APPENDICES

Appendix A

- **The standard methods used in this work**

1. Standard method for the determination of ferrous iron in natural water

The procedure for determining Fe (II) using phenantroline method was selected.

- Preparation of reagents [51]

These solutions were stored in glass-stoppered bottles. The HCl and ammonium acetate solutions are stable indefinitely if tightly stoppered. The hydroxylamine, phenanthroline, and stock iron solutions will be stable for several months. The standard iron solutions were not stable. It will be prepared daily as needed by diluting the stock solution.

a. Hydrochloric acid, conc. HCl

b. Hydroxylamine solution: Dissolved 10 g $NH_2OH.HCl$ in 100 ml water.

c. Ammonium acetate buffer solution: Dissolved 250 g $NH_4C_2H_3O_2$ in 150 ml water. After that it was added 700 ml conc. (glacial) acetic acid.

d. Phenanthroline solution: Dissolved 100 mg 1,10-phenanthroline monohydrate, $C_{12}H_8N_2.H_2O$ in 100 ml water and 2 drops conc HCl were added to the water.

e. Stock iron solution: ferrous ammonium sulfate was preferred, slowly added 20 ml conc H_2SO_4 to 50 ml water and dissolved 1.404 g $Fe(NH_4)_2(SO_4)_2.6H_2O$. Diluted to 1000 ml with water and mixed; 1.00 ml = 200 µg Fe.

f. Standard iron solution was prepared daily for use.

Working standard ferrous iron solution: Transfer 0.15, 0.25, 0.50, 1.25, 2.50, 3.75, and 5.00 ml ferrous iron standard solution to seven 25 ml volumetric flasks that containing 0.03, 0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 ppm, respectively. Dilute to volume with milli-Q water.

g. Procedure of the determination of Fe (II)

This procedure was determined for Fe (II) only; acidify a separate sample with 2 ml conc HCl per 100 ml sample at time of collection. Then was filled bottle directly from sampling source and stopper. Immediately withdraw a 50 ml portion of acidified sample and added 20 ml phenanthroline solution and 10 ml $NH_4C_2H_3O_2$ solution with vigorous stirring. Diluted to 100 ml and measured color intensity within 5 to 10 min. Do not expose to sunlight. This mixed solution was measured absorbance of each sample at 510 nm.

2. Standard method for the determination of phosphate in natural water

The procedure for determining phosphate using ascorbic acid method was selected.

a. Sulfuric acid, H_2SO_4 2.5 M: Diluted 70 ml conc. H_2SO_4 to 500 ml with distilled water.

b. Potassium antimonyl tartrate solution:
Dissolved 1.3715 g $K(SbO)C_4H_4O_6.1/2H_2O$ in 400 ml distilled water in a 500 ml volumetric flask and dilute to volume.

c. Ammonium molybdate solution: Dissolved 20 g $(NH_4)_6Mo_7O_{24}.4H_2O$ in 500 ml milli-Q water.

d. Ascorbic acid, 0.01 M: Dissolved 1.76 g ascorbic acid in 100 ml milli-Q water. The solution was stable for about 1 week at 4^oC.

e. Combined reagent: Mixed the above reagents in a-d proportions for 100 ml of the combined reagent: 50 ml 2.5 M H_2SO_4, 5 ml potassium antimonyl tartrate solution, 15 ml ammonium molybdate solution, and 30 ml ascorbic acid solution. It was mixed after addition of each reagent. If turbidity forms in the combined reagent, shake and stand for a few minutes until turbidity disappears. The reagent was stable for 4 hour.

f. Stock phosphate solution: Dissolved in milli-Q water 219.5 mg anhydrous KH_2PO_4 and dilute to 1000 ml; 1.00 ml = 50.0 µg PO_4^{3-} - P.

g. Standard phosphate solution: prepared daily for use.

