CHAPTER 3

RESULTS AND DISCUSSION

3.1 FI-colorimetric determination of Ca(II), Mg(II), Fe(II) and Zn(II)

A FI-colorimetric method using simple and common reagents (Mg(II), EDTA and EBT) was developed for the determination of Ca(II), Mg(II), Fe(II) and Zn(II) in pharmaceutical supplement samples It was based on replacement of Mg(II) in Mg(II)-EDTA complex by metal ions. The released Mg(II) reacted with EBT.

Changing of Mg-EBT color intensity was measured by colorimeter. Peak height was recorded and found to be proportional to the concentration of Ca(II), Mg(II), Fe(II) and Zn(II).

3.1.1 Preliminary studies

3.1.1.1 Absorption spectra of EBT and Mg-EBT complex

Absorption spectra of EBT and Mg-EBT complex were studied. Firstly, Solution A (see section 2.4.1) was scanned over the range of 400–800 nm using deionized water as a reference. A maximum absorption wavelength was observed at 625 nm (Figure 3.1a). This may be due to the free EBT or HIn^{2-} species (1).

Secondly, solution B (see section 2.4.1) was prepared by adding a standard solution of Zn(II) in solution A. The blue color of solution B was immediately

changed to wine-red color. It was found that a maximum absorption wavelength was at 530 nm (Figure 3.1b).

The B-solution spectrum was similar to Young's experiment, which have studied the Mg-EBT complex (1)

The stability constants (log K_f) for the Mg(II)-EDTA and Zn(II)-EDTA complexes are 8.79 and 16.5, respectively (2). This large difference is advantageous for the replacing reaction. Accordingly, an absorbed molecule in solution B was Mg-EBT, which occurred from replacing reaction.

Therefore, the measuring the absorption of Mg-EBT by a colorimeter with a green filter (maximum transmission at 530 nm) would be possible for the determination of cation, which is stronger complexation with EDTA than Mg(II).

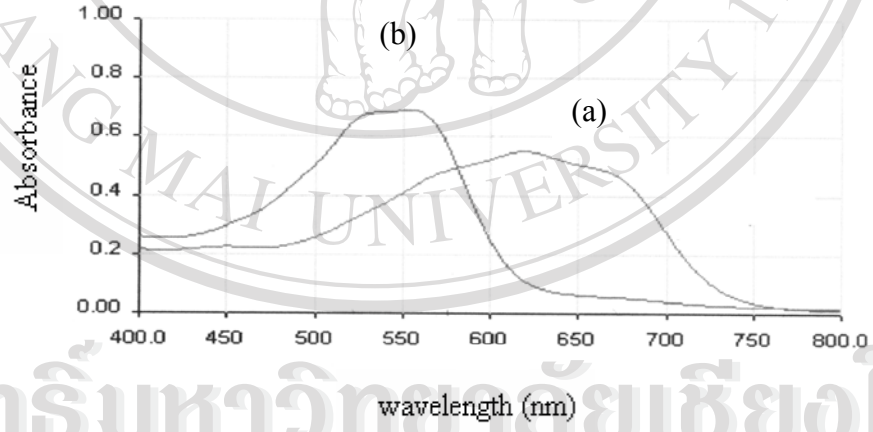

Figure 3.1 Absorption spectra of solutions recorded against deionized water as a reference. (a) 0.0012% w/v EBT + 0.05 M Mg-EDTA + 0.1 M NH_3/NH_4Cl buffer (blue solution) (b) 2.25 x10^{-3} M Zn(II) + 0.0012% w/v EBT + 0.05 M Mg-EDTA + 0.1 M NH_3/NH_4Cl buffer (wine red solution)

3.1.1.2 Study of stability of Mg-EBT complex

According to (2), it was reported that EBT indicator solution should be prepared freshly due to its instability. The stability of EBT and Mg-EBT complex were then checked.

Series of Mg-EBT solutions was prepared by using Zn(II) standard in the solution A (see section 2.4.1). Absorbance values of the solutions were measured at 530 nm, within 5 hours at 0, 30, 150 and 300 min. The results are shown in Table 3.1. No change in the absorbance of EBT (blank solution) was observed for at least throughout 300 min. Under this condition, it indicated that the EBT reagent was stable enough for FIA procedure.

However, it was found that after 150 min the absorbance (at 530 nm) of the solution similar to the solution B but with different Zn(II) concentrations slightly decreased as shown in Figure 3.2. This may be due to forming of Zn-EBT by the replacement of Mg-EBT complex for Zn(II) and releasing Mg(II).

Table 3.1 Absorbance (530 nm) values of Mg-EBT (replacement of Zn(II) with Mg(II)-EDTA complex) measured in various periods after mixing

Zn(II)(mM)	Absorbance(530nm) of Mg-EBT at a period (min) after mixing			
	0	30	150	300
0.00	0.342	0.345	0.350	0.350
1.37	0.464	0.460	0.456	0.416
1.50	0.600	0.600	0.580	0.566
2.25	0.663	0.681	0.668	0.626

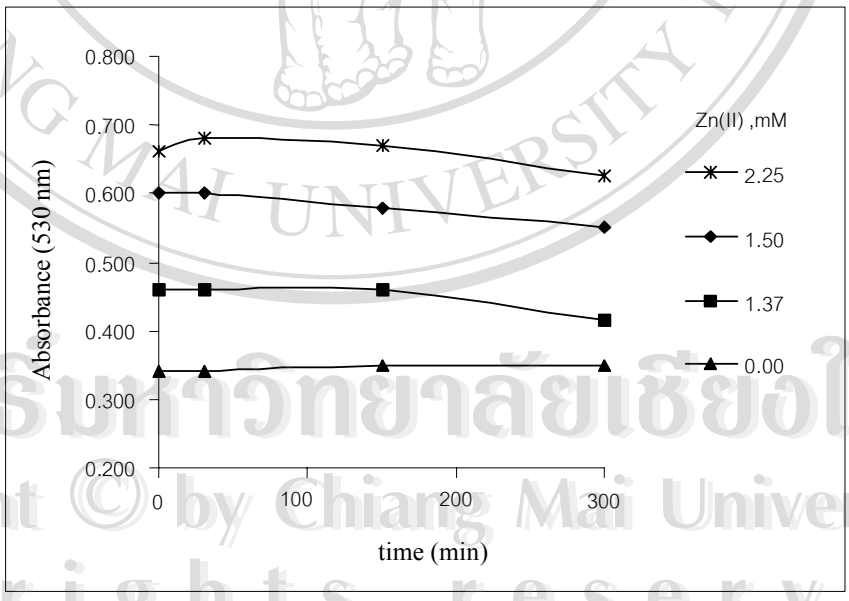

Figure 3.2 The absorbance of Zn(II) 0-2.25 mM in 0.0012% w/v EBT + 0.05 M Mg-EDTA + 0.1 M NH_3/NH_4Cl buffer pH 10 in various time (0, 30, 150 and 300 min)

3.1.1.3 Batchwise colorimetric determination of Zn(II) using Mg-EDTA and EBT

A series of standard solutions of Zn(II) was put into a volumetric flask and made up to volume 10 mL with a mixture of Mg-EDTA (0.05 M) and EBT (0.0012% w/v) in NH_3/NH_4Cl (0.1 M, pH 10) buffer. The solution was taken to measure the absorbance at 530 nm against deionized water. This would be made a series of various Zn(II) concentrations (0.75-2.00 mM). The results are summarized in Table 3.2.

It was found that the absorbance value was linearity proportional to Zn(II) concentration in the range of 1.0–1.5 mM (y = 0.5294x – 0.4682) (Figure 3.3). Although the narrow calibration range of Zn(II) was obtained, the linear range could be expanded by optimizing FIA parameters in further work.

This test indicated that it was possible to use the metal ion replacing reaction for the determination of Zn(II) and some cations e.g. Ca(II) and Fe(II) by FI-colorimetric procedure.

