CHAPTER 1

INTRODUCTION

1.1 Iron

1.1.1 The importance of iron determination

Iron is predominantly bivalent and trivalent in its compounds. It is usually of interest to evaluate the redox characteristics of the environment of natural waters [1], by considering the Fe(II) and Fe(III) concentration ratio. Its redox and physiological properties make it an important component of the biogeochemical cycles of elements such as carbon, sulfur and oxygen. Its reactivity also drives numerous chemical processes in natural waters, and it is a significant factor in the evaluation of water quality [2]. Fe(III) is the thermodynamically stable form in oxygenated water, existing predominantly as insoluble oxy-hydroxides or colloidal matter. Fe(II) is a transient species in surface oxic waters, existing via chemical or photochemical Fe(III) reduction, or via atmospheric deposition [3]. So the determination of iron is of great importance for guiding the marine scientists to control the amount of iron in natural water.

Iron is an essential micronutrient for organisms and in certain high-nutrient, low-chlorophyll areas of the world's oceans; iron appears to limit phytoplankton growth, which may have important implications for global carbon cycle [3]. Iron determination is then of important role in such a study. The amount of iron is important for controlling this cycle.

In human body iron is an essential mineral. The average adult has 4-5 g of iron, of which 60-70% is present as haem in the circulating haemoglobin, and the remainder present in various enzymes (e.g. catalase, cytochrome oxidase), in muscle myoglobin or stored. About 15% of the iron is stored in the liver as ferritin, in the other tissue as haemosiderin, and as the blood transport complex called transferin (average blood level 50-180 μg per 100 ml plasma). A human body loses iron 0.5-1.5 mg per day (in faeces 0.3-0.5 mg per day, in sweat as skin cells 0.5 mg, traces in hair and urine) so the sufficient amount should be intaken (12 mg for adults, 15 mg during pregnancy and lactation and for adolescents, 7.5-10.5 mg for children, rising to 13.5 mg in 11-14 year-old group). The human body cannot absorb all of intaken iron. Only 0.5-1.5 mg of iron from a diet containing 10-15 mg can be absorbed. Absorption of iron in the body is aided by vitamin C and reduced by phosphate and phytic acid [4]. Iron deficiencies are particularly common in premenpausal woman, owing to loss through menstruation, and often results in chronic anemia. Even college-age women should pay particular attention to the amount of iron in their diets [5]. However, one may take iron supplement from iron tablets, which are in various forms of iron compounds such as fumalate, gluconate, sulphate and citrate. An assay of iron contents such in a pharmaceutical preparation is essential.

1.1.2 Methods for iron determination

Several methods for the determination of iron have been proposed. Most of them involve spectrophotometry, chemiluminescence, atomic absorption spectrophotometry (AAS), inductively coupled plasma-atomic emission spectrophotometry (ICP-AES), or voltammetry. Of these methods, some can

determine iron in the form of Fe(II) only such as spectrophotometry using 1,10-phenanthroline except that Fe(III) is reduced with some reducing agent to give Fe(II) first and then complex with 1,10-phenanthroline. Some methods can determine iron in the form of Fe(III) such as spectrophotometry using thiocyanate (SCN^-) as a color developing agent. And some can determine total iron such as AAS and ICP-AES. The separation techniques such as ion chromatography and capillary electrophoresis can determine both forms of iron simultaneously leading to speciation of iron species.

Polarography and voltammetry are techniques, in which multi-ions can be determined simultaneously, and are often used for simultaneous determination of Fe (II) and Fe(III). Polarography and voltammetry are the world wide techniques for determination of metal ions including Fe(II) and Fe(III). These techniques involve the study of current-voltage relationships at a mercury electrode under certain controlled conditions. Investigation of Fe(II) and Fe(III) in various samples using various modes of voltammetry have been reported [6-14]. Some are for simultaneous determination of Fe(II), Fe(III) and total iron in standard rocks [6]. Pulse polarography for determination of Fe(II), Fe(III) and copper(II) in process samples was reported [7]. Simultaneous determination of Fe(II) and Fe(III) at micromolar concentrations was possible [8].

