CHAPTER 2

Experimental

2.1 Apparatus and Components

1. Syringe pump 2.5 mL syringe barrel, 6 cm moving plunger, 48000 steps motor, Model XLP-6000, Cavro, USA
2. 10-position selection valve , Valco Instrument, USA
3. Data recording system, Ecorder Model 280, eDAQ, Australia
4. A home-made light emitting diode / light dependent resister (LED/LDR) colorimetric detector
5. Flow through cell of 10 mm path length, Hellma, Germany
6. Computer

2.2 Chemicals

1. Acetic acid: CH_3COOH (99.7% purity, RCI Labscan, Thailand)
2. Ammonium ferric sulfate dodecahydrate: $FeNH_4(SO_4)_2.12H_2O$ (99 - 102% purity, Merck, Darmstadt Germany)
3. Ammonium ferrous sulfate hexahydrate: $(NH_4)_2Fe(SO_4)_2.6H_2O$ (99% purity, Merck, Darmstadt Germany)
4. Ammonium molybdate tetrahydrate: $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (81 - 83% purity, Carlo Erba, France)
5. Formaldehyde solution: CH_2O (35 - 40% purity, Merck, Darmstadt Germany)
6. Hydrochloric acid: HCl (37% purity, RCI Labscan, Thailand)
7. Hydroxylammonium chloride: $HONH_2 \bullet HCl$ (99% purity, Sigma-Aldrich, Germany)
8. L-ascobic acid: $C_6H_8O_6$ (99.7% purity, Merck, Darmstadt Germany)

9. L (+) tartaric acid: $C_4H_6O_6$ (99.5% purity, Carlo Erba, France)

10. Manganese(II) sulfate monohydrate: $MnSO_4 \cdot H_2O$ (98-102% purity, Merck, Darmstadt Germany)

11. Nitric acid: HNO_3 (65% purity, Carlo Erba, France)

12. 1,10-Phenanthroline hydrochloride monohydrate: $C_{12}H_8N_2 \cdot HCl \cdot H_2O$ (≥99% purity, Sigma-Aldrich, Germany)

13. Sodium acetate: $C_2H_3NaO_2$ (99% purity, Carlo Erba, France)

14. Sulfuric acid: H_2SO_4 (96% purity, RCI Labscan, Thailand)

15. Sodium hypochlorite: NaClO (12.5% purity, Ajax)

16. Sodium salicylate: HOC_6H_4COONa (99% purity, Carlo Erba, France)

17. Sodium hydroxide: NaOH (99% purity, Merck, Darmstadt Germany)

18. Sodium tetraborate decahydrate: $Na_2B_4O_7 \cdot 10H_2O$ (BDH, America)

19. Sodium nitroprusside: $C_5FeN_6Na_2O$ (98% purity, BHD, America)

20. Potassium dihydrogenphosphate: KH_2PO_4 (99% purity, Carlo Erba, France)

21. Potassium antimony(III) oxide tartrate: $K(SbO)C_4H_4O_6 \cdot 0.5H_2O$ (99.0-103% purity, Merck, Darmstadt Germany)

2.3. Preparation of standard solutions and reagents

2.3.1 Stock standard solution of 1000 mg Fe(II) L^{-1}

Fe(II) stock standard solution (1000 mg Fe(II) L^{-1}) was prepared by dissolving 0.7022 g ferrous ammonium sulfate hexahydrate $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$ in 50 mL of deionized water, then adding 0.25 mL of sulfuric acid $[H_2SO_4]$ (conc.) and adjusting the volume to 100 mL with deionized water.

2.3.2 Stock standard solution of 1000 mg Mn(II) L^{-1}

Stock standard solution of 1000 mg Mn(II) L^{-1} was prepared by dissolving 0.3077 g of manganese(II) sulfate monohydrate $[MnSO_4 \cdot H_2O]$ in water, adding 0.50 mL of nitric acid $[HNO_3]$ (conc.) and adjusting to the make of a 100 mL with water.

