APPENDICES

APPENDIX A

1. FIA dispersion [2, 4, 5]

The design of FIA system is based on the theory of dispersion. The shape of the resulting zone is decided by two main processes: convective transport and diffusion transport. Convective transport happens from mechanical flow driven by a propelling system. It consists of two processes: turbulent and laminar flows (Figure 1A(a)). The turbulent flow occurs in moving of liquid with air-segmentation. The laminar flow occurs for non-segmented solutions in narrow tubing. In FIA, laminar flow is predominant feature and causes the sample zone to spread in a parabolic shape due to higher velocity at the center of tubing.

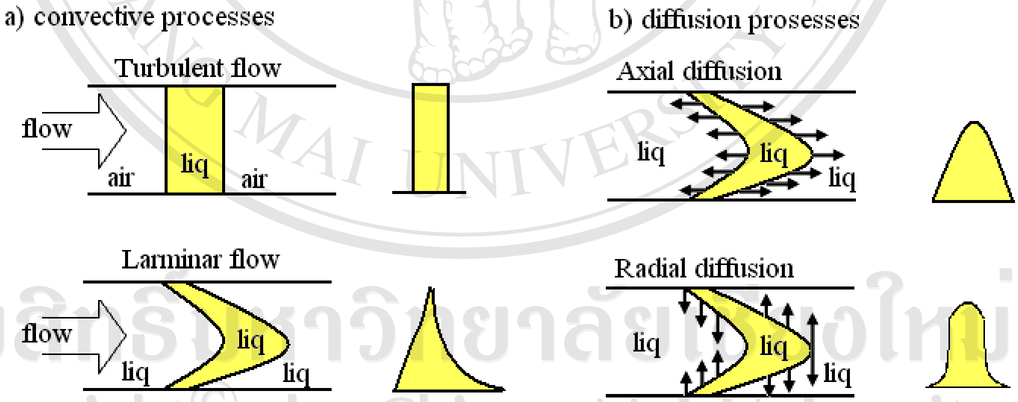


Figure 1A General types of transport in closed tubes and the recorded profiles at the detector [4].

Diffusion transport zone is caused by concentration of gradients. There are two types of diffusion processes: axial and radial, as shown in Figure 1A(b). Axial diffusion zone is insignificant compared to convective flow, but the radial diffusion zone contributes more significantly to sample dispersion. This process, termed “secondary flow”, results in a washout effect accounting for the low joint contamination of samples continuously injected into the carrier stream and also assists to limit band spreading. At low flow rate it may even be the major process for dispersion. In fact, flow injection analyses regularly accomplished under conditions in which dispersion by both convection process and radical diffusion happens as shown in Figure 2A(c).

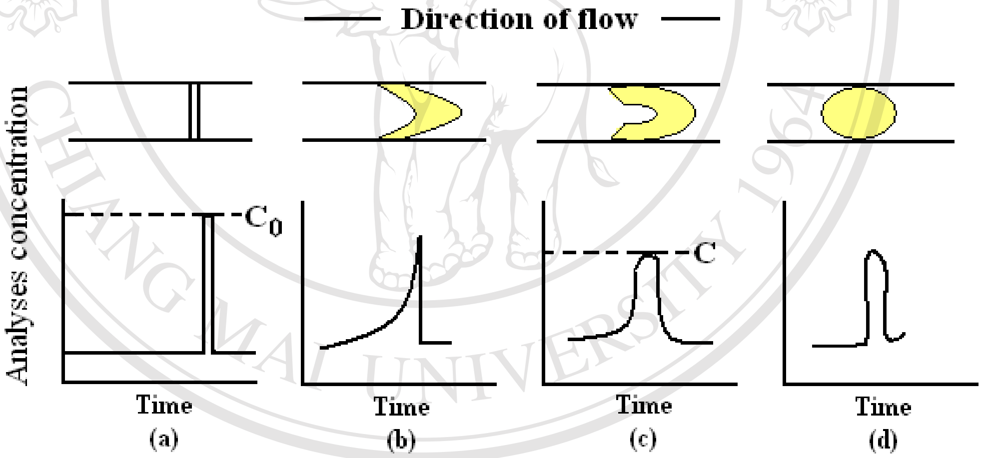


Figure 2A Effects of convection and diffusion on concentration profiles of analyses at the detector: (a) no dispersion; (b) dispersion by convective process; (c) dispersion by convective process and radical diffusion; (d) dispersion by diffusion [6].

A simple dispersion process experiment is used to follow dispersion by measure dispersion by means of the dispersion coefficient as shown in Figure 3A. A sample solution zone is homogeneous and has the primary concentration C^0 that would yield a square signal. The height of square signal could be related to the sample concentration (Figure 3A, left). When the sample solution is injected to carrier stream, it forms a dispersed zone whose form depends on the geometry of the channel and flow velocity. Therefore, the response curve has the shape of a peak reflecting a continuum of concentrations series (Figure 3A, right), which composed of a certain concentration of individual elements of fluid.

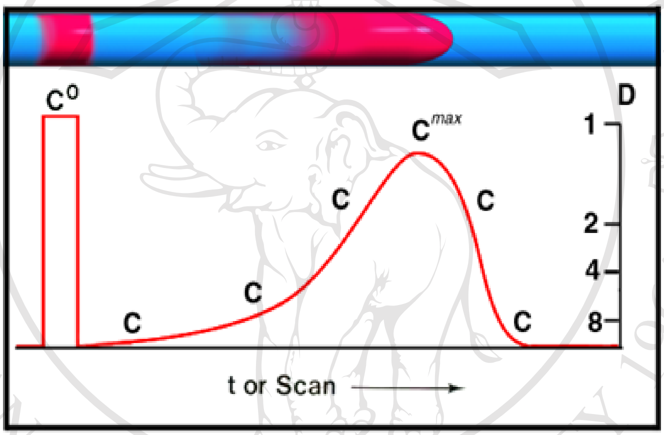


Figure 3A Dispersed sample zone in flow system; an original homogeneous sample zone (top left) disperses during its movement through a tubular reactor (top center), thus changing from an original square profile (bottom left) of original concentration C^0 to a continuous concentration gradient with maximum concentration C_{max} at the apex of the peak [2].

The dispersion coefficient (D) is defined as the proportion of the analyte concentration before and after the dispersion takes place:

$$D = C^0 / C_{max}$$

Where C^0 is the original concentration of injected sample solution and C_{max} is the concentration of dispersed sample solution in flow line.

Dispersion may be regarded in terms of the three general categories:

(1) Low dispersion systems ($D < 2$) are used when one intends to prevent the primary concentration of the analyte in the injected fluid zone being diluted by the carrier solution.

(2) Medium dispersion systems ($2 < D < 10$) are also employed in single channel FI systems, where reagents are employed as carrier streams, to attain adequate mixing of sample and reagent.

(3) Large dispersion ($D > 10$) and medium systems are employed to achieve sample dilutions, typically to bring the analyte concentration into an appropriate range for readout.

The FI experimental parameters which may influence the dispersion including sample volume, carrier flow rate and flow rate ratio between sample carrier and merging reagent and geometrical dimensions and configurations of manifold components. Varying the values of these parameters confers a significant degree of control over the dispersion characteristics and facilitates optimization of a flow injection system for many diverse applications.

2. FIA Instrumentation [6, 7]

The elementary components of FIA consist of a propelling system, a sample introduction system, a transport and reaction system and a detection system.

2.1 Propelling system

The Propelling system is a critical component to drive the solution in the FIA systems. It would be highly reproducible timing, pulse less and reproducible flow rate in fluid propulsion. There are three types of liquid transport devices which are usually employed in the FIA systems namely; pressurized bottle, peristaltic pump and syringe pump. A peristaltic pump is a extremely versatile propulsion device, which is still the most often used drives for FIA systems and also in other continuous flow analysis systems, because it may provide several channels according to diameter of tubing, equivalent or different pumping rates may be obtained. It consists of a motor-driven wheel with peripherally placed rollers and a pressure cam which is compressed against the rollers. One or several pump tubing are attached so that they rest on a minimum of the rollers at all times (Figure 4A). The flow rates can be easily adapted by rotation rate and I.D. of the peristaltic tubing. A pump has to have at least eight rollers, in order to make a flow with small regular pulses – otherwise, the irregular flow rate will affect dispersion and repeatability of the assay.

