C H A P T E R I I

E X P E R I M E N T A L

2.1 Instruments and Apparatuses

1. SIA analyzer, model FIAlab3000 (FIAlab® Instruments, U.S.A.) equipped with a syringe pump (syringe reservoir 2.5 mL)
2. Peristaltic pump EYELA micro tube pump MP_3 (Rikakikai, Japan)
3. spectropotometer, model 6400 spectrophotometer (Jenway, UK)
4. Six - port selection valve (Valco Instruments, USA)
5. Flow through cell 1 cm, 80µl (Hellma, Germany)
6. PTFE Tubing: (Anachem, UK)
7. Home-made SIA – FIA analyzer
8. High performance liquid chromatograph (HPLC) (Hewlett Packard, USA) consists of:
    a) HP 1100 Series Thermostatted Column Compartment
    b) HP 1100 Series; Quaternary Pump
    c) HP 1100 series Vacuum Degasser
    d) HP Chemstation for LC 3D systems
    e) HP 1100 series Diode Array Detector
9. Nanopure ultrapure water system (Barnstead, USA)
10. Supelco filtered system (Supelco, USA)

11. pH meter Orion model 420 A with combined Ag/AgCl/glass electrode (Orion, USA)
12. Ultrasonicator, Transonic Digitals (Elma, Germany)
13. Microsorb™ - MV C8, 5μm, 100 A^0 150 x 4.6 mm (Varian Inc, USA)

2.2 Chemicals

All chemicals were of analytical reagent grade unless otherwise specified, which are listed as follows:

1. Standard zinc(II) solution, AAS grade (Merck, Germany)
2. 1-(2-pyridylazo)-2-naphthol, PAN: $C_{15}H_{11}N_3O$ (Aldrich, UK)
3. Sodium carbonate: Na_2CO_3 (Aldrich, UK)
4. Sodium hydrogen carbonate: $NaHCO_3$ (Fisher, UK)
5. Potassium cyanide: KCN (Fluka, UK)
6. Sodium fluoride: NaF (Fluka, UK)
7. Chlortetracycline hydrochloride: $C_{22}H_{23}ClN_2O_8.HCl$ (Fluka, Switzerland)
8. Yttrium (III) nitrate hexahydrate: $Y(NO_3)_3 \cdot 6H_2O$ (Aldrich, USA)
9. Cetyltrimethylammonium bromide, CTAB: $C_{21}H_{38}NBr$ (Fluka, Switzerland)
10. Tris(hydroxymethyl) aminomethane: $NH_2C(CH_2OH)_3$ (Aldrich, UK)
11. Hydrochloric acid: HCl (J.T. Baker Inc., USA)
12. Sodium dodecyl sulfate: SDS (Sigma, USA)
13. 2-propanol: C_3H_7OH ,HPLC grade (Aldrich, USA)
14. Sodium dihydrogen phosphate anhydrous: NaH_2PO_4 (Fluka, Switzerland)
15. Triton X-100, (M.W. 646.87) (Lancaster, UK)

16. Sodium Hydroxide: NaOH (BDH, UK)

17. Pseudoephedrine hydrochloride : $C_{10}H_{15}NO \cdot HCl$ (Fluka, USA)

18. Acetaminophen : $C_8H_9NO_2$ (Sigma, USA)

19. Guaiacoh Glyceryl Ether : $C_{10}H_{14}O_4$ (Sigma, USA)

20. Methanol : CH_3OH (Sigma, USA)

2.3 Procedure

2.3.1Reverse Flow Injection Analysis for Determination of Chlortetracycline

All reagents used were of analytical reagent grade and used without further purification. Deionized water used for all solution preparation.

2.3.1.1 Standards and Reagents Preparations

a) Standard Preparation

A standard stock solution of CTC (1×10^{-3} mol l^{-1}) was prepared by accurately weighed and dissolved appropriate amount of chlortetracycline hydrochloride in a 1000 ml volumetric flask, transferred into the reagent bottle and was kept below 4 ^{o} C. The working solutions were prepared daily by diluting the appropriate volume of the stock solution.

b) Reagent Preparations

A stock solution of yttrium (III) (100 ppm) was prepared by dissolving 0.4308 g of yttrium (III) nitrate hexahydrate in 5.0×10^{-3} mol l^{-1} CTAB. Further dilution of ytrrium (III) with 5.0×10^{-3} mol l^{-1} CTAB were made for appropriate concentrations.

A stock solution of CTAB 0.10 mol l^{-1} solution was prepared by dissolving 36.45 g of cetyltrimethylammonium bromide in 1000 mL of water. Further dilutions were made for appropriate concentrations.

Tris – buffer pH 7.5 solution was prepared by dissolution of 1.21 g Tris(hydroxymethyl) aminomethane in 1000 ml of water and adjusting the pH to 7.5 with 1 mol l^{-1} hydrochloric acid.