2.3.3 Stock standard solution of 1000 mg PO_4^{3-} L^{-1}

A stock standard solution of 1000 mg PO_4^{3-} L^{-1} was prepared by dissolving 0.1447 g potassium dihydrogenphosphate [KH_2PO_4] and adjusting to volume of 100 mL with water in a volumetric flask.

2.3.4 Stock standard solution of 1000 mg NH_4^+ L^{-1}

An ammonium standard stock solution of 1000 mg NH_4^+ L^{-1} was prepared by dissolving 0.2978 g ammonium chloride [NH_4Cl] and adjusting to volume of 100 mL with water in a volumetric flask.

Working standard solution of each analyte was prepared daily by diluting the stock standard solution with water.

2.3.5 Hydroxylamine solution, 5.0% w/v

Dissolve hydroxylammonium chloride 5.00 g and diluting to 100 mL with deionized water.

2.3.6 1,10-Phenanthroline solution, 0.50% w/v

The 1,10-phenanthroline solution was prepared by dissolving of 0.50 g 1,10-phenanthroline hydrochloride monohydrate in 2 M acetic acid–acetate buffer at pH 4.8.

2.3.7 Acetic acid solution, 0.95 mol L^{-1}

The acetic acid solution of 0.95 mol L^{-1} was prepared by dissolving 5.5 mL acetic acid in water and adjusting to volume of 100 mL in a volumetric flask.

2.3.8 Sodium acetate solution, 1.1 mol L^{-1}

The sodium acetate solution of 1.1 mol L^{-1} was prepared by dissolving 14.2339 g sodium acetate in water and adjusting to the volume of 100 mL in a volumetric flask.

2.3.9 Acetate buffer solution, 2.0 mol L^{-1} pH 4.8

The acetate buffer solution was prepared by mixing 0.95 mol L^{-1} acetic acid and 1.1 mol L^{-1} sodium acetate solutions in the ratio of 1:1.

2.3.10 Ammonium buffer solution, 1.0 mol L^{-1} pH 10.2

The ammonium buffer solution of 1 mol L^{-1} was prepared by dissolving 6.75 g ammonium chloride in 40 mL of water and using ammonia solution to adjust pH to 10.2. After that, the volume of buffer solution was adjusted to 100 mL with water.

2.3.11 Formaldoxime solution, 1.2 mol L^{-1}

The formaldoxime solution of 1.20 mol L^{-1} was prepared by dissolving 5.0 g. hydroxylammonium chloride and 9.8 mL formaldehyde solution in deionized water and adjusting to volume of 100 mL in a volumetric flask. The formaldoxime solution of 0.6 mol L^{-1} was prepared daily by appropriate diluting their stock solutions with 1 mol L^{-1} ammonium buffer pH 10.2.

2.3.12 Ammonium molybdate solution, 10.0 g L^{-1}

The ammonium molybdate solution was prepared by dissolving 1 g ammonium heptamolybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\cdot 4\ H_2O$], 10 mL sulfuric acid (6 M), 0.04 g potassium antimony(III) oxide tartrate and 0.75 g tartaric acid in water and adjusting to the volume of 100 mL in a volumetric flask.

2.3.13 Ascorbic acid solution, 2.5% w/v

The ascorbic acid solution of 2.5% w/v was prepared by dissolving 2.5 g L^{-1} ascorbic acid [$C_6H_8O_6$] in water and adjusting to the volume of 100 mL in a volumetric flask.

2.3.14 Sodium hypochlorite solution, 0.5% w/v

The sodium hypochlorite solution of 0.5% w/v was prepared by dissolving 4 mL of 12.5% w/v sodium hypochlorite [NaClO], 7 mL of 0.10 mol L^{-1} sodium hydroxide [NaOH] and 20 mL of 0.05 mol L^{-1} sodium tetraborate decahydrate [$Na_2B_4O_7 \cdot 10H_2O$] in water and adjusting to the volume of 100 mL in a volumetric flask.