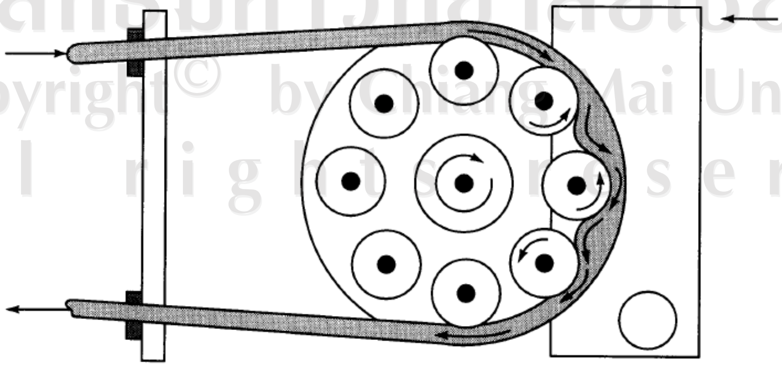


Figure 4A The rollers of a peristaltic pump and the pump tubing [7]

2.2 Sample introduction system

The sample introduction unit is the device to supply the necessary requirement to inject the precise and reproducible sample volumes into the carrier stream. To complete operation system, a clearly-stated zone of sample solution is injected rapidly as a pulse or plug of solution; in addition, the injections have to not interrupt the flow of the carrier stream. The earliest injection system employed in FIA is not use syringe, but it is difficult to guarantee a constant volume. The rotary valve (Figure 5A) was consequently used for introduction sample into flow line. The important features of valves appropriate in FIA are high precision, rapid switching, pressure limit of about 100 psi and ability to inject sample volumes from a few micro litters to several micro litters [8]. The other designs for injection system are relative injector, solenoid valve, multi-injection, selection valve and home-made low-cost rotary valve system.

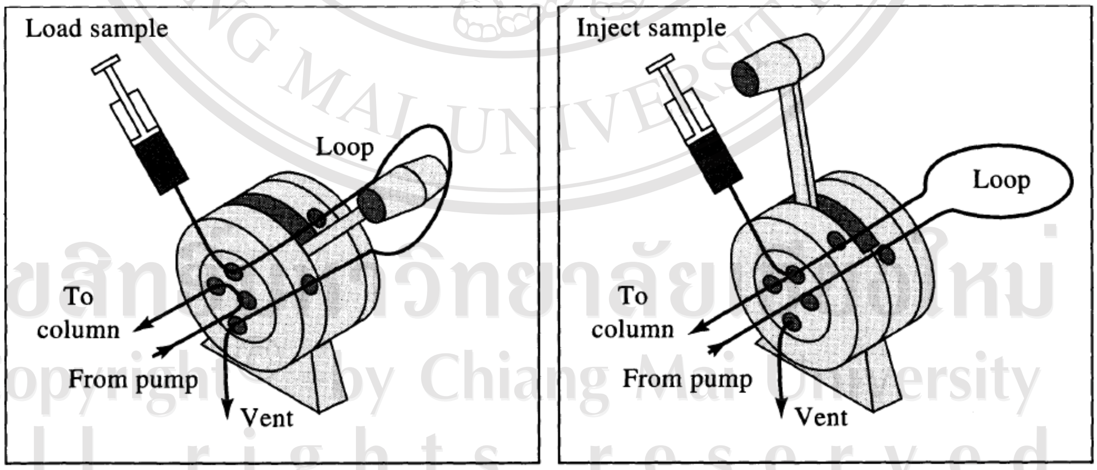


Figure 5A The system of an injection valve [8]

2.3 Transport and reaction system

The transport and reaction system consists of connectors, reactors and other manifold apparatuses (Figure 6A). In FIA, there is a wide variety of connectors, such as either dual (linear or V-shaped) or triple (T-, Y- or W-shaped) ways. They may be connected to the different components or extended either by push fitted, threaded and permanently glued. Connectors made from several polymers are fitted with ferrules that are designed to hold tubing while the connector nut is being strained. If all FIA systems work at low pressure, it is not necessary to employ connectors designed for HPLC. Nevertheless, it is very essential to use nuts, ferrules and fittings from a single industrialist as products from different sources are often mismatched and subsequent in leaks of solution in flow lines. There are many tubing materials obtainable for construction of mixing coils. The commonly used reactors are made from plastic tubing which can be coiled, knitted, or knotted e.g. Teflon (0.3-1.0 mm, i.d.) and polyethylene or polypropylene tubing. Coiled reactors are most frequently employed, being suitable to make. More efficient than coiled are hand knitted [9]. Mixing chambers of system and glass bead columns are utilized as mixing reactor. Moreover, Stainless steel is another material that has the advantages of heat conductivity, gas impermeability and surface properties that minimalize protein separation.

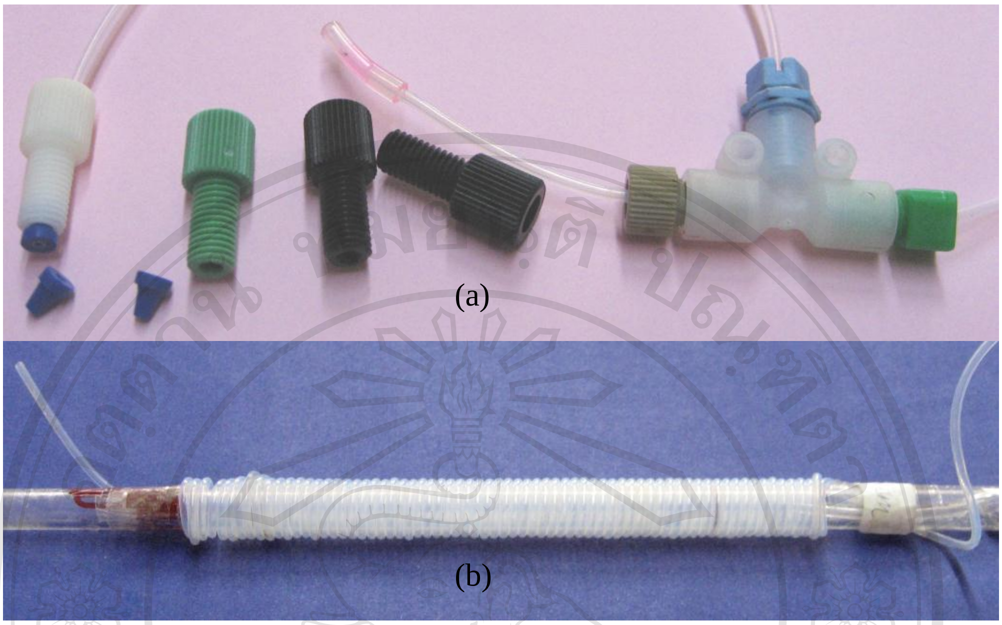


Figure 6A (a) Tubing connectors, ferrule and T-connector (b) Teflon connector coil

2.4 Flow through cell and detection methods

Flow cell are made from a variety of material such as some polymers, Plexiglas, quartz and stainless steel**.** Quartz flow cell (Figure 7A) is normally employed for FIA technique. Furthermore, Z-flow cell (Figure 8A (a)) is the normally use for UV-Vis spectrophotometry, Ion selective electrode flow cell (Figure 8A (b)) is designed for electrochemical detection system and the fused silica windows with fiber optic connection flow cell (Figure 8A (c)) is employed for fluorescence technique. Nevertheless, all of flow cell comes with Upchurch fittings, tubing and instructions.

The detection module in a FIA system depends on product that has to be detected. Usually the photometric absorption detector is used to inspection of color product. This unit permits continuous monitoring of a given property of the reaction product to offer qualitative and quantitative data of the analyte of interest. Several detection devices can be used for flow through detection of FIA such as spectrophotometer (UV-visible) and atomic absorption spectrometry. Moreover, electrochemical techniques such as amperometric and potentiometric methods, have gained new life by connection them to flow-based sample handling techniques such as FIA. Even AAS, ICP-MS and ICP-AES, and even GC have been coupled to FIA manifolds [11]. Nowadays, laptops have been combined to store measured peak heights, peak areas and peak widths of the FIA signals. The output signal from the detector is recorded as a peak by means of chart recorder, microprocessor or computer software. There are two software packages that are most popular. The first is *LabVIEW* [12] that is appropriate for the skilled programmer for controlling complex instrumentation. It's generally employed for data procurement and instrument control system for several platforms using *Microsoft*, *Unix* and *Linux* operation system. The second software is *FIAlab for Windows* [13] that is designed for FIA and SIA techniques. It is designed for controlling of FIA and SIA systems. It might control of sample injection, microfluidic manipulations, data collection and data assessment. It also controls many custom peripherals such as valves, pumps and detectors.