Table 3.2 Absorbance (530nm) of solutions containing Zn(II) and a mixture of Mg-EDTA (0.05 M) EBT (0.012% w/v) in NH_3/NH_4Cl (0.1 M, pH 10) buffer (measured at 5 min after mixing)

Zn(II) Standard, mM	Absorbance
0.00	0.000
0.75	0.030
1.00	0.050
1.12	0.138
1.25	0.200
1.50	0.320
1.75	0.330
2.00	0.332

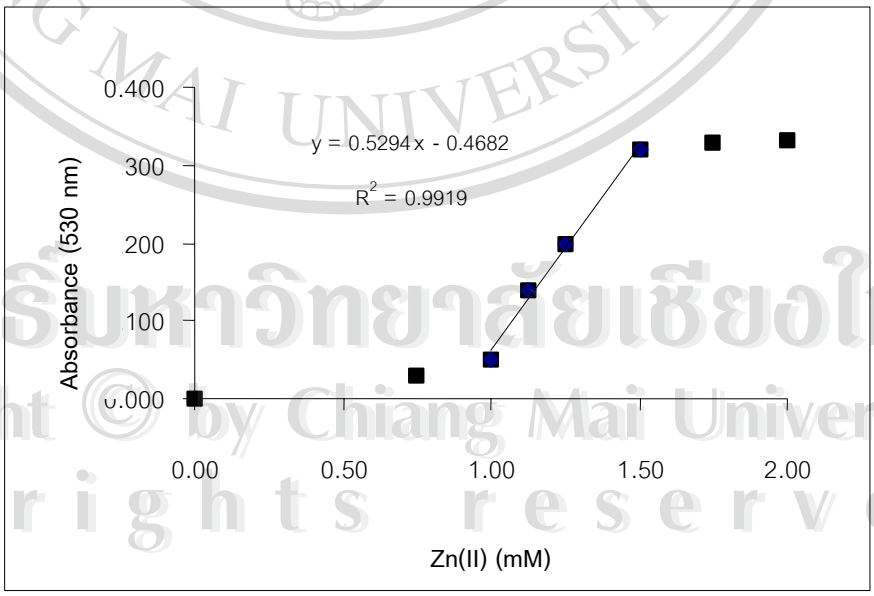

Figure 3.3 Correlation of absorbance and Zn(II) concentrations: Mg-EDTA (0.05 M) and EBT(0.0012% w/v) in NH_3/NH_4Cl (0.1 M, pH 10) buffer

3.1.1.4 Design and test of FIA system

Further studies using FI system were made by employing the replacing reactions of Mg-EBT complexes by Ca(II) , Fe(II) and Zn(II). The first trial was a single line FI system. The carrier reagent was a mixture of Mg-EDTA and EBT in NH_3/NH_4Cl buffer. A metal ion solution was injected into the carrier stream. Under a set of conditions : Mg-EDTA 0.05 M and EBT 0.05% w/v in in NH_3/NH_4Cl buffer solution the results indicated that high baseline absorption and a double peak were observed . To overcome the Schlieren effect (or refractive index effect) observed, a double-line system (Figure 2.1) was introduced by separating a Mg-EDTA (R1) and EBT (R2).

The first set of experimentals (Figure 2.1) were tried by injecting standard/sample injected into a Mg-EDTA solution before merging with EBT in NH_3/NH_4Cl buffer solution, under a set of conditions (Table 3.3).

Table 3.3 Preliminary conditions for the first set of experiments employing a double line FI manifold (Figure 2.1)

R1	$[Mg^{2+}]$ (in 0.1 M NH_3/NH_4Cl buffer)	0.008 M
	[EDTA] (in 0.1 M NH_3/NH_4Cl buffer)	0.008 M
	Flow rate	5.1 mL/min
R2	EBT (in 0.1 M NH_3/NH_4Cl buffer)	0.006% w/v
	Flow rate	2.7 mL/min
S	Injection volume	50 µl
M	Mixing coil	75 cm
D	Detection wavelength	530 nm

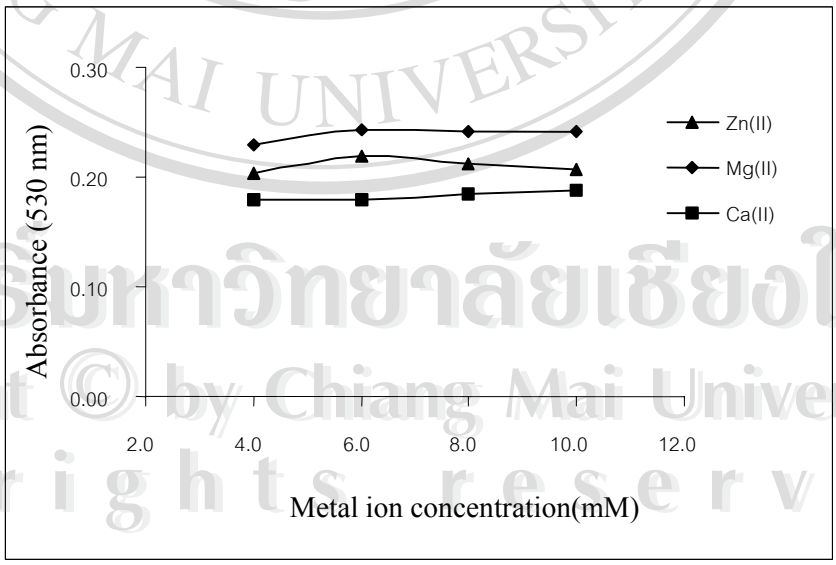

Figure 3.4 Plots of absorbance (530 nm) vs. metal ion concentration

Figure 3.4 reveals that the conditions were not suitable as absorbances of the different concentrations of the metal ions did not obey Beer's law.

3.1.2 Optimization

3.1.2.1 The Mg-EDTA solution

Using the conditions in Table 3.3, the concentration of Mg(II) was fixed at 8.0 mM with variation EDTA concentrations (Table 3.4). The results are summarized as shown in Figure 3.5.

Table 3.4 Variation of EDTA in the Mg-EDTA solutions

Mg(II) : EDTA ratio	Mg(II), M	EDTA, M
1:1.4	0.0080	0.0112
1:1.5	0.0080	0.0120
1:1.6	0.0080	0.0128

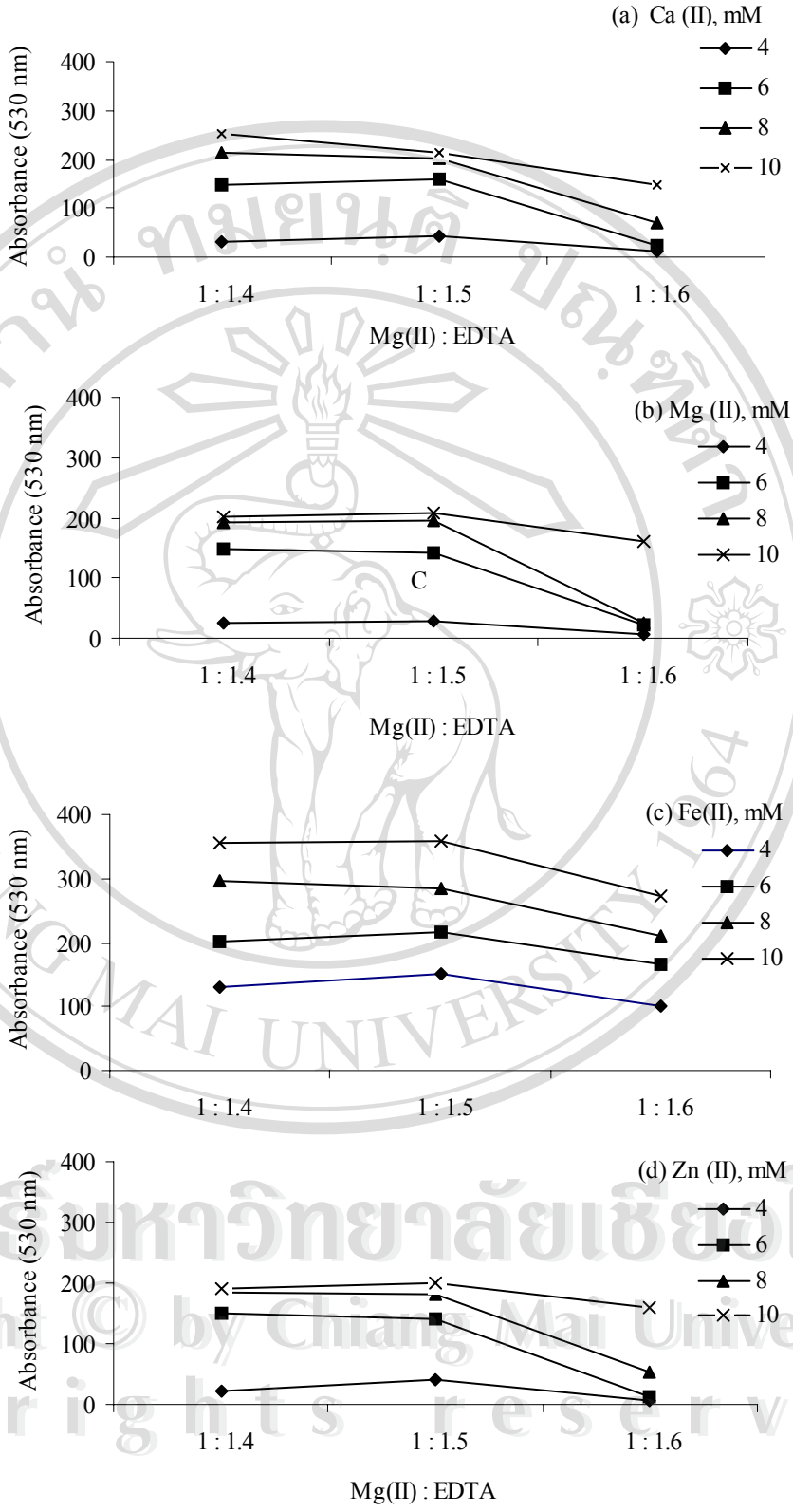

Figure 3.5 Study on variation of EDTA concentration by fixing Mg(II) at 8.0 mM for Mg-EDTA reagent for (a) Ca(II), (b) Mg(II), (c) Fe(II) and Zn(II).