2.3.15 Sodium salicylate solution, 0.4 mol L^{-1}

The sodium salicylate solution of 0.40 mol L^{-1} was prepared by dissolving 6.4044 g sodium salicylate [HOC_6H_4COONa] and 0.0456 g sodium nitroprusside [$C_5FeN_6Na_2O$] in water and adjusting to the volume of 100 mL in a volumetric flask.

2.4. Sampling sites

Water samples were collected from 9 sampling points along the Ping river in Chiang Mai, Thailand, beginning from Amphur Chiang Dao to Amphur Muang of Chiang Mai province. The samples were collected every 3 months from February to November 2015. The map of the sampling points is shown in Figure 2.1 and details of each sampling points are summarized in Table 2.1. Each sample was stored in 2 plastic bottles, the first bottles was filtered through a filter paper (Whatman No.1) before added with 1.0 mL nitric acid (conc.) to adjust pH below 2 for the determination of iron and manganese, and the second bottle was stored for the determination of phosphate and ammonium. Then, the two bottles were maintained under 4 ^oC, and water was filtered through a filter paper (Whatman No.1) before being analyzed for iron, manganese, phosphate and ammonium using the proposed SI-colorimetric system.

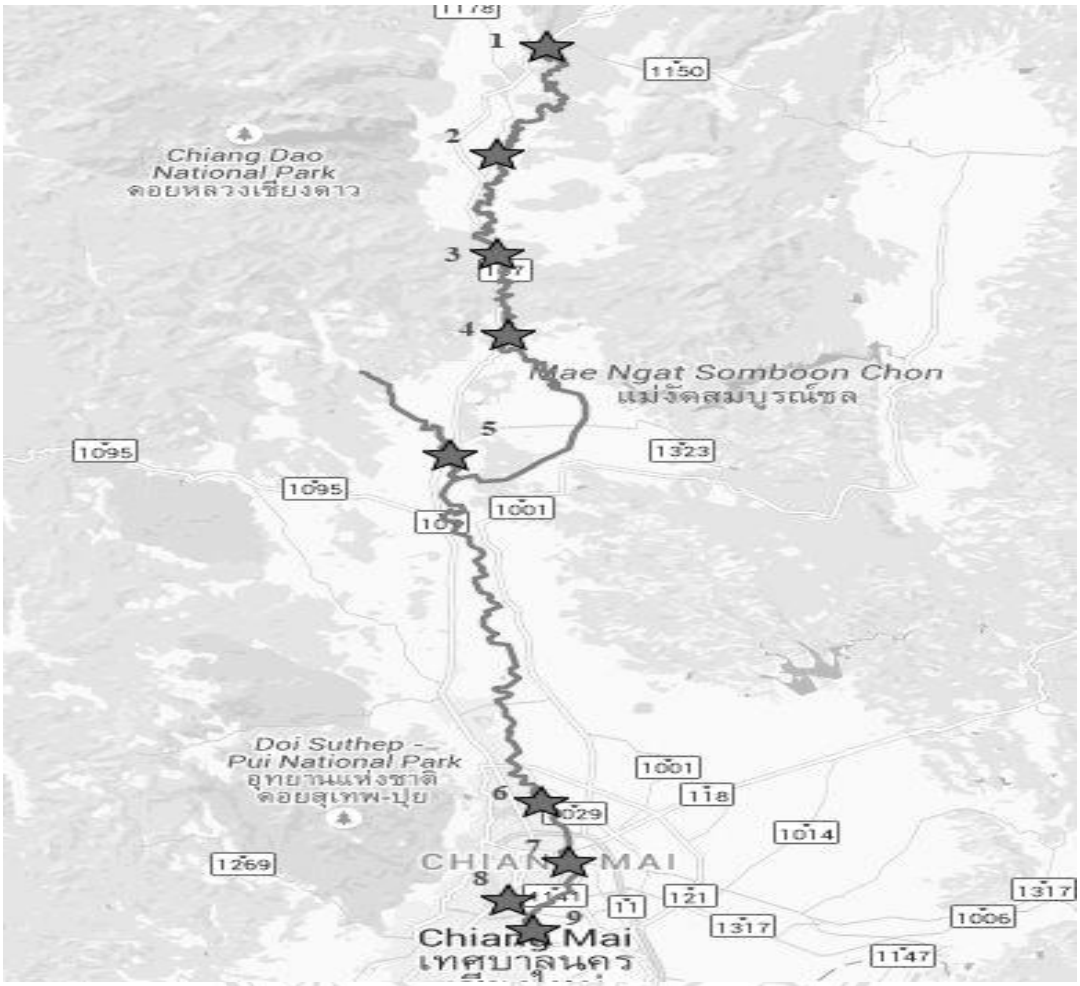

Figure 2.1 The map of the study area showing sampling points

Table 2.1 Details of various sampling points as shown in Figure 2.1

Sampling points	Name of sampling points	Coordinates	
		Latitude (N)	Longitude (E)
1	Ping Kong	19° 27′ 21′′	98° 59′ 30′′
