deionized water obtained from a

Milli-Q system (Milipore, Milford, Massachusetts, USA). Glassware and containers used were soaked in 10% (v/v) nitric acid, then rinsed with distilled and deionized water before use.

1. Sodium hydroxide solution (10^{-4} M)

Sodium hydroxide (4 g) was weighed and was dissolved with deionized water and diluted to 500 mL. The solution was then diluted to be 10^{-4} M with deionized water.

2. Stock solution of luminol (0.01 M)

The stock solution was prepared by dissolving 5-Amino-2,3-dihydro-1,4-phthalazinedione (0.1772 g) in 100 mL of phosphate buffer. Fresh stock was prepared once a month. Luminol solutions of appropriate concentrations were further diluted with the phosphate buffer.

3. Phosphate buffer (0.01M, pH 11)

Di-sodium hydrogen phosphate (1.496 g) was dissolved in deionized water and diluted to almost 1000 mL. The pH of solution was adjusted to 11 by adding 2 M NaOH. The solution was then made up to 1000 mL.

4. Hydrogen peroxide solution (10^{-3} M)

Hydrogen peroxide solution (10^{-3} M) was obtained by diluting H_2O_2 (8.8 M), supplied from the manufacturer, in phosphate buffer (0.01M).

5. Standard solution for calibration

The purchased standard solutions of 1000 mg L^{-1} of iron, manganese, zinc, copper, lead were diluted to the desired concentration.

All reagents were freshly prepared and degassed for 12-15 min by using a sonication bath before use.

2.2 Equipment

1. A Satorius balance model BP201S (Goettingen, Germany) was used for weighing requirement of the accurate amounts.
2. A Radiometer A/S model PHM61 (Copenhagen, Denmark) pH meter with a combined glass electrode was used for all pH measurements. Commercial standard buffers, pH 7.00±0.01 and 9.00±0.01, were employed for the calibration.
3. An isocratic pump (SpectraSERIES Model P100, USA) was used to deliver the carrier solution in the GrFFF system.
4. A home made injection port (Chiang Mai, Thailand) made from lucite plastic was used for sample introduction into the GrFFF channel via a septum rubber seal.
3. A hypodermic syringe (25 μL) (SGE Part no. 038110, Australia) was used to introduce the particle suspensions into the GrFFF channel.
4. A UV detector (Linear Instruments Model 200 Variable-Wavelength UV/Vis Detector, USA) at an operating wavelength of 254 nm was used to monitor particles in the GrFFF eluent.

5. A fraction collector (ISCO, Inc. Model RETRIEVER 500, Lincoln, USA.) was employed to collect the eluted particles from the FFF.
6. A six-port injection valve (Upchurch Scientific, Inc. Model V-451, USA) was employed to introduce a plug of reagent for the FI system.
7. A three-way switching valve (Upchurch Scientific, Inc. Model V100T, USA) was used for bypass of the carrier in the GrFFF system.
8. A peristaltic pump (Eyela SPM 23, Japan) was used to propel the carrier streams in the FI system.
9. Packard Radiometric Flo-one®\Beta Series A-100 Model A140K counter (Canberra, USA) with a spiral flow cell was modified for use as the chemiluminescence detector for monitoring chemiluminescence.
10. The flow cell (accessory of the Packard Radiometric Flo-one®\Beta Series A-100 Model A140K), Quick Change Flow Cell (serial number 7144) had a spiral shape and was sandwiched between the two photomultiplier tubes.
11. IBM Proprinter II (The Netherlands) was connected with the chemiluminescence detector for data read out.
12. The electrothermal atomic absorption spectrophotometer (ETAAS) was a Perkin-Elmer Model 5100 (Norwalk, Connecticut, USA.) equipped with Zeeman correction, an HGA-600 graphite furnace and AS-60 autosampler. A pyrolytically coated graphite tube with an L'vov platform was used.
13. A sonication bath (Ultrasonic Cleaner Model 8890, Cole-Palmer®, USA) was used to degas and for particle suspension dis-aggregation.
14. A Pocket Sampler (Dick Smith, Australia) with software supplied was employed as interfacing device for digitizing the GrFFF detection signal.

15. A solenoid valve (P/N 9830062, Cole-Palmer® Instrument, Inc., USA) was employed to introduce a plug of luminol to the FI system in term of time based injection.
16. The flame atomic absorption spectrometer (FAAS) was a Perkin Elmer Model 1100(Norwalk, Connecticut, USA.). The instrument equipped with deuterium background correction.
17. An IKA-VIBRO-FIX, IKA shaker (Germany), was used for agitation the particles suspensions.