2.3.1.2 Sample Preparation

A powder contents of ten capsules (Aureomycin, 250 mg, Interthai pharmaceutical manufacturing, Thailand) were transferred into a beaker then dissolved in deionized water, warmed at 60 ^{o}C for 20 min and transferred into a 1000 ml volumetric flask through a filter – paper, cooled to room temperature and diluted to the mark with the deionized water. Afterward, the samples were diluted into 100 ml volumetric flasks with deionized water to obtain solutions of about 5.2208 x 10^{-5} mol l^{-1}.

2.3.1.3 Instrument Setup and Methodology

The designs of the rFI manifold were fabricated (Figure 2.1). Using the two channels rFI manifold, a 200 µl of yttrium (III) in the CTAB medium solution was injected into the merged stream of sample/standard stream consisting of CTC solutions and Tris buffer pH 7.5 which was connected with a Y –shaped connector at the flow rate of 2.5 ml min^{-1}. Subsequently, the sample zone flowed through the reaction coil 50 cm length where the complexation reaction took place. The CTC-yttrium (III) complex was monitored by the UV-Vis spectrophotometric detector at

λ_{max} 392 nm and the FI signal was analyzed and displayed on a computer screen by the home-made software. The optimization of chemical and the flow system parameters was performed by using the so-called univariate method followed by simplex method. For the first method, a variable was modified while maintaining the other variables at their constant values. The parameters studied were the wave length, pH, concentration of yttrium (III), and concentration of CTAB, flow rate including injection volume and reaction coil lengths. In the simplex method, the summarization of the results of the five – variable optimization values were pH, concentration of yttrium (III), and concentration of CTAB, flow rate including reaction coil lengths. The points 1- 6 represent the first cycle, and the first point is the optimum condition of the univariate technique. The appropriate conditions were obtained by judging from the highest sensitivity and linearity.

2.3.2 Sequential Injection Analysis for Zinc Determination

2.3.2.1 Standard and Reagents Preparations

All chemicals used in this work were of analytical reagent grade. All solutions were prepared with deionized water

a) Standard Preparations

All chemicals were of analytical reagent grade, and used without further purification. Distilled deionized water was used throughout which was prepared by passing distilled water through a Milli – Q system.

b) Reagents

1-(2-pyridylazo)-2-naphthol (PAN) stock solution (1×10^{-2} mol l^{-1}) was prepared from the pure product by dissolving an appropriate weight in ethanol/water mixtures (9:1).

Triton X- 100 solution was prepared by an appropriate dilution with distilled deionized water.

Combined masking agent – buffer solution was prepared by adding 85 ml of 0.20 mol l^{-1} Na_2CO_3 to 15 ml of 0.20 M $NaHCO_3$ solution (pH 9.5) followed by 1.2×10^{-3} mol l^{-1} KCN and 5×10^{-3} mol l^{-1} NaF solutions.

2.3.2.2 Preparation of Pharmaceutical Sample Solutions

Ten Centrum *From A to Zinc®* multivitamin and multimineral tablets were weighed, ground, placed in a conical flask and treated with 50 ml of 0.1 mol l^{-1} HCl. The solution was shaken mechanically and filtered, the precipitate was thoroughly washed with deionized water, then the combined filtrate was transferred into a 1000 ml volumetric flask and diluted to the mark with deionized water, the final concentration of zinc (II) ions was about 6 µg ml^{-1}.

2.3.2.3 Instrumental Setup

The developed SIA manifold (Fig.1) was arranged using the following equipment: A FIAlab® 3000 system (FIAlab® Instruments, USA) consists of a syringe pump (syringe reservoir 2.5 mL) and a 6-port selection cheminert valve (Valco Instrument co., USA) which is connected to a 4 – port switching box. The 4 ports undergo the following functions:

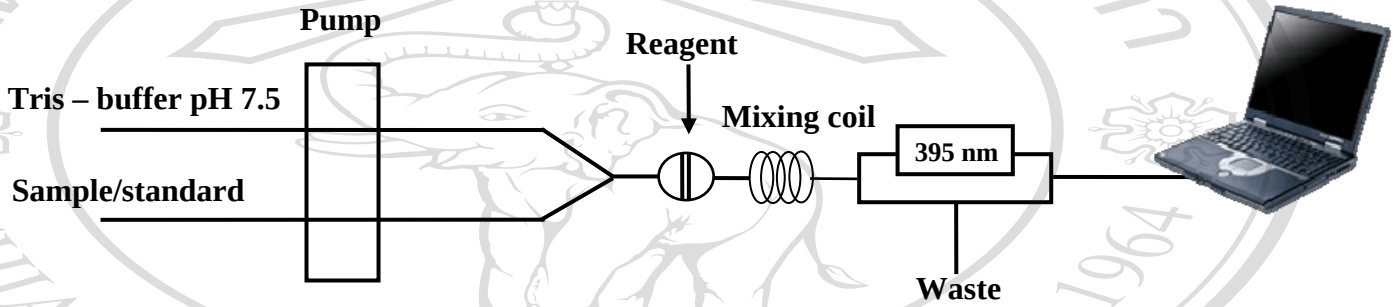

Figure 2.1 The rFI manifold designed for CTC determination by injection of the reagent solution into the merging stream of the buffer and sample/standard solution