2	Intaram Temple	19° 22′ 3′′	98° 58′ 5′′
3	Kaeng Pan Tao	19° 17′ 9′′	98° 58′ 5′′
4	Thap Dua Temple	19° 13′ 14′′	98° 58′ 21′′
5	Mae Taeng	19° 7′ 20′′	98° 56′ 40′′
6	Nawamin Bridge	18° 50′ 14′′	98° 59′ 24′′
7	Nawarat Bridge	18° 47′ 14′′	98° 0′ 13′′
8	Mae Kha Canal	18° 44′ 37′′	98° 59′ 1′′
9	Padad	18° 44′ 17′′	98° 58′ 52′′

2.5. Instrument setups

The schematic diagram of SI-colorimetric system for determination of iron, manganese, phosphate and ammonium is shown in Figure 2.1. It consisted of a syringe pump for control the flow of carrier and reagents, a 10-position selection valve for choosing appropriate reagent(s) and sample to be sucked into a holding coil, a holding coil for holding and mixing the solutions, a LED/LDR colorimetric detector, and an eDAQ recording unit with a computer. The flow lines were assembled from PTFE tubings of 0.5 mm i.d..

2.5.1 SI-colorimetric system for iron determination

The schematic diagram of SI-colorimetric for iron determination is shown in Figure 2.2. The iron determination is based on 1,10-phenanthroline method, iron(II) reacted with 1,10-phenanthroline to form orange complex. The color intensity of reaction product was detected by a LED/LDR colorimeter using green LED as light source. The hydroxylamine was used for reducing iron(III) to iron(II). The optimum condition for iron determination will be studied, including flow rate, concentration of reagent, and sample volume as shown in Table 2.2 - 2.4.