2.3 Samples Preparation

2.3.1 Preparation of Silica Gel Sample with Specific Size Ranges

The silica gel 60G (5-40 μm) from Merck, Germany for thin layer chromatography was used as a sample. The particles were irregular in shape and samples were prepared with size ranges of <10 μm and 10-20 μm (using gravitational settling). The method employs Stokes' equation:

$$t_s = \frac{18\eta w}{d^2 \Delta \rho g} \qquad (2.1)$$

where t_s : Settling time /min,

η : Viscosity of carrier liquid /Poise,

w: Depth of liquid of interest /cm,

d : Particle diameter /cm,

$\Delta\rho$: Difference in density between particle and liquid,

g: Gravitational acceleration constant /980 cm s^{-2}.

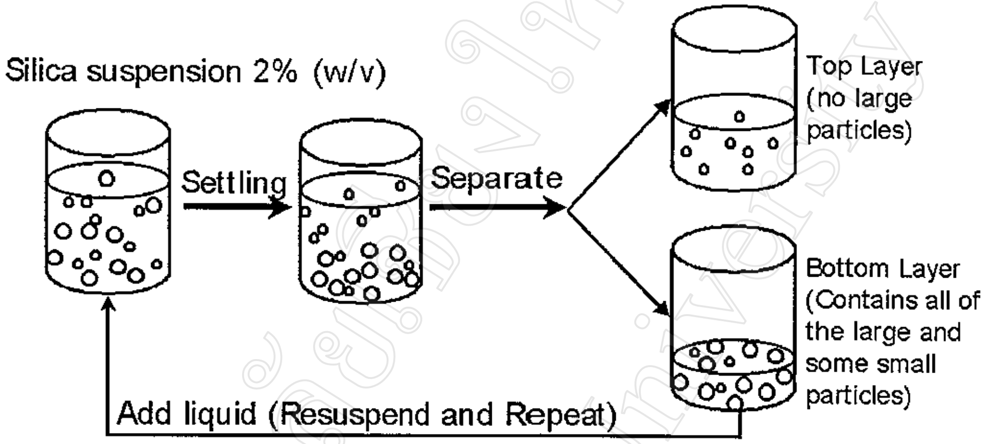

Figure 2.1 Simplified diagram of procedure of repeated settling method

The suspension of silica particles was stirred in a beaker (600 mL) then allowed to stand for a calculated settling time. The suspension was separated using a plastic syringe (100 mL). The top layer containing no large particles was transferred to another container. The bottom layer contains all of the larger particles but repeated settling is required to get rid of all of the small particles. The suspension is topped up with liquid (the same amount as the top layer was removed) and resuspended and the settling process is repeated.

In this work the suspension of silica was 2 %, w/v. The calculation of the settling time (t_s) was carried out using the following parameters; viscosity of water 0.01 Poise, depth of liquid removed 5 cm, density of silica particles 1.5 g mL^{-1} density of water 1.0 g mL^{-1}. As an example, the silica was separated at a cut off diameter of 20 µm which required 7.39 min settling time. The settling-separation cycle was repeated 10 times with the top fractions being combined.

Optical microscope photos of the original sample and two fractions are shown Figure 2.2. These demonstrate that the settling method was efficient.