It can be observed that peak height decreased significantly for Mg(II) : EDTA being 1 : 1.6 (0.008 M : 0.00128 M). The EDTA concentration at a range of 1.4-1.5 fold of 0.0080 M Mg(II) was suitable for determination of Ca(II), Mg(II), Fe(II) and Zn(II).

3.1.2.2 Effect of EBT concentration

Various concentration of EBT in R2 (0.030, 0.006, and 0.0012% w/v EBT in 0.1 M NH_3/NH_4Cl buffer) were investigated by using R1 (0.008 M Mg(II) + 0.012 M EDTA in 0.1 M NH_3/NH_4Cl buffer) with other conditions in Table 3.3.Under the conditions, standard Ca(II) solutions 2.0-6.0 mM were injected. From Figure 3.6, the higher concentrations of EBT gave higher peaks, but also higher baseline noise. An EBT concentration of 0.006% was selected.

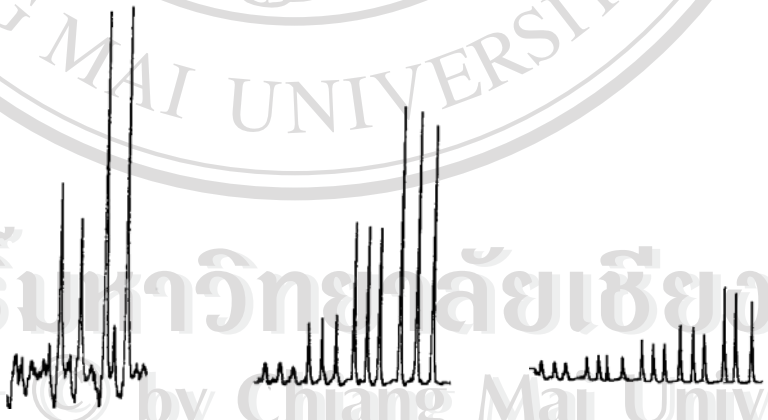

Figure 3.6 FI-grams of Ca(II) obtained

3.1.2.3 Effect of mixing coil

An effect of a mixing coil length on peak height was investigated. The results are shown in Table 3.5 and Figure 3.7. It was found that the increasing of mixing coil length, a good precision was observed. A 75-cm was chosen for optimum mixing coil length because it gave highest precision and sufficient sensitivity.

Table 3.5 Effect of mixing coil length on peak height of a series standard Ca(II) solution

Mixing coil length (cm)	Peak height, mV		
	2.0 mM	4.0 mM	6.0 mM
0	67.3 ± 25.3	230.7 ± 35.0	331.3 ± 1.2
40	70.7 ± 30.0	212.3 ± 49.7	325.3 ± 3.1
75	21.7 ± 0.6	62.0 ± 1.7	105.7 ± 1.2

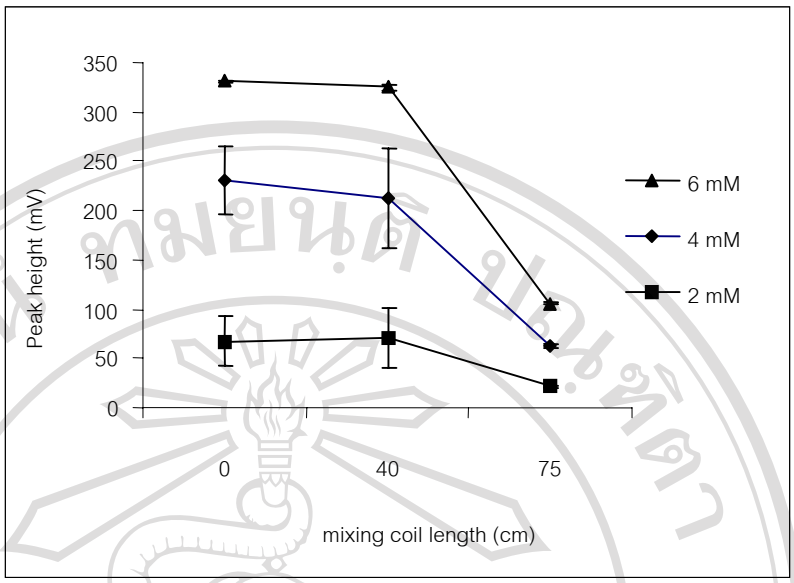

Figure 3.7 Effect of mixing coil length on peak height of a series standard Ca(II) solution.

3.1.2.4 Effect of flow rate

An effect of flow rate which is a physical parameter was checked by injecting various concentrations of Ca(II) 2.0-6.0 mM and constructing a calibration graph. Various flow rates of R1 and R2 were studied by changing the diameter of a pump tubing while keeping the speed of a peristaltic pump constant. The results obtained are summarized in Table 3.6. Condition set D was selected as good precision can be obtained.

Under the conditions, Figure 3.9 depicts FI-gram obtained and indicates sampling frequency of 200 injections per hour.

Table 3.6 Correlation of peak heights and Ca(II) concentrations at various flow rates

Condition	Flow rate (mL/min)	
	R1	R2
A	1.5	1.5
B	2.7	2.7
C	3.5	1.5
D	5.1	2.7
E	6.8	3.5

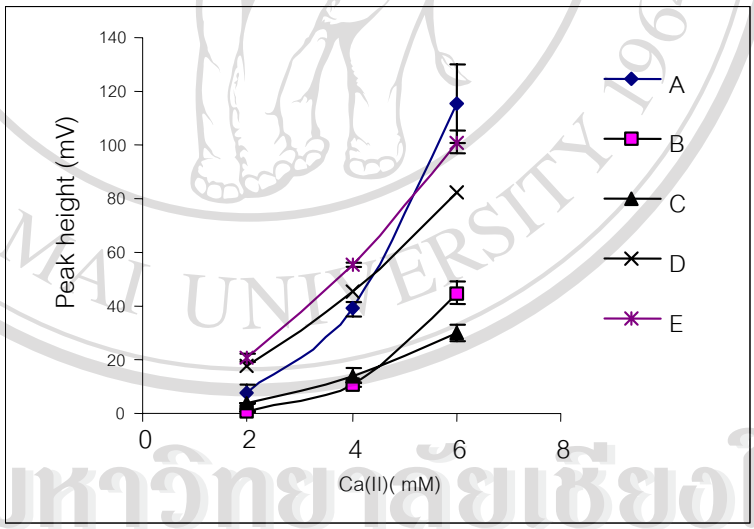

Figure 3.8 Effect of flow rate on peak height (Conditions: A, B, C, D and E) (see Table 3.6)

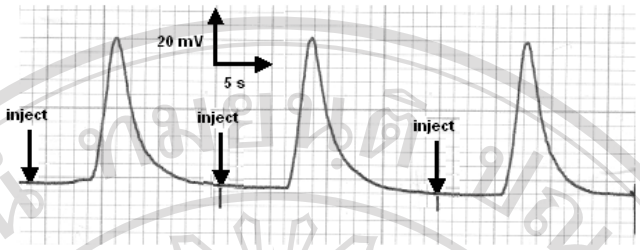

Figure 3.9 FI-gram of Ca(II) 4.0 mM under the condition set D

3.1.2.5 Effect of pH of standard solution

According to the preliminary study, the standard solutions were prepared in 0.01 M HCl (pH 2). In this experiment, effect of pH of sample solution was studied.

Adjusting solutions for various pH values (2, 3 and 5) containing 5 mM Ca(II), 5 mM Mg(II), 5 mM Fe(II) or 4 mM of Zn(II) were prepared. The solutions were injected into the FI system and evaluated for their analysis results by using calibrations prepared by employing standard solutions of pH 2. The results are shown in Table 3.7 and Figure 3.10.