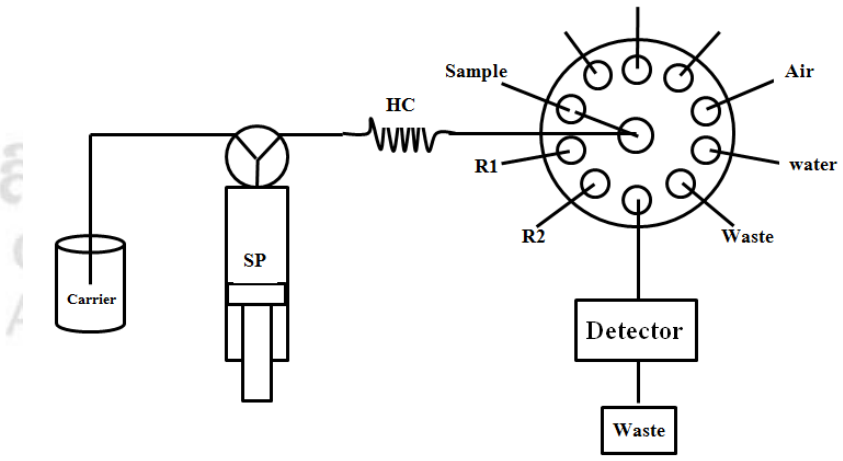

Figure 2.2 Sequential injection (SI) colorimetric system diagram for iron determination. SP = 2.5 mL barrel syringe pump, HC = holding coil, R1 = 5.0% hydroxylamine, R2 = 0.5% w/v 1,10-phenanthroline

Table 2.2 The conditions for study effect of flow rate for iron determination

Parameters	Conditions
Flow rate (mL min^{-1})	2 - 6
Volume of 2.0% 1,10-phenanthroline (µL)	50 [Mirabó, 2009]
Volume of 5.0% hydroxylamine (µL)	100 [Mirabó, 2009]
Sample volume (µL)	300 [Mirabó, 2009]

Table 2.3 The conditions for study effect of concentration of 1,10-phenanthroline for iron determination

Parameters	Conditions
Flow rate (mL min^{-1})	6
Volume of 1,10-phenanthroline (µL)	50 [Mirabó, 2009]
Concentration of 1,10-phenanthroline (% w/v)	0.1 - 2.0
Volume of 5.0% hydroxylamine (µL)	100 [Mirabó, 2009]
Sample volume (µL)	300 [Mirabó, 2009]

Table 2.4 The conditions for study effect of sample volume for iron determination

Parameters	Conditions
Flow rate (mL min^{-1})	6
Volume of 0.5% 1,10-phenanthroline (µL)	50 [Mirabó, 2009]
Volume of 5.0% hydroxylamine (µL)	100 [Mirabó, 2009]
Sample volume (µL)	100 - 800

2.5.2 SI-colorimetric system for manganese determination

The schematic diagram of SI-colorimetric for manganese determination is shown in Figure 2.3. The manganese determination is based on complexation of Mn(II) with formadoxime reagent in basic solution (pH ≥10). The hydroxylamine was used for reducing manganese(IV) to Manganese(II). The color intensity of reaction product was detected by a LED/LDR colorimeter using blue LED as light source. The optimum condition for manganese determination will be investigated, including flow rate, concentration of formaldoxime, and sample volume as shown in Table 2.5 - 2.7.

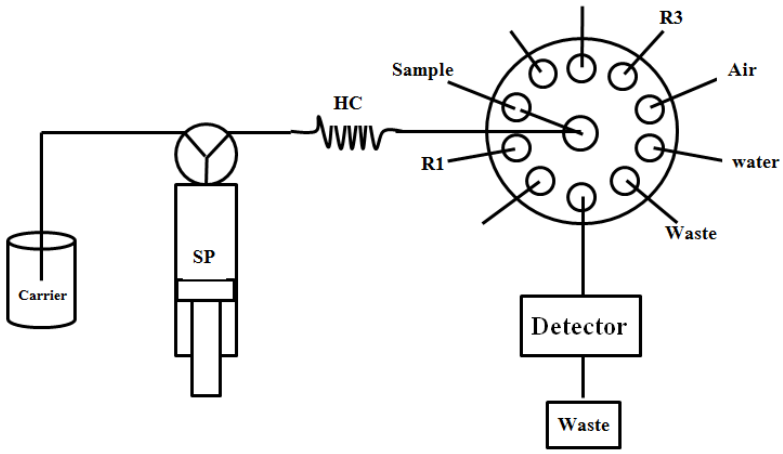

Figure 2.3 Sequential injection (SI) colorimetric system diagram for manganese determination. SP = 2.5 mL barrel syringe pump, HC = holding coil, R1 = 5.0% hydroxylamine, R3 = 0.6 mol L^{-1} formaldoxime

Table 2.5 The conditions for study effect of flow rate for manganese determination