Working standard phosphate solution: added 0.15, 0.25, 0.50, 1.25, 2.50, 3.75, and 5.00 ml Fe (II) standard solution to seven 25 ml volumetric flasks that containing 0.03, 0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 ppm, respectively. Dilute to volume with milli-Q water.

h. Procedure of the determination of phosphate

Treatment of the sample: pipette 50.0 ml sample into erlenmeyer flask. Add 0.05 ml (1 drop) phenanthroline indicator. If a red color develops add 2.5 M H_2SO_4, solution dropwise to just discharge the color. Add 8.0 ml combined reagent and mix thoroughly. After at least 10 min but no more than 30 min, measure absorbance of each sample at 880 nm, using reagent blank as the reference solution.

Appendix B

- **Apparatus and Equipment**

1. The generally method for determination of sucrose syrup

- Apparatus [52]

Refractometer

Refractometer is an optical instrument that employs the measurement of refractive index to determine the % Brix of sugar in aqueous solutions. The method is both simple and quick. Samples are measured after a simple user calibration with deionized or distilled water. Within seconds the instrument measures the refractive index of the sample and converts it to % Brix concentration units.

Principle of refractometer, refractive Index is defined as the ratio of the speed of light in empty space to the speed of light in the substance. The index of refraction is calculated from Snell's law and can be calculated from the below equation.

$$\text{Sin } \alpha_{crit} = n_{air} / n_{water}; \text{ the critical angle, } \alpha_{crit}$$

N_{air} and n_{water} were shown that refraction of the air and water, respectively. Generally this behavior can be shown down in the **Figure B-1** from as the Snell's law, with the refractive indices n_1 for water and n_2 for air.

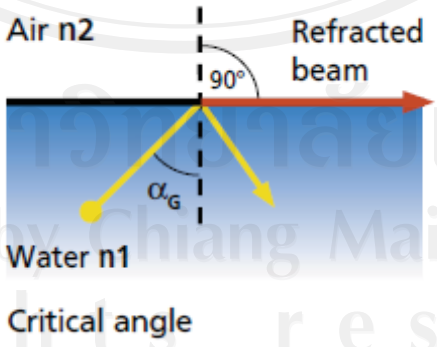

Figure B-1 Light crossing from any transparent medium into another in which it has a different speed

Appendix C

1. Reagent

1.1 Bromothymol blue (BTB) [48]

Bromothymol blue (also known as bromothymol sulfone phthalein, Bromthymol Blue, and BTB) is a chemical indicator for weak acids and bases. The chemical is also used for observing photosynthetic activities or respiratory indicators (turns yellow as CO_2 is added).

BTB acts as a weak acid in solution. It can thus be in protonated or deprotonated form, appearing yellow and blue respectively. It is bluish green in neutral solution. It is typically sold in solid form as the sodium salt of the acid indicator. It also finds occasional use in the laboratory as a biological slide stain. BTB is mostly used in measuring substances that would have relatively low acidic or basic levels (near a neutral pH) in pH 6.0-7.6 range. It is often used in managing the pH of pools and fish tanks, and for measuring the presence of carbonic acid in a liquid. The structure of BTB can be seen in the **Figure C-2.**

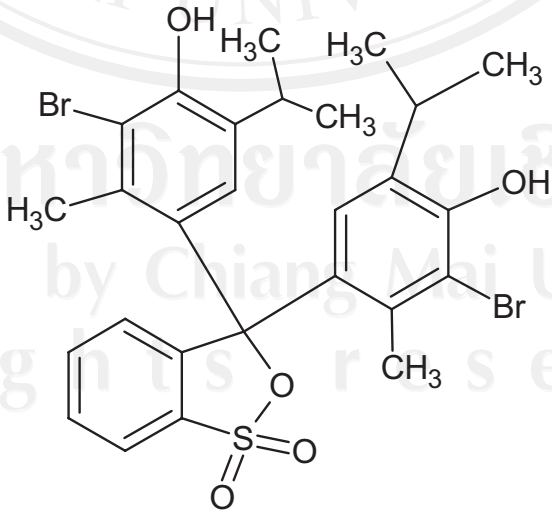

Figure C-2 The structure formula of BTB

Appendix D

1. Sucrose syrup samples

List of sucrose syrup samples

According to the list of the bottle in each of the syrup products used in this study are as follows:

- Sample 1 (Karo, original, light corn syrup with real vanilla)
- Sample 2 (Hale's blue boy brand)
- Sample 3 (nectarel, sweetener, light amber)
- Sample 4 (mountain best, mali syrup)
- Sample 5 (cisteine syrup)
- Sample 6 (Robitussin, Cough syrup)
- Sample 7 (Calpol, Cough headache)
- Sample 8 (Aparcup kids)

2. Natural water sample

The groundwater and water ponds collection were obtained from Nongprateep Road, Nongpakrung district, Muang, Chiang Mai region. This sample was collected with acid for used to studying Fe (II) determination. The water sources used in this study are as follows:

- Sample 1 (Nongprateep temple)
- Sample 2 (Nongprateep village, groundwater)
- Sample 3 (Somtum store, drinking water)
- Sample 4 (grocer's shop, water supply)
- Sample 5 (coffee shop, water supply)
- Sample 6 (Lanna karnchang, drinking water)
- Sample 7 (12 Nongprateep Road, Nongpakrung district, Muang, Chiang Mai, groundwater)
- Sample 8 (Nongprateep school, drinking water)

- Sample 9 (12 Nongprateep Road, Nongpakrung district, Muang, Chiang Mai, boiled water)
- Sample 10 (Nongprateep school, groundwater)

The pond water collection was obtained from fish pond around Chiang Mai University. The pond water samples were stored with acid in clean sample container. This sample was used to determine quantitative of phosphate with ascorbic method. The water sources used in this study are as follows:

- Sample 1 (Heaun Kasalong)
- Sample 2 (Heaun Poungchompoo)
- Sample 3 (Heaun Sritrang)
- Sample 4 (Aumong Temple)
- Sample 5 (Heaun Tongkaw)
- Sample 6 (Heaun Pongsad)
- Sample 7 (Heaun Donkkeaw)
- Sample 8 (Coffee shop at physical department)

CURRICULUM VITAE

NAME Miss Kwanhathai Ardnaree

DATE OF BIRTH April 07, 1986

ACADEMIC STATUS

2008 B.S. (Chemistry), Maejo University

2010 M.S. (Chemistry), Chiang Mai University

SCHOLARSHIPS

2009-2010 Full support from The Center of Excellence for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry PERCH-CIC.

PRESENTATION

2009 K. Ardnaree and K. Grudpan, A simple Lab-on-Chip for the Estimation of Viscosity (Oral and Poster Presentation), Research for Better Life Quality: Symposium on Flow Based Analysis, 23-24 April 2009, Chiang Mai University, Chiang Mai, Thailand.

2010 K. Ardnaree, S. Lapanantnopphakhun, W. Wongwilai and K. Grudpan, Lab-on-chip for the assay of sugar contents in syrup (Poster Presentation), 16th International Conference on Flow Injection Analysis Including Related Techniques (16th ICFIA), 25-30 April 2010, Garden Sea View Resort and Hotel, Pattaya, Thailand.

2010 K. Ardnaree, S. Lapanantnopphakhun, W. Wongwilai and K. Grudpan, Chemical analysis with kinetics using simple lab-on-chip (Poster Presentation), 16th International Conference on Flow Injection Analysis Including Related Techniques (16th ICFIA), 25-30 April 2010, Garden Sea View Resort and Hotel, Pattaya, Thailand.

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

Analytical chemistry is very important in many fields, including medical and clinical science, agriculture, metallurgy and environmental science. Many of analytical methods may involve high cost instruments, high solvent, and reagent consumption, less sample throughput. A simple Lab on Chip (LOC) should be good choice of the analytical procedure to overcome the above disadvantages.

In this research, investigation of employing simple LOC to determine sucrose syrup, iron and phosphate was made, and applications to real samples have been demonstrated.