Figure 7A Quartz flow cells for FIA

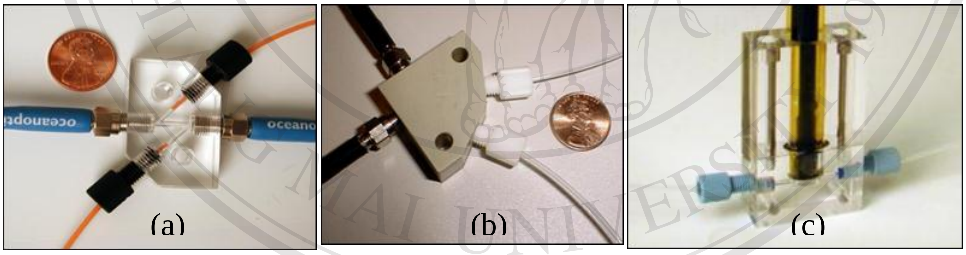
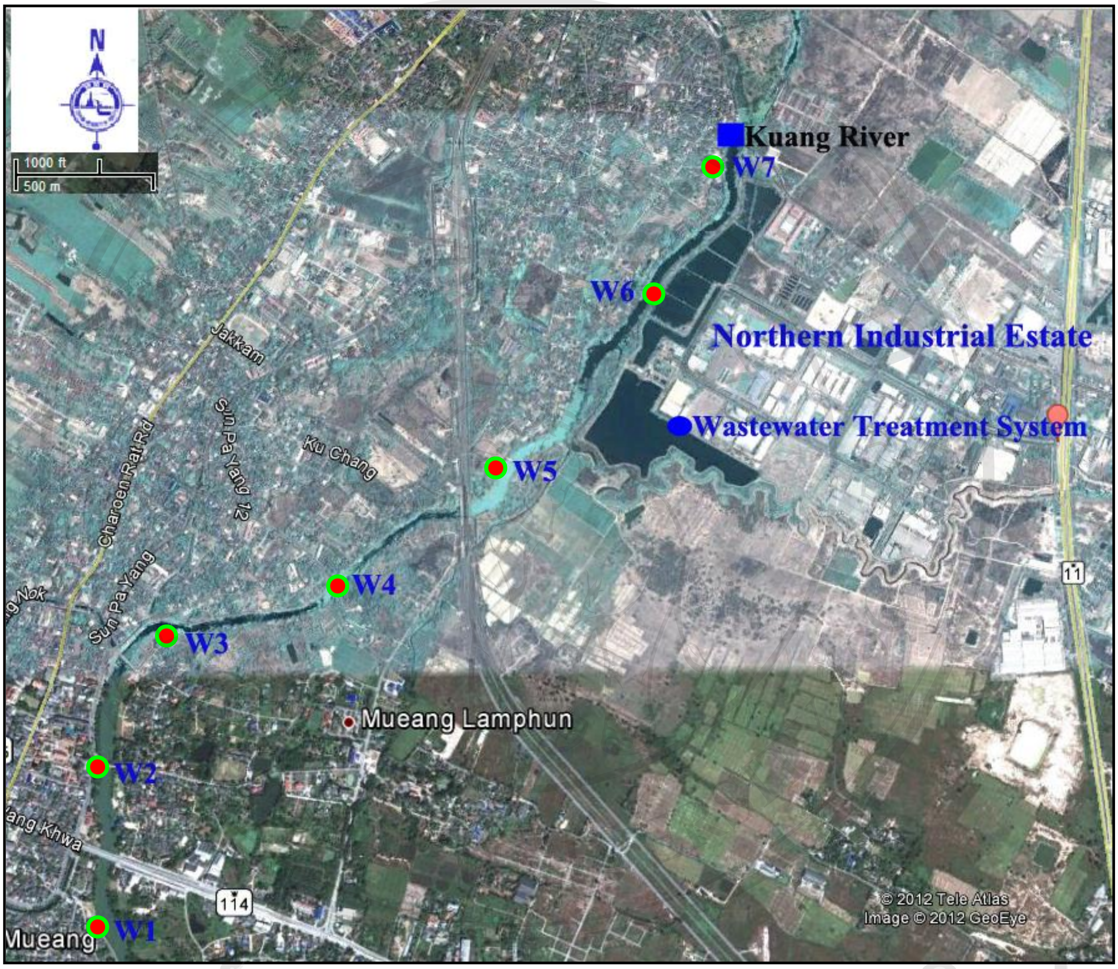


Figure 8A A variety of flow cells: (a) z-cell, (b) fluorescence flow cell and (c) ISE flow cell [10]

APPENDIX B

Water sampling site for determination of Zinc (W1-W7) (●)



APPENDIX C

1. The student t-Test [259]

$$t = \frac{\bar{x}_d \sqrt{n}}{S_d}$$

$$S_d = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n - 1}}$$

$$\bar{x}_d = \frac{\sum x_d}{n}$$

Where;	x_d	the difference between two method
	$\bar{x}_d$	the mean difference
	S_d	the standard deviation
	n	number of sample
	n-1	number of degree of freedom

The Table 1C gives the concentration of Ni(II) (mg L^{-1}) determined by the proposed FIA and FAAS methods.

Table 1C Calculation of *t*-test for Ni(II) determination of FIA.

Water samples	**Concentrations (mg L^{-1})**		$\bar{x}$	s_d	$\bar{x}_d$	$t_{calculated}$
	FIA*	**FAAS***				
E1	0.281	0.273	0.281	0.005	0.00767	2.656
E2	0.397	0.402	0.397	0.003	-0.00467	-3.080
E3	0.308	0.315	0.308	0.004	-0.00700	-2.806
E4	0.434	0.442	0.434	0.004	-0.00800	-3.893
E5	0.371	0.373	0.371	0.001	-0.00233	-3.240
E6	0.446	0.449	0.446	0.001	-0.00267	-3.703
E7	ND**	ND**	-	-	-	-
E8	0.506	0.497	0.506	0.004	0.00933	3.858

*average of triplicate results

For example:**E1**

$$\bar{x} = \frac{\sum x_i}{n}$$

$$= \frac{0.282 + 0.275 + 0.285}{3}$$

$$= 0.281$$

$$\text{Sd} = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n - 1}}$$

$$= \sqrt{\frac{0.000052667}{3 - 1}}$$

$$= 0.004$$

$$\bar{x}_d = \frac{(0.282 - 0.273) + (0.275 - 0.273) + (0.285 - 0.273)}{3}$$

$$= 0.00767$$

$$t = \frac{\bar{x}_d \sqrt{n}}{S_d}$$

$$= \frac{0.00767\sqrt{3}}{0.005}$$

$$= 2.656$$

The Ni (II) content found in water samples by the proposed FIA procedure and FAAS was compared and then the results were given in Table 1C. The calculated value of *t* are less than the *t* value from Table 3C (4.30) for two degrees of freedom signifying that results obtained by both methods display no significant difference at 95% confidence intervals.

Table 2C Calculation of *t*-test for Zn (II) determination using μFA

Water samples	**Concentrations (mg L^{-1})**		$\bar{x}$	s_d	$\bar{x}_d$	$t_{\text{calculated}}$
	μFA*	**FAAS***				
W1	0.221	0.233	0.221	0.0078	-0.01200	-2.661
W2	0.160	0.165	0.160	0.0026	-0.00500	-3.273
W3	0.345	0.341	0.345	0.0025	0.00367	2.524
W4	0.455	0.451	0.455	0.0020	0.00400	3.464
W5	0.474	0.471	0.474	0.0015	0.00267	3.024
W6	0.185	0.171	0.185	0.0089	0.01400	2.728
W7	ND**	ND**	-	-	-	-

*average of triplicate results

** not detected

For example: **W1**

$$\bar{x} = \frac{\sum x_i}{n}$$

$$= \frac{0.212 + 0.225 + 0.226}{3}$$

$$= 0.221$$

$$S = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n - 1}}$$

$$= \sqrt{\frac{0.000122}{3 - 1}}$$

$$= 0.0078$$

$$\bar{x}_d = \frac{(0.212-0.233)+(0.225-0.233)+(0.226-0.233)}{3}$$

$$= -0.0120$$

$$T = \frac{\bar{x}_d\sqrt{n}}{S_d}$$

$$= \frac{-0.0120\sqrt{3}}{0.0078}$$

$$= -2.661$$

The Zn(II) contents found in water samples by the proposed µFA procedure and FAAS was compared and then the results were given in Table 2C. The calculated value of *t* are less than the *t* value from Table 3C (4.30) for two degrees of freedom indicating that results obtained by both methods show no significant difference at 95% confidence intervals.

Table 3C Values of *t* for various levels of confidence interval.