Table 3.7 Results determined by employing a calibration graphs of standards of pH 2

Cation	Concentration (mM) obtained via the calibration graph		
	pH 2	pH 3	pH 5
Ca(II)	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.0
Mg(II)	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.0
Fe(II)	5.0 ± 0.1	5.7 ±0.1	6.1 ± 0.1
Zn(II)	3.9 ± 0.1	4.1 ± 0.1	4.1 ± 0.1

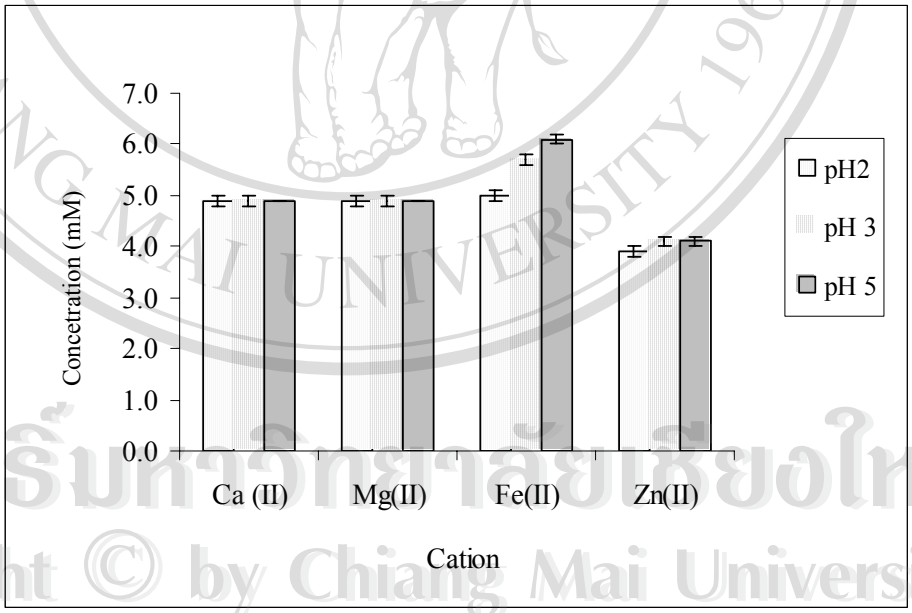

Figure 3.10 Results of the determination of cations in various pHs (2, 3 and 5) determined by using calibrations graphs prepared by standard solutions of pH 2

It was found that the concentrations of Ca(II), Mg(II) and Zn(II) at pH 2, 3 and 5 were not difference. Therefore, the pH (2-5) of standard/sample solution of Ca(II), Mg(II) and Zn(II) can be performed.

Effect of pH solution on Fe(II) solution was observed. It should be noted that for Fe(II) , higher pH values yielded higher analysis results. This may be due to another species of complex, $Fe(NH_3)_4^{2+}$ formed, as indicated in the spectra in Figure 3.11.

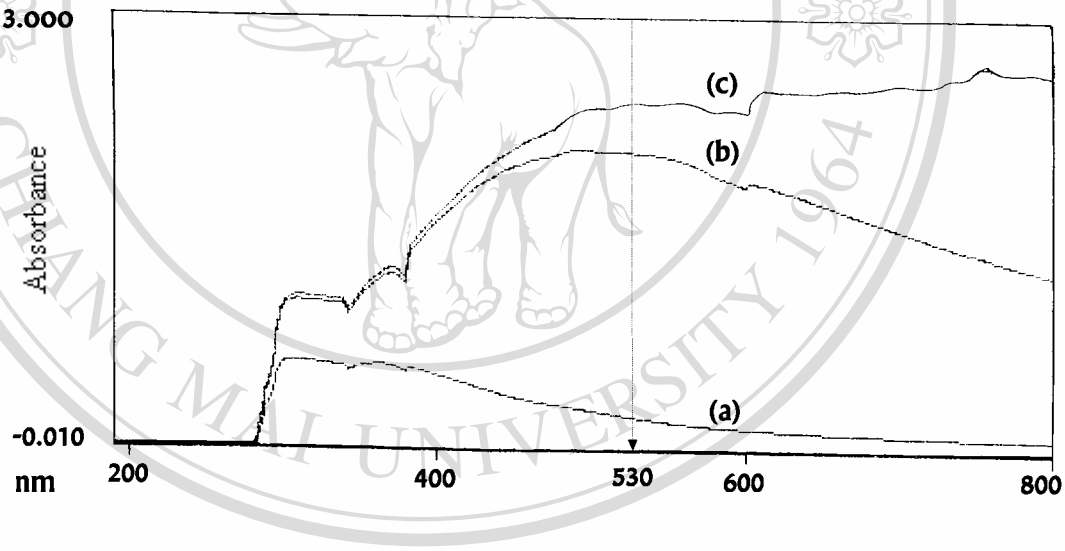

Figure 3.11 Absorption spectra of solutions containing Fe(II) (a) $5.0x10^{-4}$ M (b) $5.0x10^{-3}$ M (c) $5.0x10^{-2}$ M in 0.1 M NH_3/NH_4Cl buffer

3.1.2.6 Summary of the selected conditions

The selected conditions are summarized in Table 3.8.

Table 3.8 The optimum conditions of the FI-colorimetry procedure for the determination of Ca(II) , Mg(II), Fe(II) and Zn(II)

R1	$[Mg^{2+}]$ (in 0.1 M NH_3/NH_4Cl buffer)	0.008 M
	$[EDTA^-]$ (in 0.1 M NH_3/NH_4Cl buffer)	0.012 M
	Flow rate	5.1 mL/min
R2	Eriochrome Black T (EBT) (in 0.1 M NH_3/NH_4Cl buffer)	0.006% w/v
	Flow rate	2.7 mL/min
S.	Injection volume	50 µL
M	Mixing coil	75 cm
D	Detection wavelength	530 nm

3.1.2.7 Calibration graph and detection limit

The calibration graphs were constructed by using the selected conditions in Table 3.8. The results are represented in Table 3.9. Triplicate injections of standard solutions of Ca(II), Mg(II), Fe(II) and Zn(II) were performed. Calibration graphs were plotted between peak heights (mV) and the analyte concentrations (mM).

The detection limits (3σ) calculated from the calibration graphs (3) were 0.6, 0.7, 0.4, and 0.5 mM of Ca(II), Mg(II), Fe(II) and Zn(II) , respectively.

Table 3.9 Calibration results for the determination Ca(II) , Mg(II), Fe(II) and Zn(II)

Cation	Linear range (mM)	Calibration equation	r^2	Detection limit(mM)
Ca(II)	2.0-6.0	y = 20.13x – 18.07	0.9905	0.6
Mg(II)	2.0-6.0	y = 15.06x – 16.23	0.9890	0.7
Fe(II)	2.0-6.0	y = 31.40x – 42.41	0.9958	0.4
Zn(II)	2.0-5.0	y = 9.60x – 4.6	0.9875	0.5

3.1.2.8 Precision

Eleven injections of standard solutions of each metal ion were performed under the conditions in Table 3.8 to evaluate the precision. The results obtained are summarized in Table 3.10. The relative standard deviation is 1-2 %.

Table 3.10 Precision studies

No.	Ca(II)		Mg(II)		Fe(II)		Zn(II)	
	mV[a]	mM	mV[a]	mM	mV[a]	mM	mV[a]	mM
1	66.0	4.9	59.0	4.9	119.0	5.1	33.6	4.0
2	64.0	4.7	58.0	4.8	117.0	5.1	32.6	3.9
3	69.0	5.0	58.0	4.8	111.0	4.9	32.6	3.9
4	64.0	4.7	61.0	5.0	119.0	5.1	33.6	4.0
5	66.0	4.9	57.0	4.7	117.0	5.1	33.6	4.0
6	68.0	5.0	57.0	4.7	117.0	5.1	33.6	4.0
7	67.0	4.9	60.0	4.9	116.0	5.0	33.6	4.0
8	68.0	5.0	61.0	5.0	115.0	5.0	34.6	4.1
9	66.0	4.9	61.0	5.0	116.0	5.0	34.6	4.1
10	66.0	4.9	61.0	5.0	115.0	5.0	32.6	3.9
11	68.0	5.0	58.0	4.8	117.0	5.1	32.6	3.9
Mean		4.9		4.9		5.1		4.0
S.D.		0.1		0.1		0.1		0.1
%RSD		2		2		1		2

[a] Peak height

3.1.2.9 Applications

Applications of the proposed methods to determination of Ca(II), Mg(II), Fe(II) and Zn(II) in drug (4 samples), the mineral supplements (8 samples) were performed.

Sample preparations of different samples were investigated.