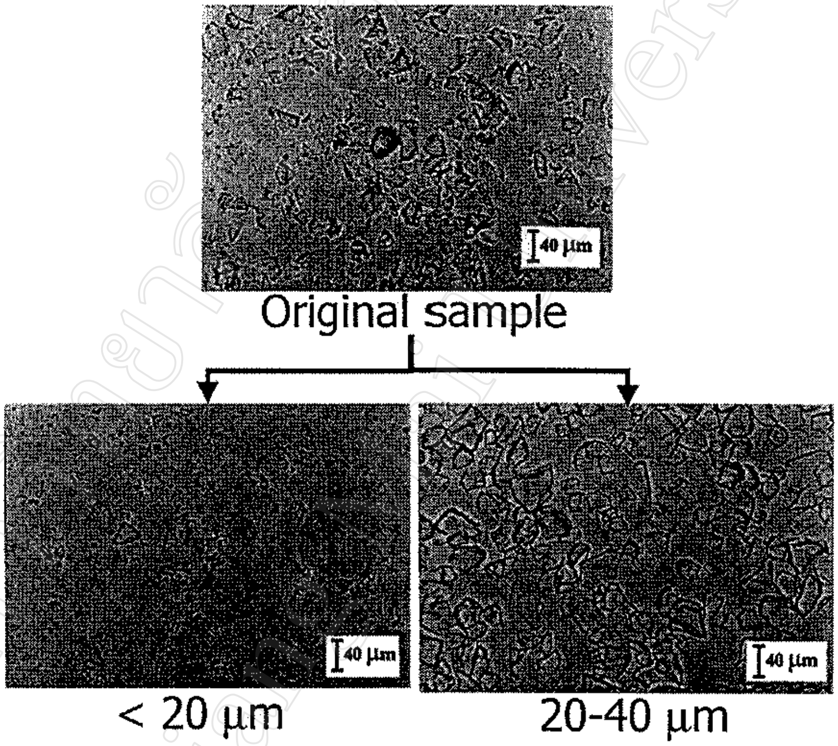

Figure 2.2 Optical microscopy photos of the original silica sample and the <20 µm fractions obtained by repeated settling

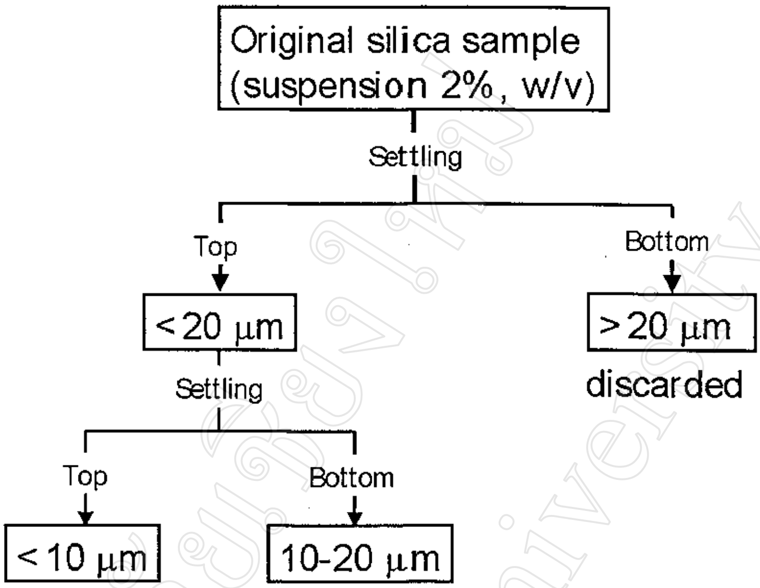

Figure 2.3 Schematic diagram of the method used for preparation of silica gel sample with size range <10 µm and 10-20 µm

In this work the settling method used to prepare the specific size ranges of the samples used for testing the performance of the GrFFF system is depicted in Figure 2.3. First the <20 µm fraction was prepared as described above. This was then divided into <10 µm and 10-20 µm fractions by repeated settling using a settling time of 30.6 min and a sample depth of 5 cm.

2.3.1 Chromatographic Silica (5 and 10 µm)

Chromatographic silica was obtained from used HPLC columns (CN packing from PARTISPHERE RTF Columns, Whatman, UK). The 5 µm particles were spherical and the 10 µm particles had irregular shapes. The particle suspension was prepared for about 2 mg mL^{-1}.

2.3.2 Preparation of the Goethite Coated Silica Particles

The goethite (FeOOH) coated silica was synthesized by a method developed based on the Fe adsorption/precipitation reported studies by Stumm and O'Melia [72]. It was used previously for the production of goethite coated kaolinite [19, 51]. A solution of iron (3.5 mM $Fe(NO_3)_3$) was gradually added to 10 mL of a suspension of silica particles (2 mg mL^{-1}). The pH was continually adjusted to 3.3 with 2 M HCl. The Fe solution addition rate was 1 mL h^{-1} for the first 2 mL and 6 mL h^{-1} for the remaining 12 mL. The mixture was continuously stirred. The suspension was then centrifuged and the silica residue was washed 4 times with Milli-Q water. The final suspension was dispersed in 10^{-4} M NaOH solution and heated in an oven at 60 °C for 24 h to convert the hydrous iron oxide coating to goethite. This step was adapted from the commonly used method of synthesis of pure goethite [73].