The preliminary studies of iron speciation in peat samples using polarography were made. Current-sampled (tast) polarography was used in the determination of the two iron oxidation states in peat samples [10]. The polarographic determination of Fe (II), Fe(III) and total iron was also studied in a zinc plant [11]. Determination of Fe (II) and Fe(III) could be made by differential pulse polarography in solutions containing ammonium tartrate and a good buffer [12]. An analytical procedure for the

determination of Fe(III) and total iron in wines based on adsorptive stripping voltammetry was described. Fe(III) was determined by using Solochrome Violet Red as chelating agent while catechol was used for the determination of the total iron content [14].

Capillary electrophoresis (CE) is being increasingly applied in the determination of alkali, alkaline earth and transition metal ions, primarily because of its great flexibility and easy implementation [15].

The process of electrophoresis is defined as 'the differential movement or migration of ions by attraction or repulsion in an electric field'. In practical terms, positive (anode) and negative (cathode) electrodes are placed in a solution containing ions. Then when a voltage is applied across the electrodes, solute ions of different charges, i.e., anions and cations, will move with the buffer flow towards the cathode (electroosmotic flow) but with different rates due to their attractions to opposite electrodes (electrophoretic flow). Capillary electrophoresis, then, is the technique of performing electrophoresis in buffer filled, narrow-bore capillaries, normally from 25 to 100 μm in internal diameter (i.d.) [16].

There are some works in the field of capillary electrophoresis, which are concerned with the separation and determination of Fe(II) and Fe(III). Using CE, direct determination of Fe(II), Fe(III) and total iron as UV-absorbing complexes using o-phenanthroline and EDTA as complexing agents has been reported [15]. Also capillary zone electrophoresis (CZE) was applied for the simultaneous determination of Fe(II) and Fe(III) selectively complexed with 1,10-phenanthroline and trans-cyclohexane-1,2-diaminetetraacetic acid (CDTA) [17]. Micellar electrokinetic chromatographic separation of Fe(II) and Fe(III) as 2-pyridylazo chelates was

achieved [18]. A more sensitive way to determine iron using an Fe(II)-1,10-phenanthroline complex and capillary electrophoresis was proposed [19].

Flow injection analysis (FIA) has increasingly been used in various fields, owing to its high throughput, cost performance and versatility. It has also been found very useful for speciation studies [22]. Two flow injection analysis systems have been developed for the simultaneous spectrophotometric determination of Fe(III) and total iron using Tiron as the color-developing reagent. The first system used a single detector with two flow cells aligned with the same optical path to yield two peaks corresponding to Fe(III) and total iron in the sample. The second system is a multi-detector system [22]. An approach for speciation of Fe(II) and Fe(III) based on integration of retention of the Fe(II)-SCN complex with detection using a conventional spectrophotometer was proposed. The device (namely, a flow-cell packed with an exchange resin) has been coupled to a flow-injection manifold with inner-couple injection valves which enables discrimination between Fe(II) and Fe(III) taking advantage of a redox minicolumn housed in the loop of one of the valves [23].

A flow-injection analysis system has been optimized for the analysis of iron in waters with high in dissolved organic carbon [24]. The method detects either dissolved Fe(II) or total dissolved iron. Simultaneous speciation of aluminium and iron using a flow-injection system was reported [25]. Oxine was used as a reagent for aluminium and Fe(III) while 1,10-phenanthroline in combination with iodide as the reagent for Fe(II).

A chemiluminescence flow system has been developed for the determination of Fe(II) and Fe(III) [1,3,26-28]. Trace amounts of Fe(II) were determined by measuring Fe(II)-catalysed light emission from luminol oxidation by oxygen [26]. A

flow-injection method was proposed for the determination of Fe(II) and for the simultaneous determination of Fe(II) and total iron based on the catalytic effect of Fe (II) on the oxidation of luminol with hydrogen peroxide in an alkaline medium [27].