1. Calcium

Calcium carbonate, $CaCO_3$

The procedures were followed (4) for calcium carbonate tablets. 20 tablets were weighed and finely powdered. A portion of the powder was weighed equivalent to about 100 mg of calcium and dissolved with 10 mL of 1 M HCl. The solution was boiled until cleared solution was observed. After that it was diluted to 250 mL with deionized water. The treated sample was analysed by the proposed method and titrimetry as a standard method.

For the capsule sample, 20 capsules were weighed and opened to remove the powder. The accurate sample powder weight was obtained by subtraction for the difference.

2. Iron

Iron amino acid, Iron citrate

20 tablets were weighed and ground into fine powder. A portion of the powder was accurately weighed equivalent to about 30 mg of Fe(II) and transferred into a porcelain crucible. It was heated by a sand bath (270-350 °C) until a dry sample was obtained. It was transferred for ashing in a furnace at 500 °C for 3 hours to destroyed any the organic matters in the sample. The yellow residue was dissolved

with concentrated hydrochloric acid, and then it was filtered and diluted to 100 mL with deionized water.

Iron sulfate $FeSO_4$ (syrup)

The syrup sample from five bottles was blended together. Density of samples was determined by weighing of 10 mL of the well-mixed sample. 30 mg of Fe(II) sample was quantitatively transferred into a porcelain crucible. It was heated by a sand bath (270-350 °C) until a dry sample was obtained. It was ashed in a furnace at 500 °C for 12 hours. The yellow residue was dissolved with concentrated hydrochloric acid, and then it was filtered and diluted to 100 mL with deionized water.

Iron sulfate capsule $FeSO_4$

20 capsules were weighed and opened to remove the powder sample. The accurate sample weight was obtained by the difference in weight. An accurate amount of 30 mg of Fe(II) sample was quantitatively transferred into a porcelain crucible. It was heated by using a sand bath until a dry sample was obtained. And then it was transferred for ashing in a furnace at 500 °C.

It was found that, the sample was coated on the support material, which could not be destroyed although the ashing time was increased to 12 hours. So this sample preparation procedure was not suitable. However, a yellow residue was done similarly to other iron samples for the assay of this sample.

It was expected that the iron in the treated solution was in the form of Fe(III) species. This was not suitable for the procedures. The treatment with Zn/Hg prior to analysis was made (5).

3. Magnesium

Magnesium hydroxide $Mg(OH)_2$ or Milk of Magnesia

A drug density was determined by weighing a well-mixed sample solution in a 10 mL volumetric flask. 80 mg of Mg(II) sample was quantitatively transferred into 100 mL volumetric flask.

5 mL of 1 M HCl was added and the mixture was shaken until a clear solution was obtained. The solution was diluted to 100 mL with deionized water. After that, the treated sample was determined by the proposed method and titrimetry as a standard method (6).

Magnesium chelated

20 tablets were weighed and ground into fine powder. A portion of the powder was weighed equivalent to about 100 mg of Mg(II) and transferred into a porcelain crucible. It was heated by a sand bath (270-350 °C) until a dry sample was obtained. It was ashed in a furnace at 500 °C for 3 hours. The gray residue was dissolved with concentrated hydrochloric and then filtered and diluted to 100 mL with deionized water. After that, the treated sample was determined by the proposed method and titrimetry as a standard method (6).

4. Zinc

Zinc sulfate

20 tablets were weighed and ground into fine powder. A portion of the powder was weighed equivalent to about 20 mg of Zn(II). It was dissolved and diluted to 50 mL with deionized water. The treated sample was determined by the proposed method and titrimetry as a standard method (6).

Zinc Chelated

20 tablets were weighed and ground into fine powder. A portion of the powder was weighed equivalent to about 20 mg of Zn(II). It was dissolved with 10 mL of 0.1 M HCl. After that it was diluted to 50 mL with deionized water. The treated sample was determined by the proposed method and titrimetry as a standard method (6).

Results (Table 3.11) were compared with those obtained with titrimetry. Evaluation by t-test (7) at a 95% confidence level indicated that there are no significant difference of the results obtained by the proposed FI-colorimetry and titrimetry. Therefore, the proposed FI-colorimetry agreed well with titrimetry.

Table 3.11 The determination results of Ca(II), Mg(II), Fe(II) and Zn(II) in drug and minerals supplements by FI-colorimetry and titrimetry

Cation	Form of sample	Label	FI-colorimetry		Titrimetry	
		mg/tab	mg/tab	%Label[a]	mg/tab	%Label[a]
Ca(II)	Carbonate	600	573.0	95.5 ± 3.2	610.2	101.7 ± 0.8
	Carbonate	250	242.8	97.1 ± 1.5	246.0	98.4 ± 0.9
Fe(II)	Chelated	18	17.8	99.1 ± 1.1	17.3	96.1 ± 1.8
	Citrate	50	50.6	101.3 ± 3.8	47.5	95.0 ± 1.8
	Sulfate(syrup)	25[b]	24.0	96.0 ± 2.0	23.8	95.0 ± 2.0
	Sulfate(capsule)	150[c]	120.0	80.0 ± 2.0	121.5	81.0 ± 1.0
Mg(II)	Chelated (Brand A)	100	93.5	93.5 ± 2.2	97.6	97.6 ± 2.0
	Chelated (Brand B)	500	453.6	90.7 ± 2.3	466.9	93.4 ± 1.2
	Hydroxide	80[b]	83.4	104.3 ± 1.4	76.6	95.8 ± 2.7
Zn(II)	Chelated (Brand C)	15	13.7	91.1 ± 2.5	14.2	94.7 ± 0.3
	Chelated (Brand D)	15	15.4	102.8 ± 4.3	14.2	94.9 ± 2.0
	Sulfate	25	27.1	108.3 ± 4.0	25.7	102.8 ± 2.0

[a] Mean ± S.D. (n=3)

[b] Label mg/mL

[c] Label mg/capsule

The precisions of overall procedures were also estimated. %RSD of the determination of Ca(II) , Mg(II), Fe(II) and Zn(II) in some samples were 2.1, 1.9, 2.4 and 1.5%, respectively.

Table 3.12 Precision of overall procedure for determination of Ca(II) , Mg(II), Fe(II) and Zn(II)

Cation	Label (mg/tab)	Found (mg/tab)			RSD (%)
		Trial 1(n=5)	Trial 2(n=5)	Average ± S.D.[a]	
Ca(II)	250	238	239	238 ± 5.0	2.1
Mg(II)	80[b]	75	74	75 ± 1.4	1.9
Fe(II)	50	48	47	48 ± 0.7	2.4
Zn(II)	25	24	24	24 ± 0.6	1.5

a $$S.D. = \sqrt{\frac{\sum (x_i - \bar{x})^2}{N - k}}$$

where N is a number of replicate data (N = 10).

K is a number of trial experiments (K = 2).

b Label mg/mL

3.2 Development of FI-voltammetry and on-line derivatization for determination of ethanol

A FI-voltammetry and on-line derivatization was developed for determination of ethanol. The accomplished procedure is based on a converting ethanol (electroinactive species) into ethyl dithiocarbonate or xanthate (electroactive species) prior to detection by Osteryoung square wave voltammetry (OSWV).

3.2.1 Preliminary studies

3.2.1.1 Investigation the reaction behavior of batchwise system

Following the reports (8, 9), the reaction behaviour of batchwise system for derivatization of ethanol was investigated. Xanthate was prepared by a procedure below :

Ethanol 1 mL + NaOH 1.0 M 1 mL + TAHS 0.2 g + CS_2 1 mL

↓

Stirred for 5 min

↓

Adding deionized water 10 mL

↓

Stirred for 1 min

↓

Waited for 3 min

↓

Withdrawn 1 mL of the aqueous solution

↓

Diluted with deionized water

An aliquot (1 mL) of the xanthate solution obtained and 10 mL of supporting electrolyte (0.15 M NH_4Cl, 0.05 M KH_2PO_4, 0.03 M NaOH and $3x10^{-4}$ M eosin) were mixed in a voltammetric cell. The solution was purged with nitrogen for 5 min. And then it was determined by OSWV using a 693 VA processor (Metrohm, Herisau, Switzerland) under the conditions in Table 3.13.

Table 3.13 OSWV conditions for batchwise analysis

Parameter	Main			Auxiliary	
HMDE	Drop size	6			
OSWV	U. ampl	20	mV	Modul. freq	50 Hz
	t. step	0.15	s	Prep. cycles	0
	t. meas	2.0	ms	Meas. cycles	4
Working potential	U. start	-100	mV	U. step	12 mV
	U. end	-500	mV	Sweep rate	80 mV/s

Under preliminary condition, an effect of ethanol concentration (5.0-60.0% v/v) on peak current was studied. It was found that peak current of xanthate exhibit at -285 mV vs. Ag/AgCl as shown in Figure 3.12. The peak current was proportional to the original ethanol concentration.