Parameters	Conditions
Flow rate (mL min^{-1})	1 - 4
Volume of 0.6% formaldoxime (µL)	250
Volume of 5.0% hydroxylamine (µL)	100 [Mirabó, 2009]
Sample volume (µL)	250

Table 2.6 The conditions for study effect of concentration of formaldoxime for manganese determination

Parameters	Conditions
Flow rate (mL min^{-1})	2
Volume of formaldoxime (µL)	250
Concentration of formaldoxime (% v/v)	0.05 - 0.6
Volume of 5.0% hydroxylamine (µL)	100 [Mirabó, 2009]
Sample volume (µL)	250

Table 2.7 The conditions for study effect of sample volume for manganese determination

Parameters	Conditions
Flow rate (mL min^{-1})	2
Volume of 0.6% formaldoxime (µL)	250
Volume of 5.0% hydroxylamine (µL)	100 [Mirabó, 2009]
Sample volume (µL)	100 - 500

2.5.3 SI-colorimetric system for phosphate determination

The schematic diagram of SI-colorimetric for phosphate determination is shown in Figure 2.4. The phosphate determination is based on molybdenum blue reaction. The orthophosphate reacted with ammonium molybdate and potassium antimonyl tartrate in acidic solution to form a molybdophosphoric acid, which was then reduced by ascorbic acid to produce molybdenum blue. The blue colored complex was detected by a colorimetric detector, employing red LED as light source. The optimum condition for phosphate determination was studied, including flow rate, concentration of ammonium molybdate, concentration of ascorbic acid, and sample volume as shown in Table 2.8 - 2.11.

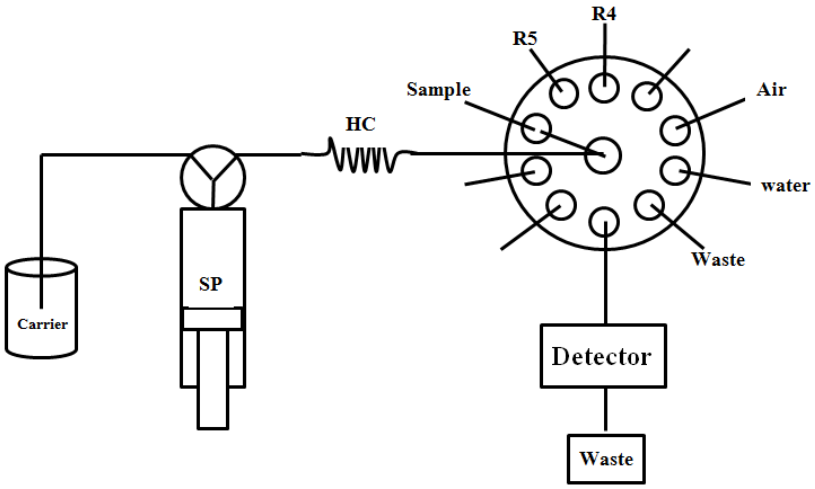

Figure 2.4 Sequential injection (SI) colorimetric system diagram for phosphate determination. SP = 2.5 mL barrel syringe pump, HC = holding coil, R4 = 10.0 g L^{-1} ammonium molybdate, R5 = 2.5% w/v ascorbic acid

Table 2.8 The conditions for study effect of flow rate for phosphate determination

Parameters	Conditions
Flow rate (mL min^{-1})	2 - 5
Volume of 16.0 g L^{-1} ammonium molybdate (µL)	72 [Mesquita,2011]
Volume of 2.0% ascorbic acid (µL)	228 [Mesquita,2011]
Sample volume (µL)	780 [Mesquita,2011]

Table 2.9 The conditions for study effect of concentration of ammonium molybdate for phosphate determination

Parameters	Conditions
Flow rate (mL min^{-1})	4
Volume of ammonium molybdate (µL)	72 [Mesquita,2011]
Concentration of ammonium molybdate (gL^{-1})	8 - 18
Volume of 2.0% ascorbic acid (µL)	228 [Mesquita,2011]
Sample volume (µL)	780 [Mesquita,2011]