2.3.3 Preparation of Clay Samples

Clay samples were obtained from different provinces in Thailand. The clays referred to Kaolin Clay (Lampang province), Red Clay (Kanchanaburi province), Ball Clay 1 and Ball Clay 2 (Payao province). The samples were kindly supplied from Dr. Ponlayuth Sooksamiti from the Office of the Mineral Resources (Region III), Chiang Mai. These clays were dry-sieved through a 45 μm sieve and dried further at 60°C for 12 h in an oven and stored in a desiccator until required.

2.3.4 Digestion of Samples

Solid particle samples (0.0500 g) were accurately weighed in a test tube (50 mL). Acid dissolution was achieved by adding 4 mL of aqua regia ($HCl:HNO_3$ 3:1 v/v) into the test tube then the mixture was heated by using a heating block (120-150 ^{o}C) until nearly dried. More aqua regia (8 mL) was gradually added and further heated until an almost clear liquid was obtained. The solution was made up to 50.00 mL.

2.4 Instrument Set up

This section describes the set ups of instruments used in this research.

2.4.1 Gravitational FFF System

Channel Construction

Two 1 cm thick clear lucite plastic blocks were cut to 40 x 8 cm and 22 bolt holes were drilled as shown in Figure 2.4(a). In addition, two holes were drilled and tapped with a ¼ inch 28 thread to take a standard flange-free nut for connecting 1/16 inch od tubing to the inlet and outlet of the channel.

A simple GrFFF channel can be assembled by using an overhead transparency sheet and two pieces of lucite blocks. The transparency sheet, usually about 0.01 cm thick, can be cut to be a spacer as shown in Figure 2.4(b).

This is clamped between the two lucite blocks so that the central hole will form the channel. Other plastic sheets can be used such as Teflon or Mylar and the thickness is usually 0.1-0.5 mm. Generally, thinner channels result in better resolution.

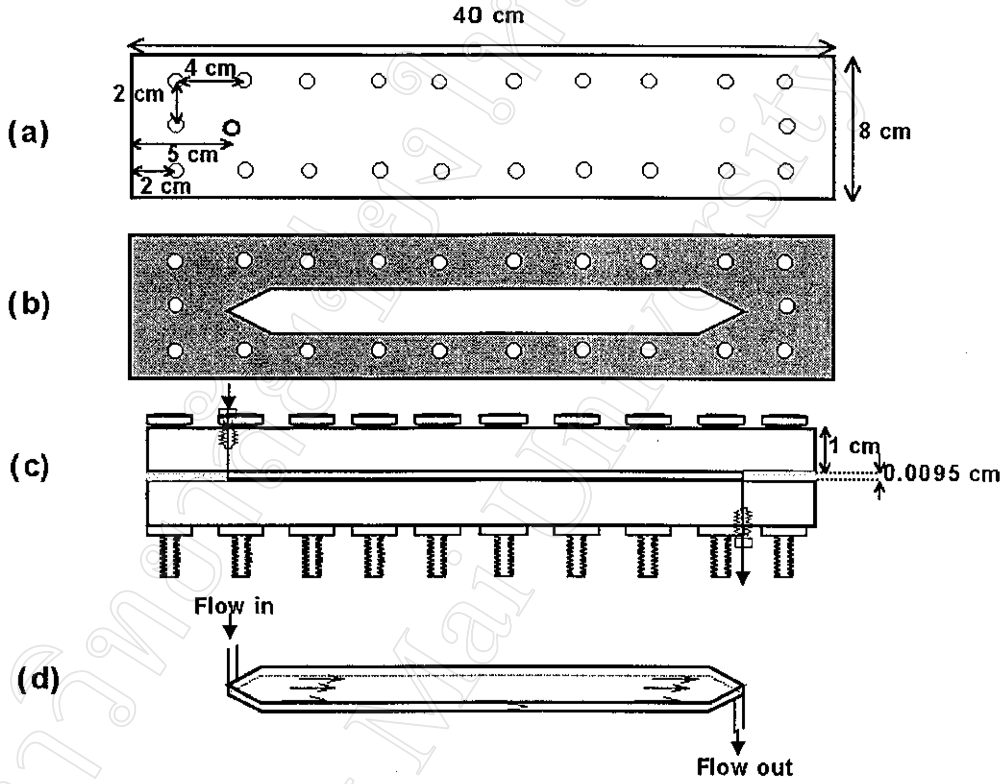

Figure 2.4 Components of a gravitational FFF channel

Carrier was pumped through the system, the suspension of particle was injected as a plug via the home made injection port through the silicone rubber septum. The 3-way valves switching were simultaneously switched manually to bypass the carrier flow around the channel if stop-flow relaxation of the particles is needed (see Figure 2.5).