Degrees of freedom	**Confidence interval**		
	90%	**95%**	**99%**
1	6.31	12.70	63.7
2	2.92	4.30	9.92
3	2.35	3.18	5.84
4	2.13	2.78	4.60
5	2.02	2.57	4.03
6	1.94	2.45	3.71
7	1.90	2.36	3.50
8	1.86	2.31	3.36
9	1.83	2.26	3.25
10	1.81	2.23	3.17
15	1.75	2.13	2.95
20	1.72	2.09	2.84
30	1.70	2.04	2.75
60	1.67	2.00	2.66
α	1.64	1.96	2.58

APPENDIX D

1. Preliminary studies

The absorption spectra of the yellow complex between Fe(III)-CTC and the reagent blank were measured over a range from 300-550 nm using Jenway 6305 spectrophotometer. CTC displayed the absorption maximum at 355 nm in phosphate buffer pH 7.5 medium. The absorption maximum of the Fe(III)-CTC complex was at 420 nm in the same medium. However, the greatest sensitivity for determination of Fe(III) using µrFA system with the USB4000 spectrometer as detector, the optimum wavelength were examined again in further studies. Moreover, the mole-ratio of Fe(III)-CTC is 1:2 (Figure 1D) [263].

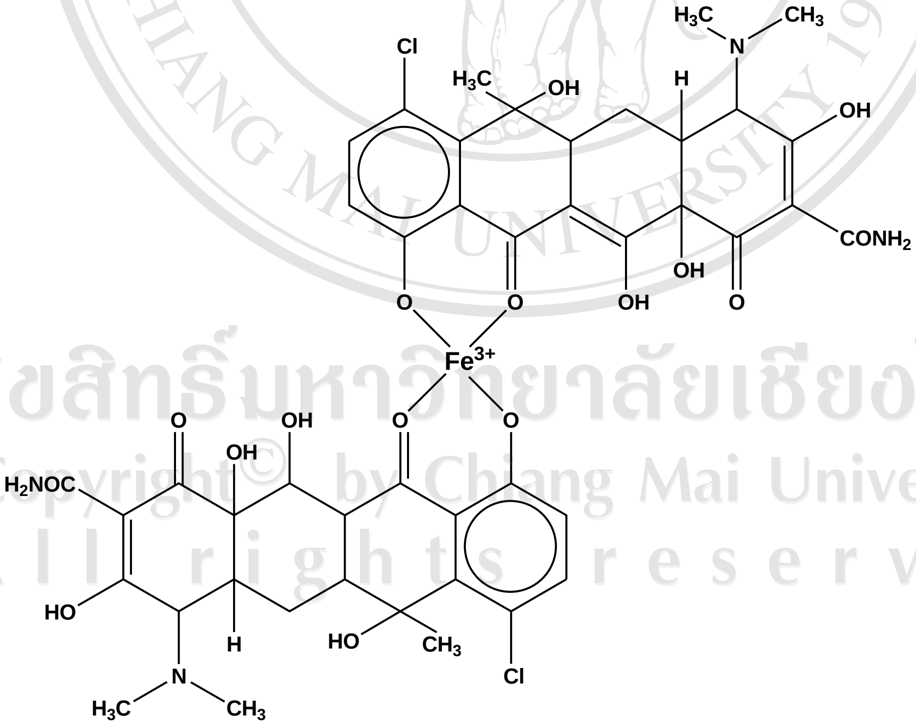


Figure 1D Complex of iron(III) ions and chlortetracycline

2. Optimum conditions for Fe(III) determination

The variable by univariate method was studied to select the optimum conditions for the micro reverse flow analysis system for determination of Fe(III). The value of one variable was varying while fixed the other variables value. The optimum value was selected form the greatest sensitivity of each parameter (slope of calibration curve). The objective of this work was to develop a novel diode laser mini-CNC machine for fabrication of PMMA chip and applied chip to µrFA spectrophotometric method for determination of Fe(III) based on reaction between Fe(III)-CTC complex in aqueous solution. It was found to be more satisfactory to inject the reagent solution into the Fe(III) streams rather injecting into the phosphate buffer stream before merging. In all experiments, five replicate were accomplished and all optimum values were selected by adjudging from the sensitivity of standard curve and reproducibility of the peak heights obtained. The parameters were studied including wavelength, pH, HNO_3 concentration in Fe(III) solution, CTC concentration, flow rate and reagent injection volume. Preliminary experimental conditions used were shown in Table 1D.

Table 1D The studied range for the optimization of all parameters of µrFA

Variable	**Studied range**
Wavelength (nm)	430-450
pH	7.0-9.5
Concentration of HNO_3 (x 10^{-2} mol L^{-1})	0.1-2.0
Concentration of chhlortetracycline (x 10^{-3} mol L^{-1})	0.4-2.0
Flow rate (µL min^{-1})	10-50
Reagent injection volume (µL)	2-10

2.1 Optimum wavelength

It is necessary to study the optimum wavelength that provide the maximum absorption of the complex between Fe(III) – CTC to obtained the greatest sensitivity. The optimum wavelength on sensitivity (slope of standard curve) for Fe(III) determination was inspected over the range 430 - 450 nm by the proposed µrFA system. The results shown in Table 2D and Figure 2D indicated that the highest sensitivity was obtained when the absorbance was measured at 440 nm. Furthermore, increasing in wavelength the sensitivity decreased gradually. Therefore, the optimum wavelength at 440 nm was selected for the further studies condition.

Table 2D Effect of wavelengths on the sensitivity of Fe(III)-CTC complex

λ (nm)	**Δ Peak height (AU) obtained from the standard Fe(III) (mg L^{-1})**					**y = mx + c**	**r^2**
	1.0	**1.5**	**2.0**	**2.5**	**3.0**		
430	0.063	0.078	0.092	0.102	0.115	0.0256x + 0.0388	0.9958
435	0.067	0.082	0.095	0.110	0.123	0.0281x + 0.0392	0.9993
440	0.066	0.083	0.100	0.116	0.128	0.0312x + 0.0365	0.9954
445	0.063	0.077	0.087	0.102	0.118	0.0272x + 0.0350	0.9939
450	0.061	0.074	0.083	0.097	0.110	0.0240x + 0.0367	0.9968

*average of five replicate results

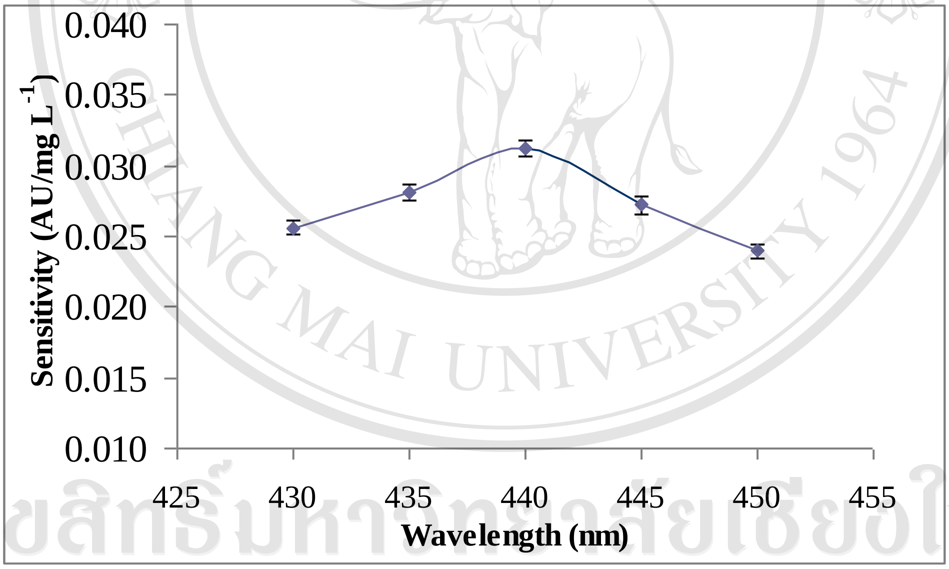


Figure 2D Effect of varying wavelengths on sensitivity for Fe(III) determination

2.2 Effect of pH on the formation of complex

In common, most complexation reactions between metal ions and any ligand are depending on pH. It is necessary to investigate the optimum pH to achieve the great selectivity for spectrophotometric determination of metal. Moreover, pH also effect on the stoichiometry of the complexes leading to occur hypsocgromic shift or bathochromic shift of the maximum absorption wavelength. Therefore, it is essential to examine the optimum pH on the absorption spectra of the reaction between Fe(III) and CTC. The complex formed by the reaction between Fe(III) and CTC in phosphate buffer medium was inspected over the pH range 7.0 – 9.5. The results were displayed in Table 3D and Figure 3D. It was found that when the pH values of phosphate buffer stream were up to 8.0, the greatest sensitivity was achieved. Subsequently, the pH exceeded 8.0, the sensitivity were slightly increased. The effect of pH by the presented procedure was in good agreement with those described by Pena et al. [264] and Liawruangrath et al. [265]. Therefore, pH 7.5 was selected as optimum to obtain the good sensitivity.