Voltammetry [29] and amperometry [30] were also employed as detection for a flow injection analysis system.

Table 1.1 summarizes the techniques for iron determination.

Table 1.1 The example methods for iron determination

Technique	Detail	Ref.
Polarography	# Analyte: Fe(II), Fe(III) and Total # Sample: Standard rocks # Direct current polarography is used to determine the ferrous/ferric ratio. The total iron is determined subsequently by ac polarography. The mercury drop, Ag/AgCl and platinum foil were used as the working, reference and auxiliary electrodes, respectively. 0.5 M oxalate was used as supporting electrolyte. # LR: 10^{-5} - 10^{-3} M	6
	# Analyte: Fe(II), Fe(III) and Cu(II) # Sample: Process streams # A form of pulse polarography was used for automated analysis of process streams. Pyrophosphate solution was used as the medium for Fe(II), Fe(III) and copper(II). The mercury drop, SCE and platinum were used as the working, reference and auxiliary electrodes, respectively.	7

Table 1.1 (Continue)

Technique	Detail	Ref.
Polarography	# Analyte: Fe(II) and Fe(III) # Sample: Synthetic samples # Fe(II) and Fe(III) were extracted into chloroform of tris (8-quinolinate) Fe(III) and tris (4,7-diphenyl-1,10-phenanthroline) Fe(II) prior to their electrochemical reduction in propylene carbonate by differential pulse polarography at –0.55 and –1.25 V vs. SCE, respectively. # LR: 2 - 200 μM	8
	# Analyte: Fe(II) and Fe(III) # Sample: Anoxic waters # Using special sampling and handling procedures for polarographic determinations in anoxic waters. The mercury drop, Ag/AgCl and SCE and platinum were used as the working, reference and auxiliary electrodes, respectively. # DL: 0.1 μM	9
	# Analyte: Fe(II) and Fe(III) # Sample: Peat # Using current-samples (tast) polargraphy mode. 0.1 M citrate was used as supporting electrolyte for speciation Fe (II) and Fe(III). Using dropping mercury, Ag/AgCl and glassy carbon as working, reference and auxiliary electrode, respectively.	10
	# Analyte: Fe(II) and Fe(III) # Sample: Zinc plant electrolyte # By adding zinc plant electrolyte to a 0.1 M citrate-0.1M EDTA solution and adjusted to pH 6.0 with ammonia. A differential pulse polarogram was recorded over the potential range of 0.10 to –0.2 V vs. Ag/AgCl. # LR: 30-100 mg l^{-1}	11
	# Analyte: Fe(II) and Fe(III) # Sample: Zinc plant electrolyte # Using supporting electrolyte solutions containing ammonium tartrate (0.10 M) and sodium chloride (0.15 M) and buffered by zwitterionic good buffers from pH 5.4 to 8.2. A differential pulse polaragram was recorded using DME and Ag/AgCl as working and reference electrode.	12

Table 1.1 (Continue)