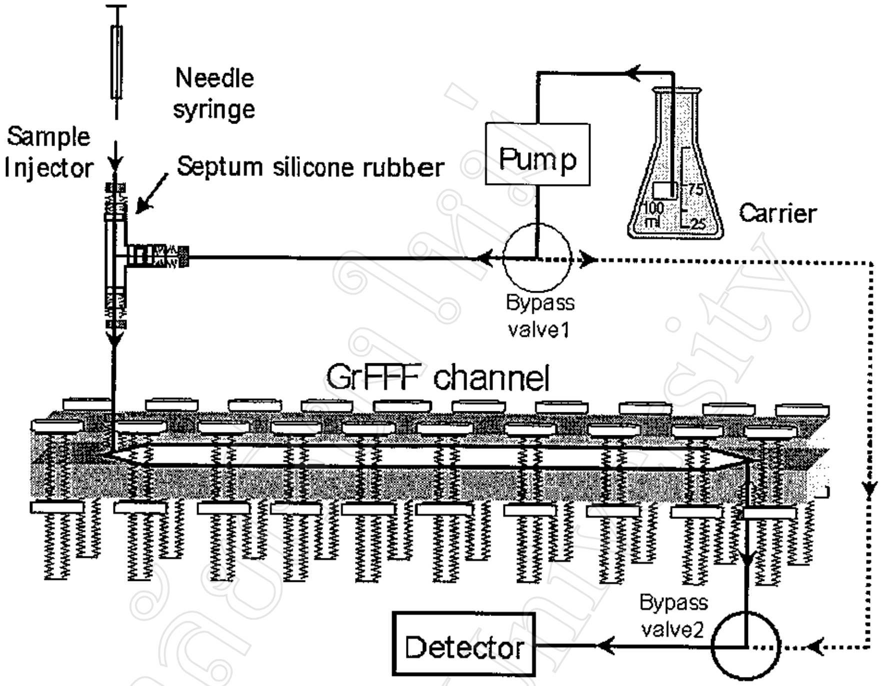

Figure 2.5 Schematic diagram of a GrFFF system showing the channel and auxiliary equipment. Arrow lines indicate the flow directions in different positions

2.4.2 Reverse Flow Injection Analysis with Chemiluminescence Detection (r-FIA-CL) for Trace Iron Determination

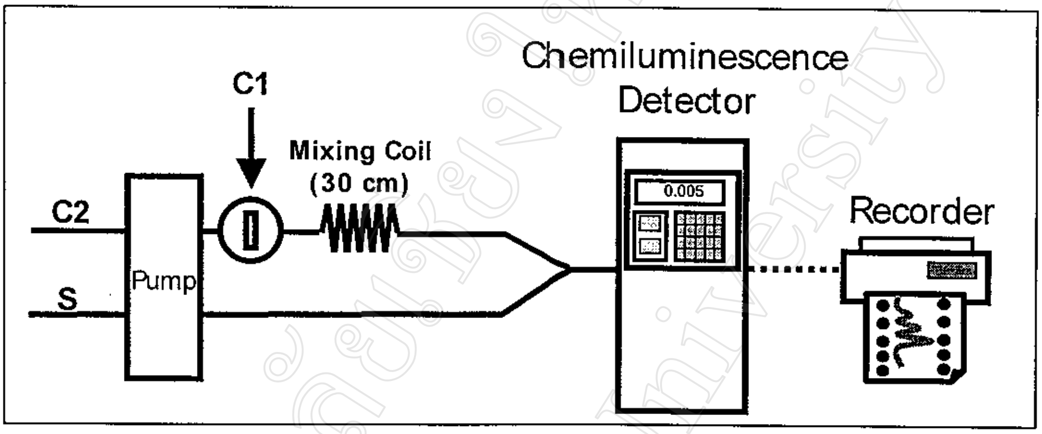

Figure 2.6 Schematic diagram of a reverse flow injection system with chemiluminescence detection for trace iron determination, the reagents used were C1 8×10^{-6} M luminol, C2 10^{-3} M H_2O_2 (Flow rate 1.0 mL min^{-1}), S: Sample/standard of trace iron (Flow rate 1.0 mL min^{-1})