Table 3D Effect of pH on the sensitivity of Fe(III)-CTC complex

pH	Δ Peak height (AU) obtained from the standard Fe(III) (mg L^{-1})					y = mx + c	r^2
	1.0	1.5	2.0	2.5	3.0		
7.0	0.067	0.083	0.100	0.116	0.129	0.0314x + 0.0365	0.9974
7.5	0.075	0.100	0.125	0.145	0.171	0.0474x + 0.0248	0.9986
8.0	0.088	0.118	0.144	0.171	0.198	0.0546x + 0.0346	0.9994
8.5	0.087	0.117	0.145	0.170	0.197	0.0546x + 0.0340	0.9989
9.0	0.089	0.118	0.147	0.172	0.199	0.0548x + 0.0354	0.9991
9.5	0.090	0.118	0.148	0.173	0.200	0.0550x + 0.0358	0.9992

*average of five replicate results

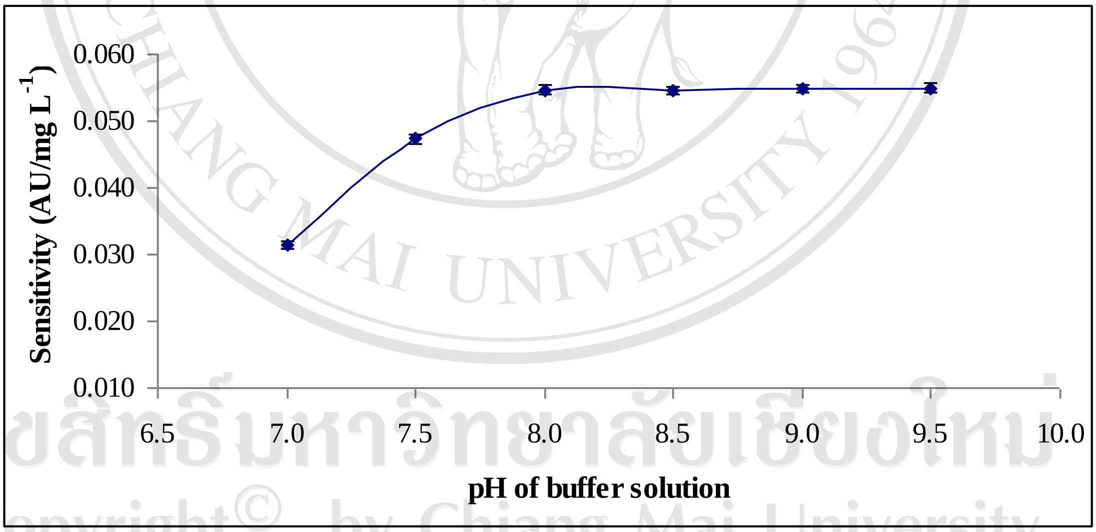


Figure 3D Effect of varying pH on sensitivity for Fe(III) determination

2.3 Effect of HNO_3 concentration in Fe(III) standard solution

The HNO_3 concentration in Fe(III) standard solution on the sensitivity was investigated. Several concentrations over the range 0.1 x 10^{-2}-2.0 x 10^{-2} mol L^{-1} were studied. The results were shown in Table 4D and Figure 4D. It was seen that, the sensitivity increased with increasing in HNO_3 concentration up to 0.5 x 10^{-2} mol L^{-1} further increase in HNO_3 concentration gave rise to slightly decrease in sensitivity due to the stability of CTC was reduced under strong acid and alkaline solution [265]. Hence, the concentration of HNO_3 0.5 x 10^{-2} mol L^{-1} was selected as optimum to obtain the good sensitivity.

Table 4D Effect of HNO_3 concentration on the sensitivity of Fe(III)-CTC complex

[HNO_3] (x 10^{-2} mol L^{-1})	**Δ Peak height (AU) obtained from the standard Fe(III) (mg L^{-1})**					**y = mx + c**	**r^2**
	1.0	**1.5**	**2.0**	**2.5**	**3.0**		
0.1	0.088	0.117	0.146	0.170	0.198	0.0546x + 0.0346	0.9989
0.3	0.093	0.125	0.156	0.190	0.220	0.0638x + 0.0292	0.9997
0.5	0.099	0.135	0.167	0.200	0.233	0.0665x + 0.0339	0.9997
1.0	0.094	0.129	0.159	0.195	0.227	0.0664x + 0.0280	0.9994
2.0	0.090	0.124	0.155	0.187	0.220	0.0646x + 0.0260	0.9998

average of five replicate results

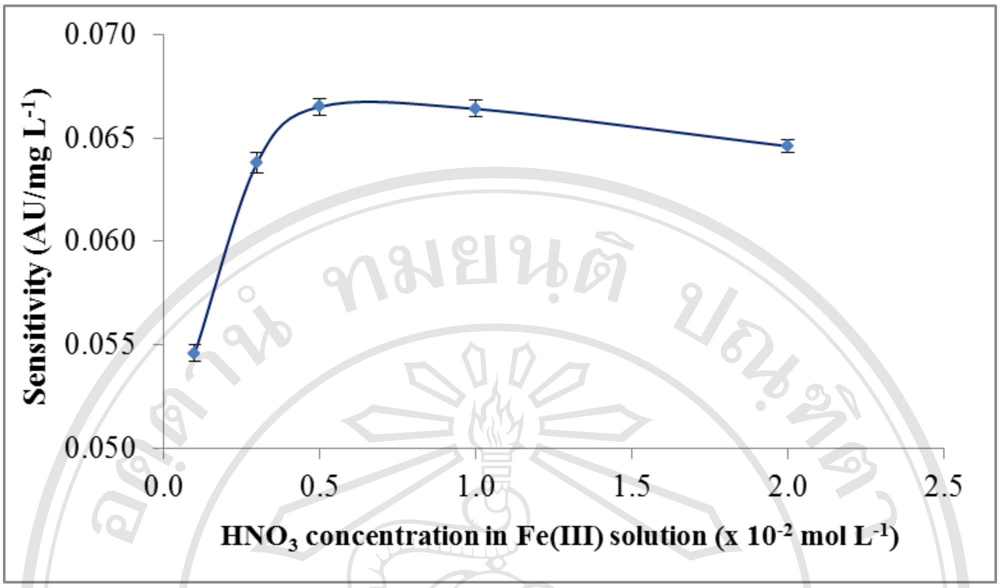


Figure 4D Effect of HNO$_3$ concentration on the sensitivity of Fe(III)-CTC complex

2.4 Effect of chlortetracycline concentration

Generally, the amount of reagent is demanded for complete color development of the complex and it is necessary to study the optimum concentration of CTC to achieve the required stoichiometry of the complex as metal to ligand ratio. The effect of varying concentrations on the sensitivity of chlortetracycline reagent solution between 0.4 x 10^{-3} and 2.0 x 10^{-3} mol L^{-1} was investigated. The results are displayed in Table 5D and Figure 5D. The highest sensitivity was obtained when the concentration of CTC solution was 1.2 x 10^{-3} mol L^{-1}, so this concentration was selected as the optimum concentration. After that, the sensitivities remained constant in CTC reagent concentration to 2.0 x 10^{-3} mol L^{-1}.

Table 5D Effect of CTC concentration on the sensitivity of Fe(III)-CTC complex

[CTC] (x 10^{-3} mol L^{-1})	**Δ Peak height (AU) obtained from the standard Fe(III) (mg L^{-1})**					**y = mx + c**	**r^2**
	1.0	**1.5**	**2.0**	**2.5**	**3.0**		
0.4	0.098	0.135	0.167	0.199	0.232	0.0664x + 0.0334	0.9992
0.8	0.105	0.141	0.182	0.213	0.251	0.0728x + 0.0328	0.9987
1.2	0.111	0.152	0.190	0.229	0.269	0.0784x +0.0332	0.9998
1.6	0.112	0.154	0.192	0.232	0.271	0.0792x + 0.0342	0.9996
2.0	0.113	0.155	0.193	0.233	0.273	0.0796x + 0.0342	0.9998