Technique	Detail	Ref.
Voltammetry	# Analyte: Fe(II) and Fe(III) # Sample: Moss peat # Using special designed electrode system	13
	# Analyte: Fe(III) and Total # Sample: Wines # The method was based on adsorptive stripping voltammetry. Solochrome Violet Red was used as chelating agent for Fe(III) while catechol was used for the determination. Each chelate was adsorbed on the hanging mercury electrode and the reduction current of the accumulated chelate was measured vs Ag/AgCl. # DL: 1 μg l^{-1}	14
Capillary Electrophoresis	# Analyte: Fe(II), Fe(III) and Total # Sample: Wet chemistry process # Using o-phenanthroline and EDTA to form complex with Fe(II) and Fe(III), respectively before injecting (hydrodynamic injection) to a capillary filled with 100 mM borate pH 9.2 and detecting with UV detection at 200 nm. # DL: 0.3 mg l^{-1}, Fe(II) : 0.6 mg l^{-1}, Fe(III)	15
	# Analyte: Fe(II), Fe(III) and Total # Sample: Tap water # Using 1, 10-phenanthroline and CDTA to form complex with Fe(II) and Fe(III), respectively before injection to a fused silica capillary (47cm × 75 μm i.d.) filled with a borate buffer (100 mM, pH 9.0) and with direct UV detection at 254 nm. # DL: 0.06 mg l^{-1}, Fe(II) : 0.1mg l^{-1}, Fe(III)	17
	# Analyte: Fe(II), Fe(III), Cu(I) and Cu(II) # Sample: - # Fe(II)/Fe(III) and Cu(I)/Cu(II) are electrophoretically separated as chelates of 4-(2-pyridylazo) resorcinol (PAR), 2-(5-bromo-2-pyridylazo)-5-diethylamino-phenol(5-Br-PADAP), and 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-(3-sufloropropyl) aniline(3,5-diBr-PAESA). Micellar media was used for the preservation of the original oxidation states of metal ions during the separations.	18

Table 1.1 (Continue)

Technique	Detail	Ref.
Capillary Electrophoresis	# Analyte: Fe(II) # Sample: Water, serum # Using an Fe(II)-1, 10-phenanthroline complexing system and filling a capillary with ammonium acetate-acetic acid (50 mM pH 5.0) as a running buffers and the detection wavelength is set at 270 nm instead of 508 nm. # DL: 5×10^{-9} M	19
Ion Chromatography	# Analyte: Fe(II) and Fe(III) # Sample: Geological materials # Using ion-chromagraphy with a post-column derivatization. Fe(II) and Fe(III) were separated with CS-5 column (250 × 4 mm i.d.). The eluent use was pyride-2,6-dicarboxylic acid (PDCA). The detection was operated at 520 nm.	20
FIA/Spectro-photometry	# Analyte: Fe(II) and Fe(III) # Sample: Pharmaceutical samples # Using FI method, bases on the reaction of Fe(II) with 2, 2'-dipyridyl-2-pyridylhydrazone (DPPH) in acidic medium to form a water-soluble reddish complex (λ_{max} = 535 nm). Using a double-injection valve, which enabled the simultaneous injection of two sample volumes in the same carrier stream. # LR: 0-30 mg Fe l^{-1} : 0-50 mg Fe l^{-1}	21
	# Analyte:Fe(III) and Total # Sample: Synthetic mixtures # Tiron was used as color developing reagent. The first system used a single detector with two flow cells to yield two peaks of Fe(II) and total iron. The second system is a multi-detector system. # TP: 60 samples h^{-1}	22

Table 1.1 (Continue)

Technique	Detail	Ref.
FIA/Spectro-photometry	# Analyte: Fe(II) and Fe(III) # Sample: Natural water # The method based on integration of the Fe(III)-SCN complex with detection using a conventional spectrophotometer. A flow-cell packed with an ion exchange resin was coupled to a FI manifold with inner-coupled injection valves. The detection was operated at 480 nm. Fe(II) was converted to Fe(III) for total Fe. # LR: 80-500 μg l^{-1} # DL: 80 μg l^{-1}	23
	# Analyte: Fe(II) and Total # Sample: High DOC water # Fe(II) is detected using ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p-disulfonic acid). Electrothermal atomic absorption spectrometry of iron at 248.3 nm was conducted. Using Zeeman background correction and graphite furnace. # LR: 10 nM-300 μM # DL: 10 nM	24
	# Analyte: Fe(II), Fe(III) and Al(III) # Sample: Natural water samples # Oxine is used as the reagent for Al and Fe(III), and 1,10-phenanthroline in combination with iodide as the reagent for Fe(II). The reaction products were extracted into chloroform. A diode array detector is used. # DL: 0.06 mg l^{-1} (for all) # TP: 85 samples h^{-1}	25
Chemilumi-nescence	# Analyte: Fe(II) # Sample: Water # By measuring Fe(II)-catalyzed light emisson from luminol oxiolation by oxygen. The reaction is carried out in a flow system driven by infusion pump. The reactants are continuously mixed in a cell positioned directly in front of a photomultiplier tube, which measures light emission. # LR: 0.005-50 μg l^{-1} # DL: 0.005 μg l^{-1}	26