The chemiluminescence detector was equipped with a coincident circuit, (Packard Radiometric Flo-one®\Beta Series A-100 Model A140K), and recorder (Printer, IBM Proprinter II). Using the rFIA-CL system (Figure 2.6), a standard or sample was continuously pumped and forced to merge with an H_2O_2 stream. An aliquot of a buffered luminol solution was injected into the H_2O_2 stream via an injection valve placed before the junction with the sample. The merged stream then passed through the detector where the chemiluminescence light was continuously monitored.

2.4.3 Combination System of GrFFF and r-FIA-CL Detection

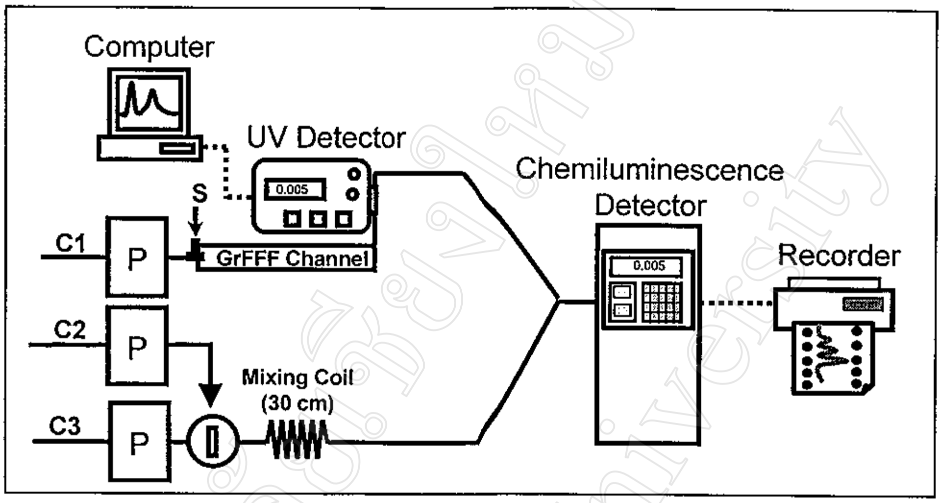

Figure 2.7 Schematic diagram of the GrFFF coupled with rFIA-CL for iron determination. The reagents used were C1: 10^{-4} M NaOH (Flow rate 1.00 mL min^{-1}), C2: $8x10^{-6}$ M luminol (Flow rate 1.0 mL min^{-1}), C3: 10^{-3} M H_2O_2 (Flow rate 3.0 mL min^{-1})

For the GrFFF-rFIA-CL system (Figure 2.7) the H_2O_2 was mixed with a plug of luminol which was pumped rather than the luminol being injected at regular intervals as in the previous section. The FFF eluent was first passed through a UV detector and then merged with the stream of the mixture of reagents, followed immediately by CL detection.