average of five replicate results

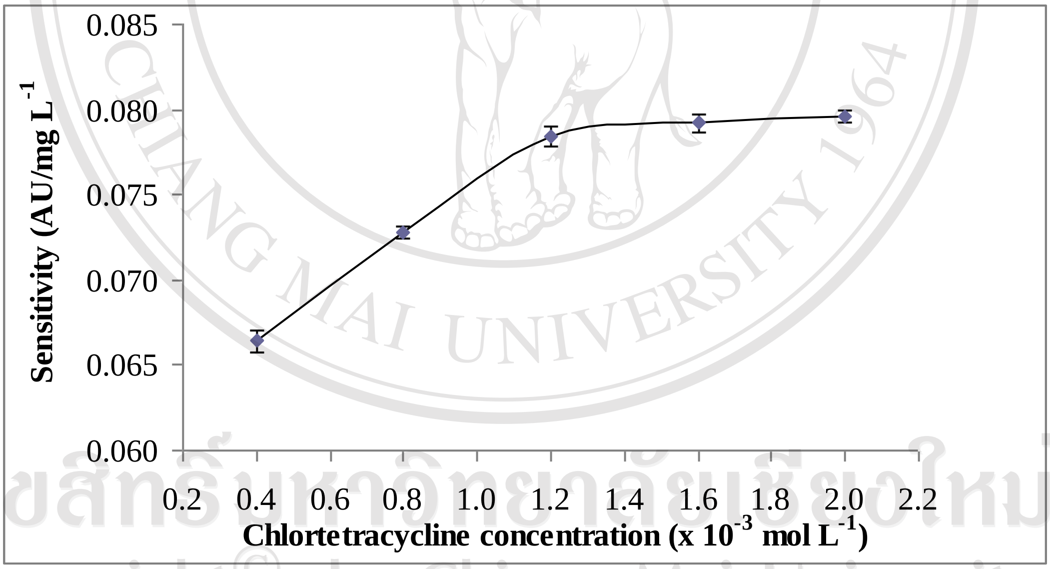


Figure 5D Effect of CTC concentration on the sensitivity of Fe(III)-CTC complex

2.5 Effect of reagent flow rate

The effect of the flow rates of phosphate buffer solution (pH 8.0) and Fe(III) standard solution were performed over the range 10–50 μL min^{-1} for both streams. The results were shown in Table 6D and Figure 6D. It was seen that the sensitivities increased with increasing flow rate up to 30 μL min^{-1}. After that, the sensitivities were decreased. The reaction time is short at high flow rate leading to increases dispersion, a poorer the ratio of sample peak to blank peak and a high sample and high sample and carrier solution consumption. However, the large dispersion zone was occur at lower flow rate giving to low sampling rate. Therefore, a flow rate of 30 μL min^{-1} for each stream was chosen with the greatest sensitivity and reasonable low reagent consumption (chemical waste < 4 mL h^{-1}). In addition, a flow rates of 30 μL min^{-1} provided a dramatically sample throughput (40 h^{-1}).

Table 6D Effect of reagent flow rate on the sensitivity of Fe(III)-CTC complex

Flow rate (μL min^{-1})	**Δ Peak height (AU) obtained from the standard Fe(III) (mg L^{-1})**					**y = mx + c**	**r^2**
	1.0	**1.5**	**2.0**	**2.5**	**3.0**		
10	0.112	0.153	0.192	0.231	0.272	0.0796x + 0.0328	0.9999
20	0.117	0.158	0.206	0.245	0.288	0.0858x + 0.0312	0.9992
30	0.122	0.169	0.213	0.259	0.306	0.0911x + 0.0315	0.9998
40	0.118	0.162	0.206	0.252	0.296	0.0892x + 0.0284	0.9999
50	0.115	0.158	0.202	0.246	0.291	0.0880x + 0.0264	0.9999

average of five replicate results

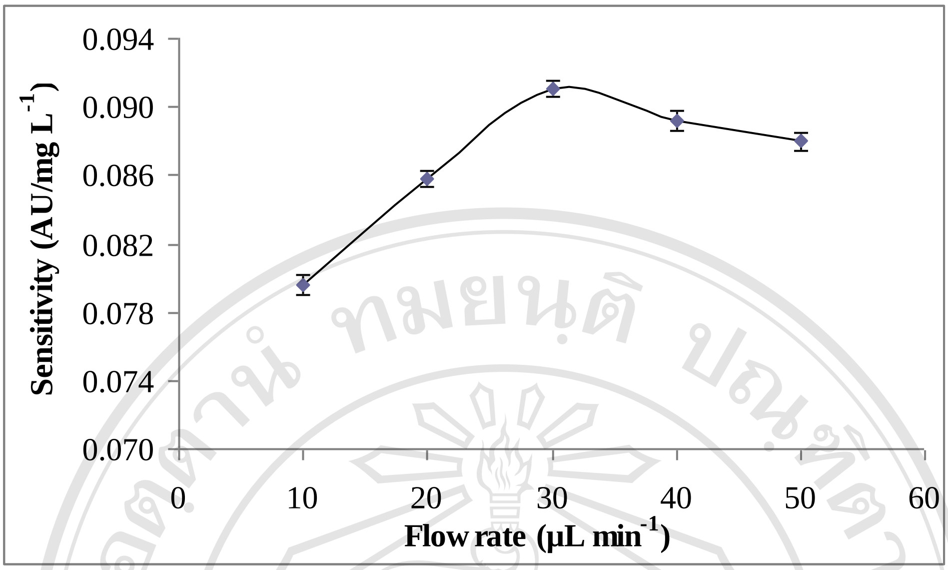


Figure 6D Effect of flow rate on the sensitivity of Fe(III)-CTC complex

2.6 Effect of injection volume

The effect of the reagent volume on the sensitivity was examined by injecting volumes in the range 2–10 µL chlortetracycline reagent solution into the Fe(III) stream via with selection valve. The results were displayed in Table 7D and Figure 7D. It was found that the sensitivity climbed on increasing the injection volume from 2 to 10 µL. Moreover, injection volume over 6 µL produced the slightly increased sensitivity. Therefore, the suitable injection volume of CTC for further apply was 6 µL.

Table 7D Effect of injection volume on the sensitivity of Fe(III)-CTC complex

Injection volume (μL)	**Δ Peak height (AU) obtained from the standard Fe(III) (mg L^{-1})**					**y = mx + c**	**r^2**
	1.0	**1.5**	**2.0**	**2.5**	**3.0**		
2	0.122	0.169	0.213	0.259	0.306	0.0911x + 0.0315	0.9998
4	0.127	0.176	0.223	0.276	0.328	0.1004x + 0.0252	0.9995
6	0.134	0.187	0.238	0.291	0.345	0.1053x + 0.0284	0.9999
8	0.129	0.181	0.228	0.288	0.339	0.1054x + 0.0222	0.9988
10	0.127	0.180	0.226	0.286	0.338	0.1056x + 0.0202	0.9987

average of five replicate results

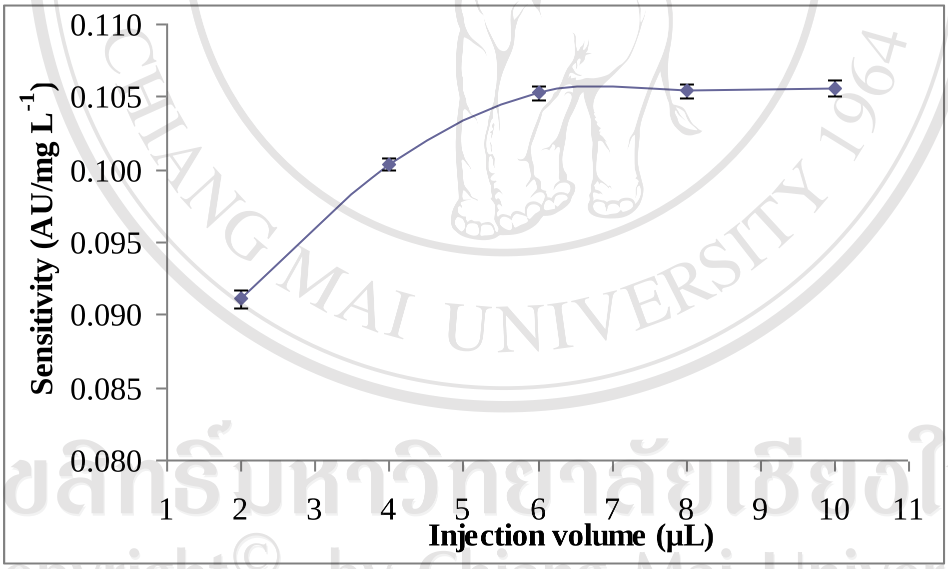


Figure 7D Effect of injection volume on the sensitivity of Fe(III)-CTC complex

2.7 Summary of the optimum condition using univariate method

The proposed μrFA system for determination of Fe(III) is displayed in Figure 2.8. Table 8D shown the studied range and the optimum value of all conditions using univariate method.

Table 8D The studied range and the optimum value of all parameters of μrFA

Variable	Studied range	Optimum
Wavelength (nm)	430-450	440
pH	7.0-9.5	8.0
Concentration of HNO_3 (x 10^{-2} mol L^{-1})	0.1-2.0	0.5
Concentration of chhlortetracycline (x 10^{-3} mol L^{-1})	0.4-2.0	1.2
Flow rate (μL min^{-1})	10-50	30
Reagent injection volume (μL)	2-10	6

2.8 Analytical characteristics

2.8.1 Calibration curve and detection limit

Using the μrFA system (Figure 2.8) and the optimum conditions in Table 8D, linear range and calibration curve were constructed by using series of standard Fe(III) in the range of 0.1-5.0 mg L^{-1}. All measurements were performed in five replication injection, the results are shown in Table 9D and Figure 8D. It was seen that the linear calibration curve over the range of 0.5 – 4.0 mg L^{-1} Fe(III) was established that can be presented by the regression equation y = 0.1056x + 0.0254 (r^2 = 0.9997) where y expresses the absorbance of Fe(III)-CTC yellow complex and x is Fe(III) concentration in mg L^{-1} after subtraction of blank as displayed in Figure 9D. In addition, The μrFI-gram is displayed in Figure 10D.