Table 1.1 (Continue)

Technique	Detail	Ref.
FIA/Chemilumi-nescence	# Analyte: Fe(II) and Total # Sample: Human hair, water # A flow-injection method based on the catalytic effect of Fe(II) on the oxidation of luminol with hydrogen peroxide in an alkaline medium is proposed. Using silver column to reduce Fe(III) to Fe(II), which allows total iron determination. # LR: 5×10^{-9} - 1×10^{-6} M # TP: 18 samples h^{-1}	27
	# Analyte: Fe(II) and Fe(III) # Sample: Water # Using flow system for the sequential determination of Fe (II) and Fe(III). Fe(II) was detected by its catalytic effect on the CL reaction between luminol immobilized on an anion exchange resin column and dissolved oxygen. Fe (III) was determine by difference measurement after on-line conversion to Fe(II) in a reducing mini-column packed with Cu plated Zn granules. # LR: 1×10^{-9} - 1×10^{-6} g ml^{-1} # DL: 4×10^{-10} g ml^{-1}	1
	# Analyte: Fe(II) and Fe(III) # Sample: Seawater # The technique is based on a flow injection method coupled with luminol chemiluminescence detection. Using sulphite and in-line matrix elimination/preconcentration on an 8-hydroxyquinoline (8-quinolinol) chelating resin column for Fe(III) reduction. # DL: 40 pM	3
	# Analyte: Fe(II) and Fe(III) # Sample: Seawater # The FI-analyzer based on in-line preconcentration and luminol chemiluminescence detection was used for Fe(II). The device contained a column with the chelating resin MAF-8HQ and was designed to measure Fe(III). # LR: 0.05-0.3 nM # DL: 0.022 nM, Fe(II) 0.021 nM, Fe(III)	28

Table 1.1 (Continue)

Technique	Detail	Ref.
FIA/Voltam-metry	# Analyte: Fe(II) and Fe(III) # Sample: Wine and tap water # Using differential pulse anodic stripping voltammetry in a flow-through configuration on a glassy carbon electrode. Pyrophosphate buffered at pH 9.0 with ammonia ammonium chloride buffer was used as carrier and supporting electrolyte. # LR: 10^{-6} -10^{-3} M # DL: 1×10^{-6} M	29
FIA/Ampero-metry	# Analyte: Fe(II) and Fe(III) # Sample: Standard rock # Flow injection analysis with amperometric detection was used. The flow-through cell contains a glassy carbon electrode. The applied potential selected were +0.8V vs SCE for Fe(II) and +0.2V vs SCE for Fe(III). The carrier steam was a 0.1M perchloric acid –0.061 M potassium chloride solution. # LR: 10^{-5} -5×10^{-4} M, Fe(II) 10^{-5} -10^{-3} M, Fe(III) # DL: 5×10^{-7} M (both)	30

1.1.3 Needs for method development for iron determination

Although there are many methods for iron determination, each method is appropriate for a specific sample condition. Apart from other main analytical characteristics such as sensitivity, precision, accuracy and sample throughputs, cost of an analysis is also to be considered. Development of a method or procedure is still essential, especially in a place with conditions like in Thailand.

FIA with various advantages could be appropriate to fulfill the requirements mentioned above. ICP-AES may be very sensitive but due to its high cost in operation, the technique may not be suitable for the assay of iron contents in pharmaceutical preparations.

A new reagent which is cheap should also be developed especially to complement to a FIA procedure.