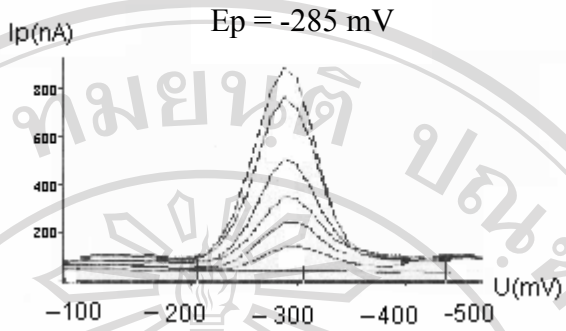

Figure 3.12 Osteryoung Square Wave voltammogram of ethyl dithiocarbonate from ethanol 5.0-60.0% v/v

- *Effect of phase transfer catalyst*

Chan and Lee (9) have been discussed that TAHS can be used for derivatization of ethanol with carbon disulfide and it did not exhibit any interference wave within the scanning potential range of the determination. For that, the maximum conversion of ethanol (0.1-0.5 g) required 6 hours.

Two series (with and without TAHS) of derivatized ethanol (0-60.0 % v/v) were investigated. The linear equations and correlation coefficients were $y = 21.639x - 71.104$ ($r^2 = 0.947$) and $y = 22.45x - 48.495$ ($r^2 = 0.9905$), respectively for with and without TAHS. From the results, it indicated that derivatization reaction without TAHS should be performed because it gave enough sensitivity and good correlation coefficient for determination ethanol in the working range.

-Effect of sodium hydroxide

Ethoxide was produced (see section 1.1.2.2) in the first step of the derivatization reaction of ethanol. A higher sodium hydroxide concentration should give a higher ethoxide concentration. A higher signal should then be observed.

An aliqout of 1 mL of individual concentration of NaOH solution (1.0, 2.0,3.0, 4.0 and 5.0 M) was used for derivatization reaction of ethanol (20% v/v) (as described in the section 3.2.1.1.). The results are represented in Figure 3.13. A higher concentration of NaOH gave a higher peak current.

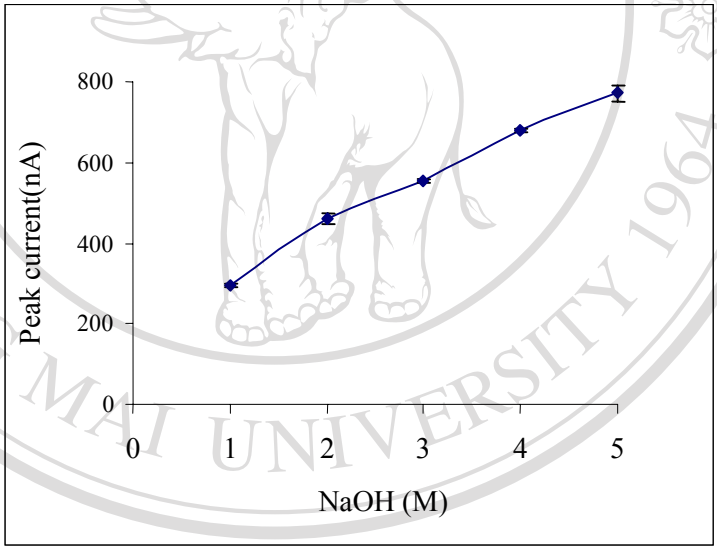

Figure 3.13 Effect of NaOH concentration

- *Effect of carbon disulfide volume*

Carbon disulfide is an organic reactant for derivatization reaction of ethanol. Various volume of CS_2 (1.0-8.0 mL) were using for the derivatizations of ethanol 15.0% v/v. The result (Figure 3.13) indicate that under the CS_2 volumes tried there was no significant difference in observed signal.

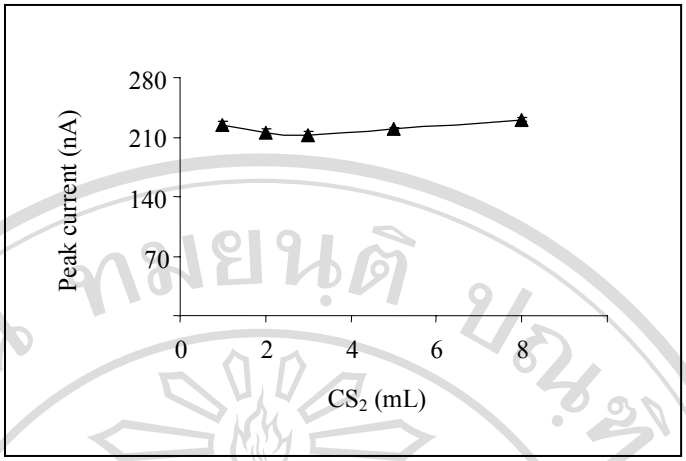

Figure 3.14 Effect of CS_2 volume

3.2.1.2 Study of OSWV parameters

For batchwise analysis, default parameters of OSWV could be employed. However some of parameters will be unsuitable for flow system. So each adjustment parameters were re-studied in the flow system. The expected potential waveform of OSWV is demonstrated in Fig 3.15.

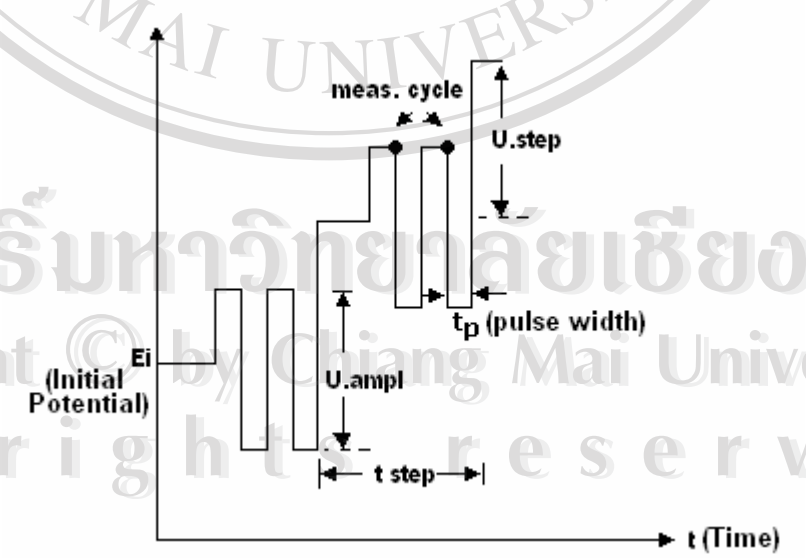

Figure 3.15 Potential waveform for OSWV

The selected values of some OSWV parameters are summarized in Table 3.14.

Table 3.14 Adjustment parameters of OSWV

Parameter	Default value	Variable value	Selected value for FI system	Figure
U. amp	20 mV	5-45 mV	45 mV*	3.16
U. amp	12 mV	4, 6, 12 mV	12 mV*	3.17
Modul. Freq.	50 Hz	50, 100 , 150 Hz	100 Hz**	3.18

* Selected from the experimental data which gave a highest current

** Selected from the experimental data which gave a good peak shape

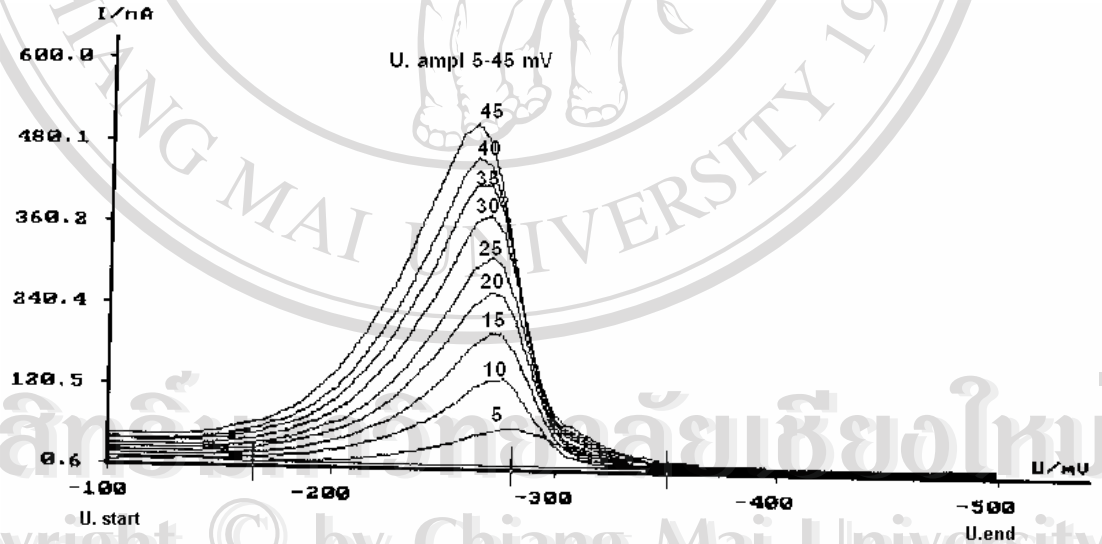

Figure 3.16 Voltammograms of derivatized ethanol 15% v/v at various potential amplitude (U. amp) 5-45 mV (Modul. freq = 50 Hz, U. step = 12 mV and Sweep rate 80 mV/s) (see Table 3.13)