Table 9D Peak height for linear range of calibration curve

[Fe(III)] (mg L^{-1})	**Peak height (AU)**					$\overline{X}$	**Δ P.H. (AU)**
	1	**2**	**3**	**4**	**5**		
0	0.032	0.031	0.032	0.033	0.032	0.032	0.000
0.1	0.043	0.042	0.042	0.043	0.043	0.043	0.011
0.2	0.057	0.057	0.058	0.058	0.058	0.058	0.026
0.3	0.065	0.065	0.064	0.065	0.066	0.065	0.033
0.5	0.107	0.107	0.107	0.109	0.107	0.107	0.075
1.0	0.167	0.167	0.167	0.169	0.167	0.167	0.135
1.5	0.219	0.217	0.219	0.221	0.220	0.219	0.187
2.0	0.270	0.268	0.268	0.270	0.269	0.269	0.237
2.5	0.323	0.323	0.323	0.325	0.325	0.324	0.292
3.0	0.373	0.373	0.372	0.374	0.373	0.373	0.341
3.5	0.426	0.427	0.428	0.426	0.427	0.427	0.395
4.0	0.479	0.479	0.479	0.481	0.480	0.480	0.448
4.5	0.511	0.510	0.511	0.511	0.511	0.511	0.479
5.0	0.531	0.531	0.530	0.530	0.530	0.530	0.498
5.5	0.541	0.540	0.540	0.540	0.541	0.540	0.508

* average of five replicate results

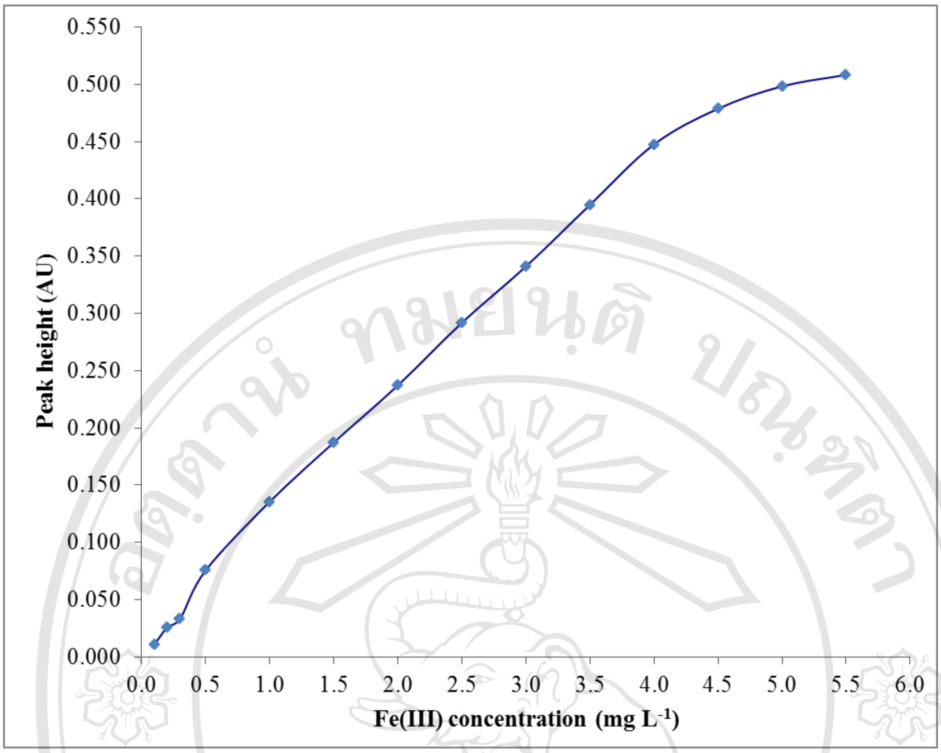


Figure 8D Relation between peak height (AU) and standard Fe(III) solution

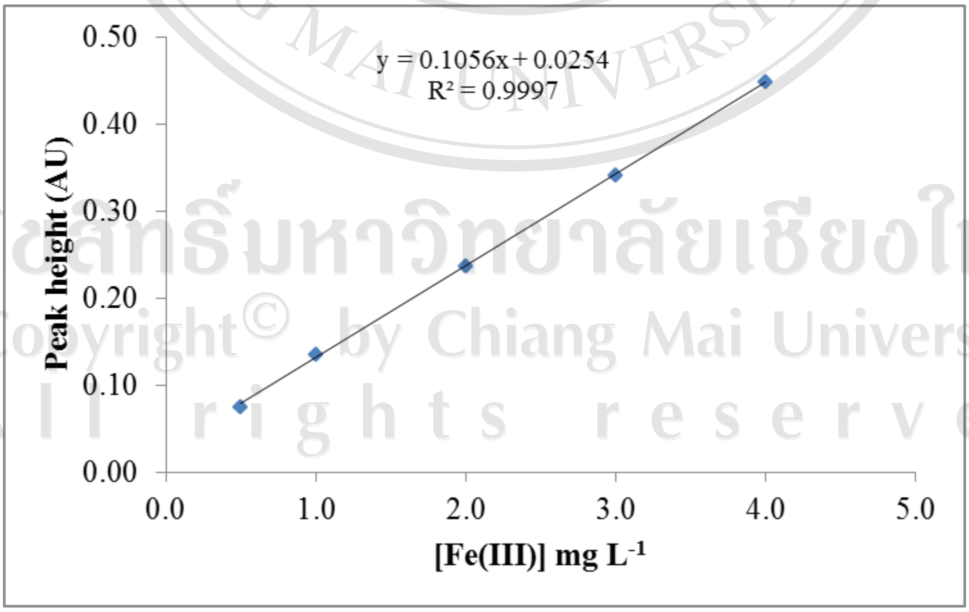


Figure 9D Calibration curve for Fe(III) determination

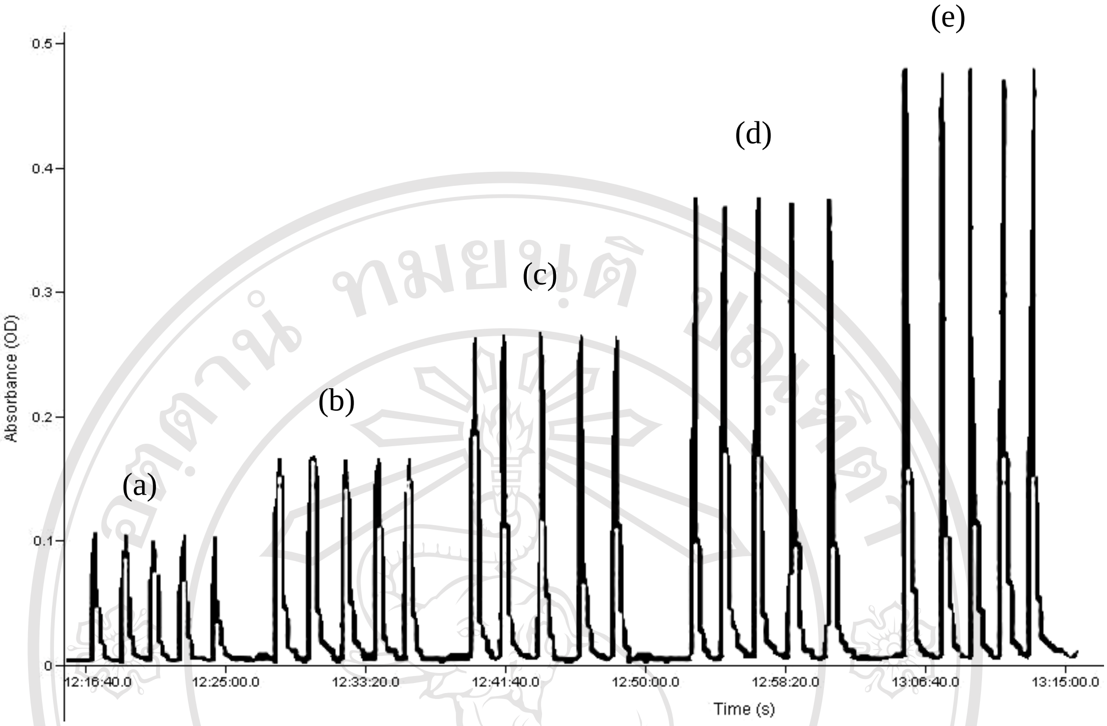


Figure 10D The μrFI-gram of standard Fe(III) solution; a) 0.5 mg L^{-1}, b) 1.0 mg L^{-1}, c) 2.0 mg L^{-1}, d) 3.0 mg L^{-1}, e) 4.0 mg L^{-1}

The detection limit was determined from the regression equation with the calculated parameters of the intercept of the straight line and three-times the standard deviation of the regression time (3σ) [259]. The detection limit of the proposed method was found to be 0.17 mg L^{-1} Fe(III). In addition, the quantitation limit (10σ) was found to be 0.55 mg L^{-1} Fe(III).