A procedure with capillary electrophoresis should be developed for a possibilities for simultaneous determination and/or specification for Fe(II) and Fe(III). Voltammetry should offer a sensitivity method of Fe(II) and Fe(III) which should also result in simultaneous determination and/or speciation of Fe(II) and Fe(III).

1.2 Phosphate

1.2.1 The importance of phosphate determination

Apart from others, in aquatic systems, phosphorus is exhibited in various inorganic and organic forms. While these may exist in dissolved, colloidal, or particulate form, the predominant species is orthophosphate in either the mono or diprotonated form (HPO_4^{2-}, $H_2PO_4^-$). The dissolved component is operationally defined by filtration, and for this reason the term filterable is used in preference to either dissolved or soluble, both of which are used extensively and interchangeably in the literature [31].

Phosphate is of great interest in environmental monitoring as it is limiting for nutrient for plant and algal growth. Increases in phosphate can result in eutrophicaton. Eutrophication is the enrichment of surface waters with nutrients. The problem of the eutrophication results entirely from increased productivity of plants or algal bloom within the waters as a result of increased nutrient [32]. To know the amount of phosphate in water is useful for controlling this problem of pollutant.

1.2.2 Methods for phosphate determination

The determination of phosphorus in waters has historically been based on the photometric measurement of 12-phosphomolybdate or the phosphomolybdenum blue species produced when phosphomolybdate is reduced. Phosphorus species that are determined in this manner are referred to as reactive, and much of the nomenclature of phosphorus speciation derives from this origin [31]. The reaction may be represented as followed [33].

$$7PO_4^{3-} + 12(NH_4)_6Mo_7O_{24} + 36H_2O \rightarrow 7(NH_4)_3PO_4 \cdot 12MoO_3 + 51NH_4^+ + 72OH^-$$

$$(NH_4)_3PO_4 \cdot 12MoO_3 + \text{mild reducing agent} \rightarrow \text{Mo (IV) species (blue)}$$

Many proposed flow-based methods have been developed by using these reactions.

Voltammetry and amperometry have been used as the detection systems of FIA methods for phosphate determination [34-37].

As summarized in Table 1.2, flow-based analysis systems with spectrophotometric detection and electrochemical detection have been applied for phosphate determination in various types of samples.

Table 1.2 Analytical methods for determination of phosphate

Technique	Detail	Ref.
FIA/Poten-tiometry	# Analyte: Phosphate # Sample: Soil extracts # Using the direct potentiometric flow injection analysis with a cobalt wire electrode. The electrode response to phosphate is a result of $Co_3(PO)_2$ precipitate formation at the electrode surface. The carrier used contained 25 mM potassium hydrogen phthalate. # LR: $1.0x10^{-5}$ –$5.0x10^{-3}$ M # DL: $1x10^{-6}$ M	34
FIA/Voltam-metry	# Analyte: Phosphate # Sample: Blood serum and hydroponic fluids # Phosphate was determined as molybdophosphate by FIA using a glassy carbon electrode as a voltammetric detector. The eluent used was 2% ammonium molybdate in 0.6% (v/v) sulfuric acid. The applied potential was +0.22 V. # LR: 10^{-6} –10^{-4} M	35
	# Analyte: Phosphate, silicate # Sample: Washing powders # Static differential-pulse voltammetric and flow injection voltammetric procedures have been developed for the determination of total phosphate and soluble silicate. Glassy carbon, platinum and calomel were used as working, auxiliary and reference electrode.	36
	# Analyte: Phosphate, silicate, arsenate and germanate # Sample: - # Phosphate, silicate, arsenate and germanate were determined as molybdovanadophosphate at a glassy carbon electrode by a static and flow injection system. Heteropolyacids pre-formed in various aqueous media before injecting into eluents consisting of the reagent blank. # DL: 10^{-7} M Silicate, 10^{-6} M Phosphate	37