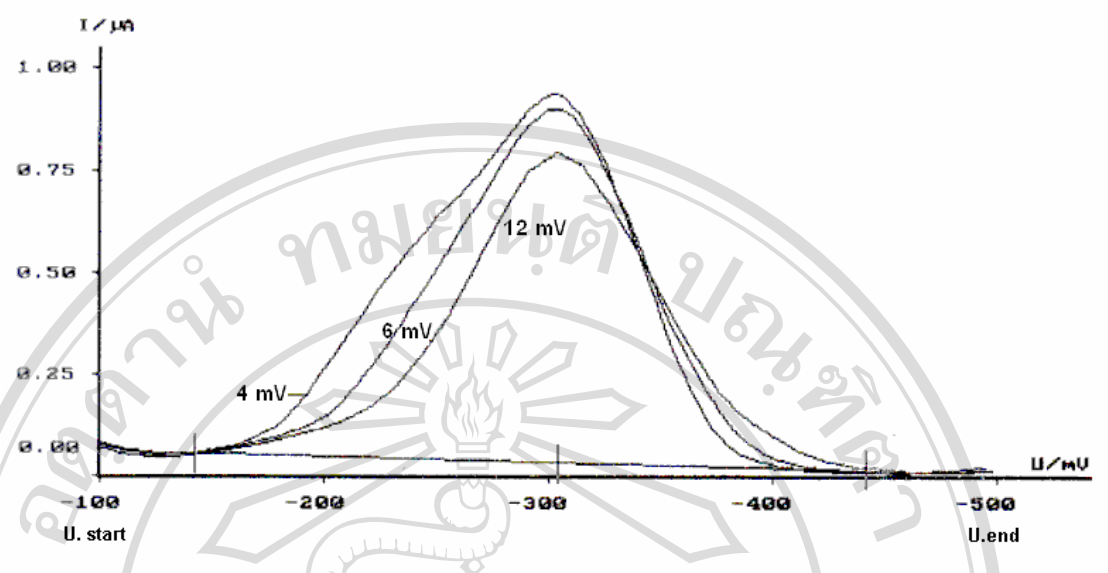

Figure 3.17 Voltammograms of derivatized ethanol 15% v/v at various potential step (U. step) 4, 6 and 12 mV (Modul. freq = 50 Hz, U. amp=20 mV and Sweep rate 80 mV/s) (see Table 3.13)

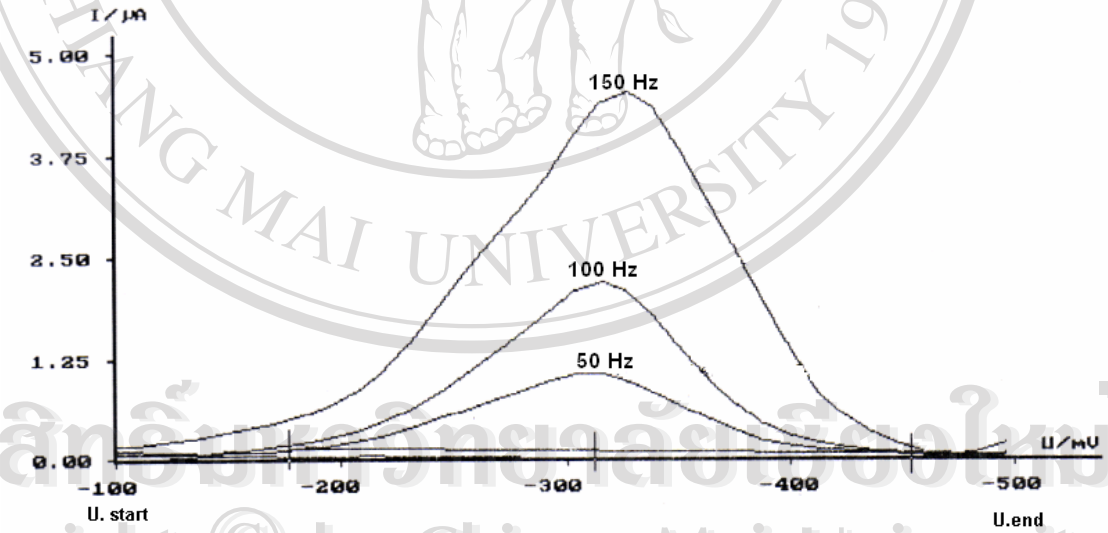

Figure 3.18 Voltammograms of derivatized ethanol 15% v/v at various Modul. freq = 50, 100 and 150 Hz (U. amp = 45 mV , U. step = 12 mV and Sweep rate 80 mV/s) (see Table 3.13)

The study of measuring cycle value, an auxiliary parameter of OSWV, is represented in Table 3.15.

Table 3.15 Size of data file from using various measuring cycle

Meas. Cycle	Ip(nA)	Size of data file (Bytes)
2	532.96 ± 5.4	3,796
4	540.06 ± 5.6	3,799
6	529.09 ± 5.5	3,795

Various measuring cycles (2, 4 and 6) were tested. It was found that no difference of peak current and data file size therefore measuring cycle 4 was selected.

3.2.1.3 Design and construction laboratory made HMDE flow cell

A laboratory made flow cell with HMDE has been reported by many workers (10-12). An adapted voltammetric cell and using an housing for HMDE (10), or using a flow adapter (59) were complicated and high volume of container. Other design, a capillary of HMDE was inserted in the flow cell and used a silicone tubing for tight fit at the tip of capillary (12). This may break the capillary. The designed flow cell is shown in Figure 2.3.

3.2.1.4 Testing the developed flow cell by analyzing zinc analyte model

A laboratory made flow cell for HMDE was tested by set up a flow system in Fig 3.18. Zinc was chosen as an analyte model. A measurement of diffusion current using HMDE (one drop of mercury) and scanning potential from -100 to -1000 mV. The peak potential at -630 mV vs. Ag/AgCl was observed .

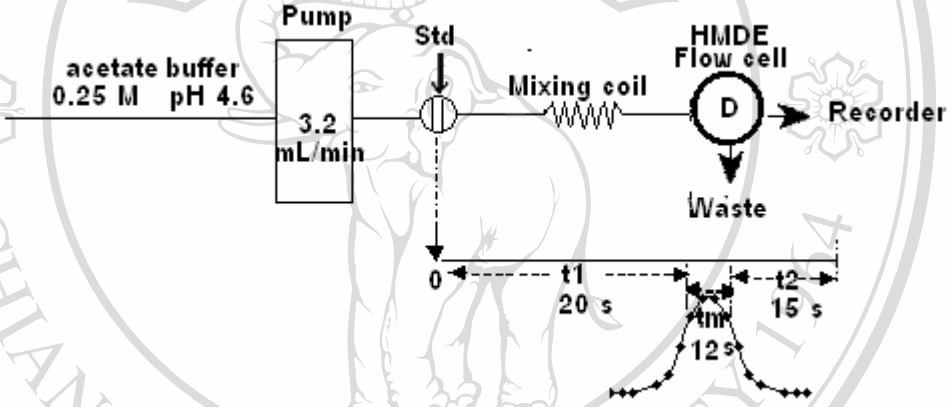

Figure 3.19 A manifold and of Zn(II) determination using a laboratory made HMDE flow cell and a time diagram of one injection when t1=waiting time 1 (injected zone move to flow cell) , tm = measured time (press start button for operate 693VA processor) and t2=waiting time 2 (time for signal down to baseline)