Table 2.10 The conditions for study effect of concentration of ascorbic acid for phosphate determination

Parameters	Conditions
Flow rate (mL min^{-1})	4
Volume of 10.0 gL^{-1} ammonium molybdate (µL)	72 [Mesquita,2011]
Volume of ascorbic acid (µL)	228 [Mesquita,2011]
Concentration of ascorbic acid (%)	1 - 3
Sample volume (µL)	780 [Mesquita,2011]

Table 2.11 The conditions for study effect of sample volume for phosphate determination

Parameters	Conditions
Flow rate (mL min^{-1})	4
Volume of 10.0 g L^{-1} ammonium molybdate (µL)	72 [Mesquita,2011]
Volume of 2.5% ascorbic acid (µL)	228 [Mesquita,2011]
Sample volume (µL)	100 - 400

2.5.4 SI-colorimetric system for ammonium determination

Ammonium determination is based on Berthelot method, consisting of 2 steps. Firstly, ammonium reacted with hypochlorite to form monochloramine in basic solution. Then, monochloramine reacted with salicylate, having sodium nitroprusside as catalyst, to produce indophenol blue. The complex was detected by a LED/LDR colorimeter, with red LED light source. The schematic diagram of SI-colorimetric for phosphate determination is shown in Figure 2.5. The optimum condition for ammonium determination will be investigated, including flow rate, concentration of sodium hypochlorite, concentration of sodium salicylate, sodium nitropusside and sample volume as shown in Table 2.12 - 2.16.

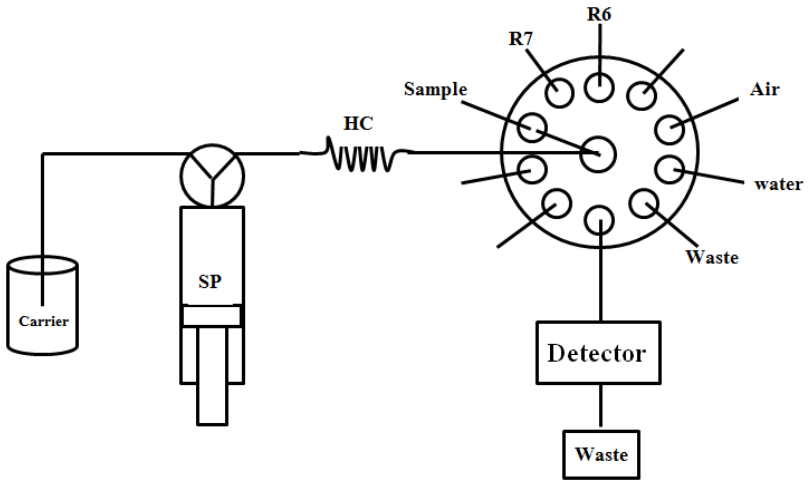

Figure 2.5 Sequential injection (SI) colorimetric system diagram for ammonium determination. SP = 2.5 mL barrel syringe pump, HC = holding coil, R6 = 0.5% w/v sodium hypochlorite and R7 = 0.4 mol L^{-1} sodium salicylate

Table 2.12 The conditions for study effect of flow rate for ammonium determination

Parameters	Conditions
Flow rate (mL min^{-1})	2 - 5
Volume of 0.05% sodium hypochlorite (µL)	350
Volume of 0.5 mol L^{-1} sodium salicylate (µL)	350 [Mirabó, 2009]
Concentration of sodium nitroprusside (mol L^{-1})	0.0032 [Mirabó, 2009]
Sample volume (µL)	350 [Mirabó, 2009]

Table 2.13 The conditions for study effect of concentration of sodium hypochlorite for ammonium determination