Table 1.2 (Continue)

Technique	Detail	Ref.
FIA/Ampero-metry	# Analyte: Phosphate # Sample: - # On-line reactions developed for use with flow injection was adapted for use in capillary-fill device (CFD). By reduction of 12-molybdophosphate at a screen-printed carbon electrode in a CFD, 0.1M potassium chloride was used as electrolyte and amperometric detection at 65 mV was performed. # LR: $5x10^{-6}$ M - $2x10^{-3}$ M # DL: $5x10^{-6}$ M)	38
FIA/Spectro-photometry	# Analyte: Phosphate, silicate, arsenate # Sample: - # Silicate, phosphate and arsenate were determined simultaneously by using FIA with on-line column separation. The method was based on measurement of the absorbance at 810 nm of the heteropoly blue formed with ascorbic acid as reducing agent.	39
	# Analyte: Phosphate # Waste water samples # Using on-line microwave-induced digestion and FIA for determination of total phosphorus. The method was based on the formation of molybdenum blue. # LR: 0-18 mg l^{-1} # DL: 0.09 mg l^{-1} # TP: 7 samples h^{-1}	40
	# Analyte: Phosphate # Sample: Foodstuffs # A spectrophotometric method for phosphate, which is based on the stopped-flow technique and involves recording the progress curve for formation of the colored complex of 12-molybdophosphate with malachite green. # LR: 5-55 μg P l^{-1} and 100-1000 μg P l^{-1}	41

Table 1.2 (Continue)

Technique	Detail	Ref.
FIA/Spectro-photometry	# Analyte: Phosphate # Sample: Estaurine waters # Solving the problem of Schlieren or refractive index (RI) effect in a conventional FI manifold by using sodium chloride solution of similar refractive index as a carrier instead of water. # DL: 6 μg P l^{-1}	42
	# Analyte: Organic phosphate # Sample: Natural waters # Flow injection with in-line photochemical digestion for the dissolved organic phosphorus (DOP) is proposed. Using ultraviolet photoreactor and tin(II) chloride reduction of phosphomolybdate for the spectrophotometric determination of the reactive phosphorus produced by the photo-oxidation. # LR: 0.02-6 mg P l^{-1} # DL: 0.01 mg P l^{-1} # TP: 75 and 50 sample h^{-1}	43
	# Analyte: Phosphate # Sample: Waste waters # The method is based on reduction of phosphoantimonylmolybdic acid method coupled with FIA, which kinetics were faster than the original procedure. The conditions were re-examined for the analysis of phosphate.	44
	# Analyte: Phosphate # Sample: Natural waters # Using an inexpensive FI instrument for determining low concentration of dissolved reactive phosphorus. An inexpensive detector consists of a flow cell and a simple photometer that incorporates a super-bright light-emitting diode as the source and a photodiode as the detector. The tin(II) chloride-molybdate method coupled with in-line preconcentration anion-exchange column were used. # LR:0-100 μg P l^{-1} # DL: 0.6 μg P l^{-1} # RSD: 2.9% at 2.0 μg P l^{-1} and 0.5% at 50 μg P l^{-1}	45

Table 1.2 (Continue)