Port A is connected to a syringe control (CAVRO XL 3000 stepper motor – driven syringe pump).

Port B is available for other instruments (where necessary)

Port C is connected to a valve control unit

Port D is connected to Jenway 6400 Spectrophotometer

A Jenway 6400 Spectrophotometer (Jenway, Dunmow, Essex, UK) equibed with a 1 cm path-length cell (Hellma, Germany) and a monochromator which can be scaned over the wavelength range 360 – 800 nm. The flow system used PTFE tubes as the liquid channels. The holding coil was constructed by winding the PTFE tubing around the small test tubes (1/16 in. × 0.023 in., O.D. x I.D.). An absorbance signal can be retrieved directly from a Jenway 6400 spectrophotometer via the RS-232 interface. The absorbance of the colored Zn(II)– PAN complex was monitored at 553 nm through a 1 cm path length flow cell. All electrical devices of the manifold were computer controlled by means of a home – made program written in Microsoft Visual Basic 6.0.

2.3.2.4 Sequential Injection Method

A 4 – port RS 232 switching box receives an activation command from the PC through the master port. When the system is initialized, it activates port A (Fig. 2.2) move the piston of the syringe to zero position. It also activates port C to actuate with the valve at position 6. Then, it activates port A to drive the syringe to aspirate the carrier (water) with the desired volume. After that, it activates port C to actuate the valve at position 1 (combined masking agent – buffer solution). Then, it activates port A to drive the syringe to aspirate the desired volume of combined solution. It

again activates port C to actuate the valves at position 2 (Triton X – 100), 3 (sample) and 4 (PAN reagent) , respectively followed by activating port A to drive the syringe to aspirate the desired volume of solution. While the PC is sending the empty syringe command through port A, it activates port D and receives an absorbance signals from the spectrophotometer and drives the plot module to plot the SIA grams on the screen. The maximum peak heights are also detected at 553 nm and displayed in this process. The time required to analyze one sample is approximately 1.5 min. Table 2.1 lists the steps of the procedure entered to the FIAlab for windows software.

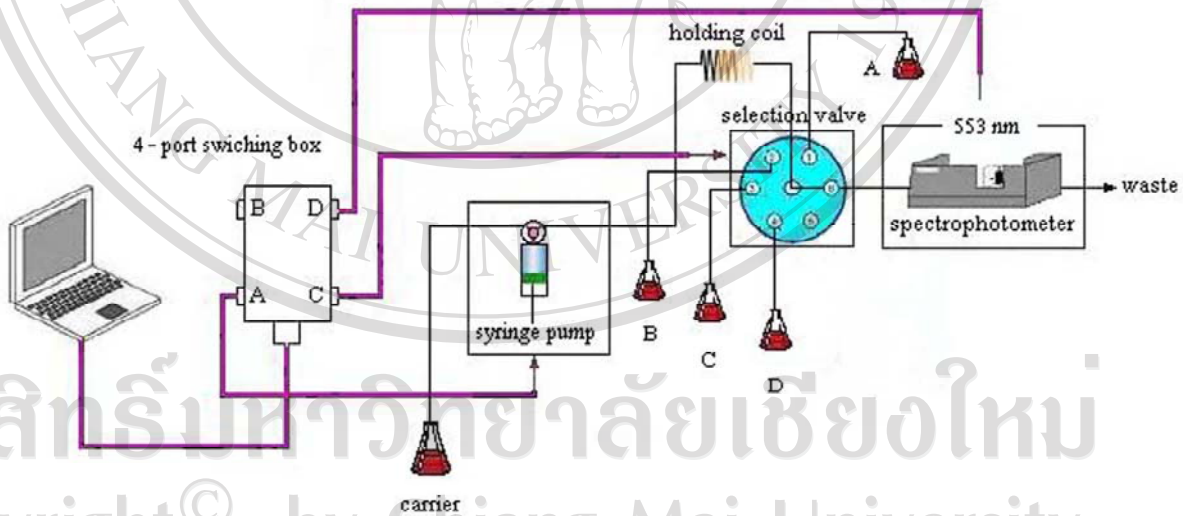

Figure 2.2 SI manifold for the determination of zinc(II) : A (valve 1), a combined buffer solution and masking agent ; B(valve 2), Triton X – 100 ; C(valve 3), sample ; D(valve 4), PAN solution ; E(valve 5) water and 150 cm /0.7 mm (length/i.d.) holding coil.