Parameters	Conditions
Flow rate (mL min^{-1})	4
Volume of sodium hypochlorite (µL)	350
Concentration of sodium hypochlorite (%)	0.05 - 0.8
Volume of 0.5 mol L^{-1} sodium salicylate (µL)	350 [Mirabó, 2009]
Concentration of sodium nitroprusside (mol L^{-1})	0.0032 [Mirabó, 2009]
Sample volume (µL)	350 [Mirabó, 2009]

Table 2.14 The conditions for study effect of concentration of sodium salicylate for ammonium determination

Parameters	Conditions
Flow rate (mL min^{-1})	4
Volume of 0.5% sodium hypochlorite (µL)	350
Volume of sodium salicylate (µL)	350 [Mirabó, 2009]
Concentration of sodium salicylate (mol L^{-1})	0.1 - 0.5
Concentration of sodium nitroprusside (mol L^{-1})	0.0032 [Mirabó, 2009]
Sample volume (µL)	350 [Mirabó, 2009]

Table 2.15 The conditions for study effect of concentration of sodium nitroprusside for ammonium determination

Parameters	Conditions
Flow rate (mL min^{-1})	4
Volume of 0.5% sodium hypochlorite (µL)	350
Volume of 0.4 mol L^{-1} sodium salicylate (µL)	350 [Mirabó, 2009]
Concentration of sodium nitroprusside (mol L^{-1})	0.001 - 0.003
Sample volume (µL)	350 [Mirabó, 2009]

Table 2.16 The conditions for study effect of sample volume for ammonium determination

Parameters	Conditions
Flow rate (mL min^{-1})	4
Volume of 0.5% sodium hypochlorite (µL)	350
Volume of 0.4 mol L^{-1} sodium salicylate (µL)	350 [Mirabó, 2009]
Concentration of sodium nitroprusside (mol L^{-1})	0.0032 [Mirabó, 2009]
Sample volume (µL)	1 - 500

2.6 Linearity of calibration graph

Under the optimum condition, the standard solutions at various concentrations of each analyte were injected into the SI-colorimetric system. Peak height was examined and used to plot a calibration graph for the determination of iron, manganese, phosphate and ammonium in water samples. Linear range of calibration graph in each case was evaluated.

2.7 Limit of detection

The limit of detection (LOD) is the limit of detection is the lowest concentration level that can be determined to be statistically different from an analytical blank [Deming, 2003], at the LOD defined as 3 * standard deviation of blank signal divided by the slope of calibration graph.

2.8 Recovery

The recovery was performed by spiking 0.5 mg L^{-1} iron, 0.5 mg L^{-1} manganese, 0.3 mg L^{-1} phosphate and 0.3 mg L^{-1} ammonium standard solutions into water samples, and determined by SI-colorimetric system. Peak height obtained was subtracted by peak height of water samples without added the standard solution, and used for calculation of the analyte concentration from the calibration equation. The data obtained were calculated as a percentage of recovery.

2.9 Precision

The precision was examined from the injection of 1 mg L^{-1} standard solution of each analyte into the SI-colorimetric system for 9 times under the optimum conditions, and calculated as a percentage of relative standard deviation (RSD) of peak height.

2.10 Interferences

The study of interferences for determination of iron, manganese, phosphate and ammonium in surface water was carried out for some cations and anions potentially presented in water, such as K^+, Na^+, Ca^{2+}, Mg^{2+}, NO_2^-, NO_3^-, Cl^-, and some heavy metals. The experiment was performed by spiking the interfering ion into the standard

solution containing 1 mg L^{-1} of iron, manganese, phosphate, and ammonium. The solution was then determined by SI-colorimetric system, and peak height obtained was compared with that of 1 mg L^{-1} of each standard solution without spiking the interfering ion. The result was reported in the term of a percentage of difference.

2.11 Analysis of real water sample

Under optimum condition for the determination of each analyte, the developed method was applied for determination of iron, manganese, phosphate and ammonium in water samples collecting from the Ping river in Chiang Mai Province.