2.4.4 Combining System of GrFFF and ETAAS Detection

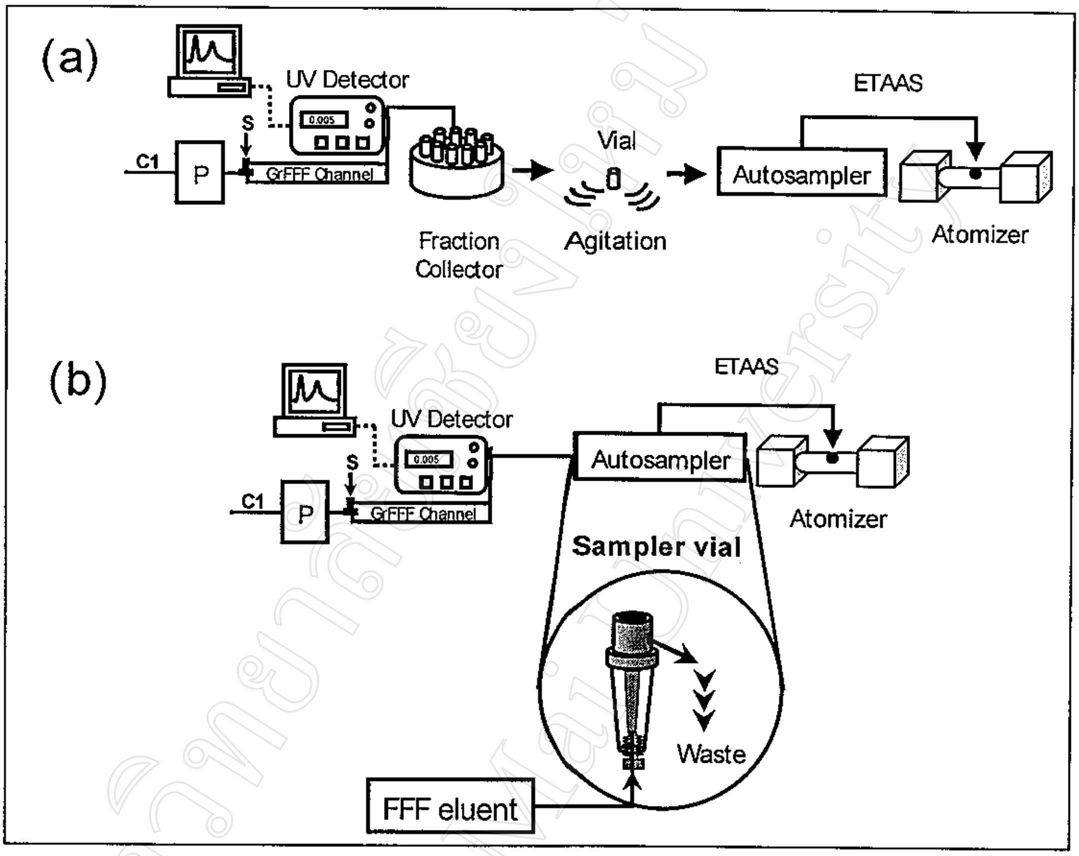

Figure 2.8 Schematic diagram of the GrFFF coupled with ETAAS, C1: 10^{-4} M NaOH (Flow rate 0.20 mL min^{-1}), (a) Off-line GrFFF-ETAAS, (b) On-line GrFFF-ETAAS

Figure 2.8(a) indicates the off-line analysis with ETAAS done by collecting the fractions of the GrFFF eluent via a fraction collector. All fractions were homogenized with a vortex mixer before introduction as a slurry into atomizer of the ETAAS system for iron determination.

Figure 2.8(b) shows a diagram of on-line GrFFF with ETAAS detection. The eluent of the GrFFF was passed into a flow through sampler vial (Chiang Mai, Thailand) mounted in the autosampler of the ETAAS. The vial design was a modified version that used by Chen and Beckett (2001) [51]. The FFF eluent was connected to the bottom sampler vial, via the nut with ferrule (Figure 2.8). The vial was designed to take the eluent continuously and overflow to waste (see arrow direction). The modified sampler vial was connected to the GrFFF eluent with a ¼ inch 28 thread to take the standard flange free fitting for connecting $^{1}/_{16}$ inch od to inlet (outlet of GrFFF) of the continuous flowing. The vial sampler was put into the tray of the autosampler unit of the ETAAS instrument where was programmed to sample only from the GrFFF eluent. This one position particle fractions were directly introduced as a slurry on-line into the ETAAS system for iron determination. Therefore, the ETAAS measurement can be performed on-line.

2.4.5 Electrothermal AAS Instruments and Conditions

A calibration with iron standards (20, 40, 60, and 80 μg L^{-1}) was performed. Table 2.2 gives the temperature program used for the Fe analyses. A wavelength of 248.3 nm, slit width of 0.2 nm, operating lamp current of 30 mA and sample volume of 10 μL were employed.

Table 2.2 Graphite furnace temperature program 1 used for Fe analysis

Step	Furnace Temperature(°C)	Ramp Time (s)	Hold Time (s)	Internal Ar gas flow (mL min^{-1})
1	110	1	15	300
2	130	7	5	300
3	20	7	10	300
4	1000	1	10	300
5	2400	2	15	0
6	2600	1	5	300

In this work, the fast heating program 2 for the GFAAS shown in Table 2.3, was also used to speed up the run cycle of the analysis. This enables more analysis data to be collected across the GrFFF fractogram.

Table 2.3 Fast heating graphite furnace temperature program 2

Step	Furnace Temperature(°C)	Ramp Time (s)	Hold Time (s)	Internal Ar gas flow (mL min^{-1})
1	140	1	15	300
2	2400	3	10	0
3	2600	1	5	300