Table 2.1 experimental protocols as shown in the FIAlab for Windows software

```
Loop Start (#)  3

' Fill Syringe
   SyringePump Flowrate (µl/s) 100
   SyringePump Valve In
   SyringePump Delay Until Done

   SyringePump Aspirate (µl) 1500
   SyringePump Valve Out
   SyringePump Delay Until Done

' standard to holding coil
   Valve port 1
   SyringePump Valve Out
   SyringePump Flowrate (µl/s) 25
   SyringePump Aspirate (µl) 30
   SyringePump Delay Until Done

   Valve port 2
   SyringePump Valve Out
   SyringePump Flowrate (µl/s) 25
   SyringePump Aspirate (µl) 30
   SyringePump Delay Until Done

   Valve port 3
   SyringePump Flowrate (µl/s) 25
   SyringePump Aspirate (µl) 50
   SyringePump Delay Until Done

   Valve port 4
   SyringePump Flowrate (µl/s) 25
   SyringePump Aspirate (µl) 30
   SyringePump Delay Until Done

' Send sample to detector
   Valve port 6
   SyringePump Valve Out
   SyringePump Flowrate (µl/s) 40
   SyringePump Empty
   SyringePump Delay Until Done

' Clean detector
   SyringePump Flowrate (µl/s) 100
   SyringePump Valve In
   SyringePump Delay Until Done

   SyringePump Aspirate (µl) 250
   SyringePump Valve Out
   SyringePump Delay Until Done

   Valve port 6
   SyringePump Valve Out
   SyringePump Empty
   SyringePump Delay Until Done

   Loop End
```

2.3.3Micellar Liquid Chromatography for Some Pharmaceutical Determination

2.3.3.1 Standard, Reagent and Sample Preparations

The drugs of interest were Acetaminophen, Guaifenesin, Phenylephrine, Pseudoephedrine and Phenylpropanolamine.

a) Standard Preparations

All stock drug solutions were prepared in the concentration of 1000 μg ml^{-1} in 0.05 mol l^{-1} of SDS + 2.5 % v/v of 1-pentanol. The further dilution was done in micellar mobile phase for each drug.

b) Reagent Preparations

A 0.5 mol l^{-1} of sodium dodecylsulfate (SDS), was prepared by weighing powder of SDS 144.19 g and dissolving in milli-Q water and making up to 1000 ml in a volumetric flask.

A 0.1 mol l^{-1} sodium phosphate buffer solution was prepared by dissolving 15.6 g of sodium hydrogen phosphate in 1000 ml of milli – Q water.

Mobile phase consisting of 0.025 – 0.15 mol l^{-1} of SDS and 1 – 5 % v/v 1-pentanol and 0.05 mol l^{-1} phosphate buffer adjusting pH 3 with 0.1 mol l^{-1} of HC was prepared. The mixed mobile phase was filtered by vacuum through 0.45 μm Nylon membrane of 45 mm i.d., and then degasified in ultrasonicator for 15 minutes.

c) Sample Preparations

Tablets and capsules

Ten commercial tablets of sample were accurately weighed, ground and mixed homogeniusly, several portions were taken and weighed, dissolved in 0.05 mol l^{-1} SDS solution containing a small amount of ethanol, and diluted finally with 0.05 mol l^{-1} SDS to an adequate concentration. This preparation was also treated similarly with ten capsules.

Oral syrup

A 5 mL syrup was taken, a small amount of ethanol was added and diluted to adequate concentration with 0.05 mol l^{-1} SDS.

All sample solutions needed to be filtered using 0.45 μm Nylon membrane filter and sonicated for 15 minutes prior to injection into the chromatographic system.

2.3.3.2 Determination of Some Cough-Cold Pharmaceutical Preparations

The chromatographic conditions were carried out with can isocratic mode using mixture of aqueous SDS solution and pentanol as mobile phase. The C8 column was equilibrated with the mobile phase for 30 minutes at a flow rate of 1.0 ml min^{-1}. Peak identification and peak purity were performed by comparison of their retention time and the UV absorption spectra of the chromatographic peaks with those of reference compounds previously recorded by injection of each one individually. Analysis was carried out at 210 nm with three replicated injections (20 μl loop injector) of each sample and/or standard solution.

For the analysis of the cough – cold pharmaceuticals containing acetaminophen, guaifenesin, phenylephrine, pseudoephedrine and phenylpropanolamine, a 0.150 mol l^{-1} SDS – 2.0 % v/v pentanol mobile phase at pH 7 was used.