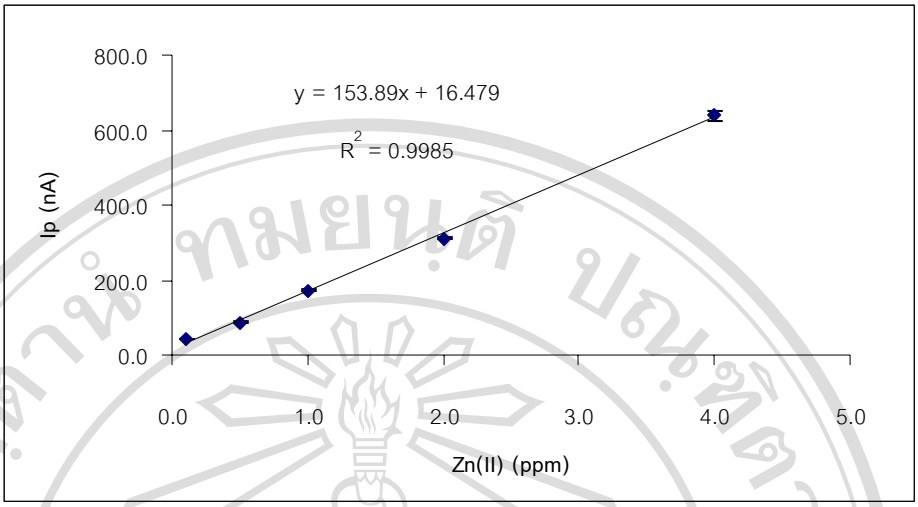

Figure 3.20 Calibration graph of Zn(II) 0.1-4.0 ppm using FI-voltammetry with a laboratory made flow cell

From Figure 3.20, it could be evaluated and the FI procedure with a laboratory made flow cell could be used to determination of zinc successfully. A good correlation coefficient and RSD values (1.7-5.0 %) were obtained. An on-line derivatization combine with this flow cell is left for a further work.

3.2.2 On-line derivatization combine with HMDE flow cell

The system is a double line system (Figure 2.2). A gas-diffusion is shown in Figure 3.21.

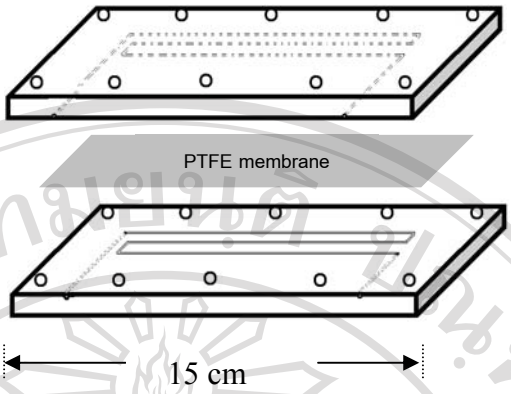

Figure 3.21 Gas- diffusion unit for carbon disulfide using in on-line derivatization system

Carbon disulfide was transported and diffused through PTFE membrane leading to the reaction in the other stream. The carbon disulfide stream was designed to flow back the same reservoir which was covered with deionized water.

3.2.2.1 Set up system

FIA manifold (Figure 2.2) was set up. Preliminary operation of the whole system was checked. A leakage of carbon disulfide to aqueous phase problem was solved by using the equal flow rate of each line and using long back pressure coil (270 cm). Carry over was also checked by observation of dye color prior to operation of the on-line derivatization experiment. The values of starting time (t_1) and waiting line t_2 (similar to that in section 3.2.1.4) were investigated.

Table 3.16 Starting time (t1) and waiting time(t2)

Equal flow rates of NaOH and CS_2 (mL/min)	Time (s)	
	t_1	t_2
0.6	140	50
1.1	90	30
2.0	50	20

3.2.2.2 Effect of delay of starting time

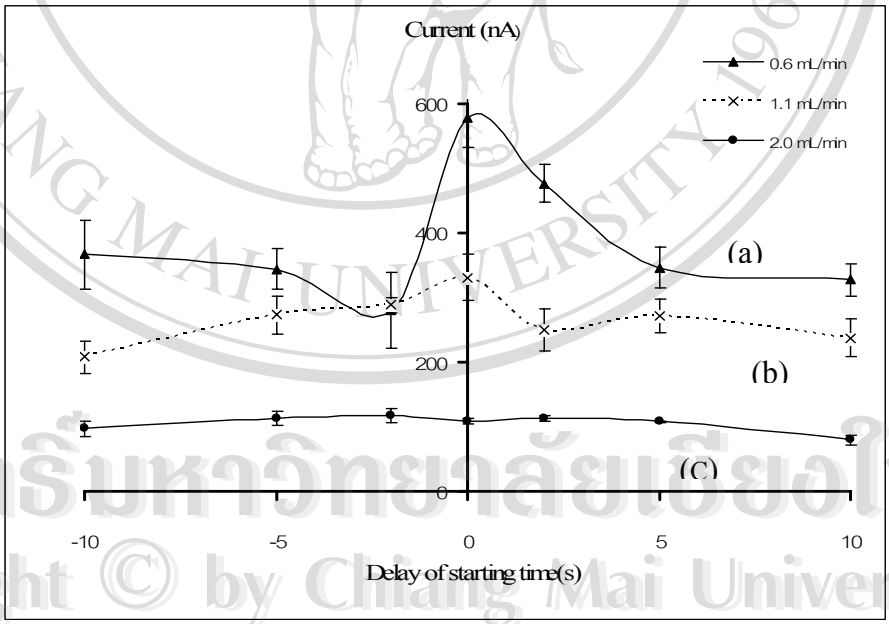

Figure 3.22 Plots of delayed starting time vs. peak current of derivatized ethanol 25 % v/v from on-line system using equal flow rate of (a) 0.6 mL/min (b) 1.1 mL/min and (c) 2.0 mL/min

The currents due to derivatized ethanol (xanthate) 25% v/v using equal flow rates of CS_2 and NaOH (0.6, 1.1 and 2.0 mL/min) were plotted in the Figure 3.22. A lower flow rate (0.6 mL/min) gave a higher signal because of a longer reaction time of the derivatization before flowing through a flow cell. However, a flow rate of 0.6 mL/min was not suitable because a higher of a higher deviation. The flow rate of 2.0 mL/min was selected because enough sensitivity could be obtained and with less deviation.

The voltammetric detection for current with HDME can be made by pressing a start button of the processor. Due to manual operation, it was necessary to check the signal if the time delayed for ± 10 s. The currents due to derivatized ethanol(xanthate) 25% v/v measured at 0, ±2, ± 5 and ±10 s. The results are summarized in Figure 3.22. It was found that a consistent current was observed in system C (2.0 mL/min). It could be concluded that the appropriate system for the on-line derivatization and FI-voltammetry at 2.0 mL/min of NaOH and CS_2.

3.2.2.3 Calibration graph

Figure 3.23 depicts a calibration graph.

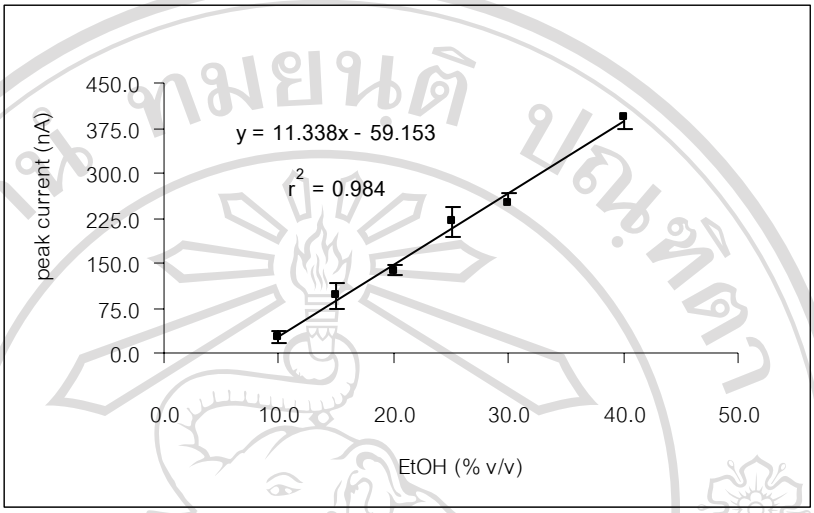

Figure 3.23 Calibration graph

3.2.2.4 Precision

Precision was studied as summarized in Table 3.17.

Table 3.17 RSDs (%) for the determination of ethanol by FI- on-line derivatization voltammetry

Ethanol (%v/v)	RSD (%) (n=3)
10.0	33.4
15.0	22.0
20.0	5.4
25.0	10.9
30.0	7.3
40.0	5.1

3.2.3 Suggestion for FI- on-line derivatization voltammetry for determination of ethanol

The proposed procedure gave higher RSD (maximum value 33%). The sample rate for duplicate injections was 10 samples per hour.

Precautions and further investigation should be considered.

- Design of HMDE and flow cell chamber and its positioning.
- Type of pump tubing (organic resistance) for carbon disulfide.
- Type of membrane (PTFE membrane) for gas diffusion device.
- Waste due to carbon disulfide and mercury as they are hazardous substances.