Technique	Detail	Ref.
FIA/Spectro-photometry	# Analyte: Phosphate # Sample: - # Fabrication of micro FIA system based on glass substrates using lithographic techniques and etching methodology was used for orthophosphate analysis based on colorimetric detector, which was facilitated through the use of fiber optics coupled to a LED photodiode system. # LR: 10-100 μg P l^{-1} # DL: 0.7 μg P l^{-1} # RSD: 5% at 10 μg P l^{-1} and 0.8% at 100 μg P l^{-1}	32
	# Analyte: Phosphate # Sample: Industrial raw phosphoric acid # The method is based on the injection of sample into a stream of $FeCl_3$ and subsequent measurement of the decrease in absorption at 334 nm due to the formation of an Fe(III) phosphate complex. # LR: 0.1 to 20 g l^{-1} H_3PO_4 # TP: 30 samples h^{-1} # RSD: 1.4%	46
	# Analyte: Phosphate, silicate # Sample: Sunflower # The continuous addition of reagent technique was used as an effective means of improving the simultaneous reactor-rate determination of phosphate and silicate based on the formation of the corresponding heteropolymolybdated. # RSD: 2.5-4.5%	47
	# Analyte: Phosphate and silicate # Sample: Waste water samples # The method is based on formation of vanadomolybdate and molybdosilicate. Eliminate interference of silicate over phosphate by adding oxalic acid before forming molybdophosphate. Eliminate interference of phosphate over silicate by adding 5.6% oxalic acid after forming molybdosilicic acid. # LR: 12 mg P l^{-1}, 30 mg l^{-1}Si # DL):0.2 mg P l^{-1}, 0.9 mg l^{-1}Si # RSD: < 1.4% (phosphate), < 4% (silicate) # TP: 23 sample h^{-1}	48

1.1.3 Need to develop method for phosphate determination

As phosphate is an important parameter of water quality, a procedure, which should be cost effective, simple, accurate, precise and fast would be appropriate for such a determination purposes. Spectrophotometry is usually a powerful technique for marine environments and water quality monitoring. Various FIA procedures have been proposed for determination of phosphate in fresh waters but its application to estuarine waters have been very limited due to the so-call " refractive index " or Schlieren effect. Besides, sulfide and silicate are the main interferences of these methods. To develop FIA procedures, which can eliminate interference or matrix effect or have less of these effects for phosphate determination, are of interest.

Also sulfide and silicate are usually found to interfere in phosphate determination by the molybdenum blue spectrophotometric method. Certain naturally occurring chemical species are known to interfere with the detection of phosphate, resulting in under or over estimation of phosphate concentration in waters. Of these naturally occurring chemical species, sulfide has been suggested as a potential interferent but this is yet to be quantified and studies extensively. Sulfide is a commonly found species in anoxic sediments, especially in estuarine and marine environments. Bacterial reduction of sulfate to oxidize organic matter is frequently a major process leading to the production of hydrogen sulfide in anoxic sediments [49].

$$SO_4^{2-} + 2C_{organic} + H_2O \xrightarrow[\text{Bacteria}]{} H_2S + 2HCO_3^-$$

The Standard Methods for the Examination of Water and Wastewater [50] lists sulfide as a potential interference and provides advice of elimination interference

from sulfide by adding an excess of bromine water or a saturated potassium permanganate solution.

Attempts should then be given to develop procedures to overcome the cumbersome mentioned above. Electrochemical detection in a flow injection system should overcome the problems on refractive index observed in a system with spectrophotometric detection. Due to sulfide interference, on-line oxidation should be studied. Due to differences in kinetic behaviors of phosphate and silicate, simultaneous determination of both the species should be possible by using a stopped-FI procedure.

1.3 Aims of these studies

The aims of these studies are as follows:

1. To develop FIA method for iron determination by introducing a new cheap color reagent, salicylate, for Fe(III). Assay of iron in pharmaceutical preparations is then its application.

2. To investigate possibility for a method for speciation of Fe(II) and Fe(III) using capillary electrophoresis by using PAR as a complexing ligand to differentiate their mobilities.

3. To investigate a possibility of speciation of Fe(II) and Fe(III) by employing voltammetry using a suitable supporting electrolyte/complexing ligand, namely pyrophosphate and phosphate.

4. To develop a novel FIA method with electrochemical detection for phosphate determination in water samples. On-line column pretreatment is also

incorporated for preconcentration, preseparation and single standard calibration proposes.

5. To develop an on-line sulfide removal system for phosphate determination using a molybdenum blue spectrophotometric method.

6. To develop a method for simultaneous determination of phosphate and silicate using molybdenum blue reactions with stopped-flow injection technique.