2.8.2 Interference studies

Effects of some possible interfering ions on the determination of Fe(III) that are known to react with tetracycline [266] were investigated. The tolerance limit of an ion was taken as the maximum amount (mg L^{-1}) causing an error not greater than 10% for determination of target analytes. Synthetic sample solutions containing 1.0 mg L^{-1} of Fe(III) and different amounts of some metal ions were tested under the optimum conditions in Table 8. The results are shown in Table 10D. The most cations and anions tested caused interference < ±10% for determining of Fe(III). It was seen that only Sn(II) ions had serious interfere for the determination of Fe(III). Commonly, there are very small amount of Sn(II) in canal and river, hence, it does not effect to iron analysis. However, Sn(II) can be reduce with trisodium citrate as masking agent [267]. Therefore, the developed method is very potential for determination of Fe(III) in water samples.

Table 10D Tolerable levels of interferences ions effect on the signal obtained from 1.0 mg L^{-1} Fe(III)

Interference ion	**Tolerable concentration ratio**[a] **(mg L^{-1}) ions/1.0 mg L^{-1} Fe(III)**
Na^+, K^+, Ca^{2+}, Cu^{2+}, Mn^{2+} , Pb^{2+} Zn^{2+} Al^{3+}, Pb^{2+}, Mg^{2+}, Cd^{2+},	200
SO_4^{2-}, NO_3^-	100
Sn^{2+}	20([b])

[a] The foreign species concentration causing error smaller than ±10% for request to the signal Fe(III) alone

[b] 5% trisodium citrate as masking agent

Table 11D Concentrations of Fe(III) in water sample analyzed by using the proposed μrFA system

Water samples	**Fe(III) found* (mg L^{-1})**	**Spiked Fe(III) (mg L^{-1})**	**Total Fe(III) (mg L^{-1})**	**% Recovery**
Ang-Keaw	1.695 ± 0.021	0.5	2.230 ± 0.002	105.1
Ang-Karset	1.787 ± 0.031	1.0	2.793 ± 0.001	101.0
Tap water	1.925± 0.035	0.5	2.441 ± 0.002	103.3
Ping River	0.988± 0.006	1.0	2.006 ± 0.001	101.8
Chon Pra Than Canal	0.926 ± 0.005	0.5	1.439 ± 0.002	102.6

*average of triplicate results

APPENDIX E

1. Interferences and masking reagents studies for Ni(II) determination

Table 1E Effect of masking agent for determination of 1.0 μg mL^{-1} standard Ni(II)

Interference	Concentration of masking agent	Concentration ratio (Ni(II) :Interference)	Peak height (AU) (n=5)	% Recovery
Fe^{3+}	3.0% Sodium fluoride	1 : 0	0.062	-
		1 : 1	0.064	103
		1 : 5	0.065	105
		1 : 10	0.068	110
	4.0% Sodium fluoride	1 : 0	0.060	-
		1 : 10	0.062	103
		1 : 15	0.065	108
		1 : 20	0.068	113
	5.0% Sodium fluoride	1 : 0	0.059	-
		1 : 15	0.062	105
		1 : 20	0.066	112
		1 : 25	0.068	115
Co^{2+}	0.50% Potassium oxalate	1 : 0	0.061	-
		1 : 5	0.063	103
		1 : 10	0.065	107
		1 : 15	0.068	111
	1.0% Potassium oxalate	1 : 0	0.062	-
		1 : 15	0.065	105
		1 : 20	0.067	108
		1 : 25	0.071	114

Table 1E (Continued)

Interference	Concentration of masking agent	Concentration ratio (Ni(II) :Interference)	Peak height (AU) (n=5)	% Recovery
Co^{2+}	1.5% Potassium oxalate	1 : 0	0.059	-
		1 : 20	0.063	107
		1 : 25	0.066	112
		1 : 30	0.069	117
Cu^{2+}	0.10% Thiourea	1 : 0	0.060	-
		1 : 5	0.063	105
		1 : 10	0.064	107
		1 : 15	0.066	110
	0.30% Thiourea	1 : 0	0.061	-
		1 : 15	0.064	105
		1 : 20	0.066	108
		1 : 25	0.070	115
	0.50% Thiourea	1 : 0	0.062	-
		1 : 20	0.065	105
		1 : 25	0.069	111
		1 : 30	0.072	116

2. Interferences and masking reagents studies for Zn(II) determination

Table 2E Effect of masking agent for determination of 0.5 mg L^{-1} Zn(II)

Interference	Concentration of masking agent	Concentration ratio (Zn(II) : Interference)	Peak height (AU) (n=5)	% Recovery
Al^{3+}	0.10% Sodium fluoride	1 : 0	0.183	-
		1 : 5	0.186	102
		1 : 10	0.189	103
		1 : 15	0.193	105
	0.20% Sodium fluoride	1 : 0	0.180	-
		1 : 15	0.183	102
		1 : 20	0.196	109
		1 : 25	0.212	117
	0.30% Sodium fluoride	1 : 0	0.184	-
		1 : 20	0.193	107
		1 : 25	0.210	114
		1 : 30	0.215	117
Fe^{3+}	0.10% Sodium fluoride	1 : 0	0.183	-
		1 : 5	0.186	102
		1 : 10	0.189	103
		1 : 15	0.196	107
	0.20% Sodium fluoride	1 : 0	0.181	-
		1 : 15	0.192	106
		1 : 20	0.197	109
		1 : 25	0.205	113
	0.30% Sodium fluoride	1 : 0	0.179	-
		1 : 20	0.191	107
		1 : 25	0.199	111
		1 : 30	0.208	116

Table 2E (Continued)

Interference	Concentration of masking agent	Concentration ratio (Zn(II) : Interference)	Peak height (AU) (n=5)	% Recovery
Cu^{2+}	0.02% Sodium thiosulfate pentahydrate	1 : 0	0.182	-
		1 : 5	0.184	101
		1 : 10	0.190	104
		1 : 15	0.193	106
	0.05% Sodium thiosulfate pentahydrate	1 : 0	0.180	-
		1 : 10	0.182	101
		1 : 15	0.186	103
		1 : 20	0.183	102
	0.08% Sodium thiosulfate pentahydrate	1 : 0	0.181	-
		1 : 20	0.171	94
		1 : 25	0.162	89
		1 : 30	0.160	88

CURRICULUM VITAE

Name	Mr. Narabhats Rannurags
Date of birth	February 15, 1977

Academic Background

Present Study	Doctor of Philosophy in Chemistry (Analytical Chemistry)
2006-	Chiang Mai University
Master degree	Master of Science in Analytical Chemistry
2002-2006	Prince of Songkla University
Bachelor degree	Bachelor of Science in Chemistry
1996-2000	Prince of Songkla University
1988-1994	Radsada School, Radsada, Trang, Thailand

Scholarship/Awards during Enrolment

1. Center for Innovation in Chemistry: Post Graduate Education and Research Program in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education
2. The Thesis Research Fund through the Graduate School, Prince of Songkla University.
3. Teaching Assistance (CMU, PSU)

Working Experiences

2000-2001: Research Assistance, The Project of ACIAR: Determination of Herbicide And Pesticide in Soil With HPLC Technique, Under Supervision of Assoc. Prof. Dr. Sanan Subhadhirasakul, Faculty of Pharmaceutical Science, Prince of Songkla University

2001-2002: Chemist at The Fish Inspection and Quality Control Division, Analysis of Lead, Cadmium and Copper in Sea Food with AAS, 79/2 Vichianchom Road, Muang, Songkhla, Thailand, 90000

2009-2012: Special Lecturer in Department of Chemistry, Faculty of Science and Technology, Chiang Mai Rajabhat University**,** Chiang Mai, Thailand

Presentations/Publications

1. **Voltammetic Determination of Silver (I) Using Carbon Paste Electrode Modified with 1, 8-dihydroxyanthraquinone.** Naraphat (Jaray) Rannurak, Piyarat Sukhotu, Pipat Chooto. Poster presentation at the 205th ECS Meeting, 9-13 May 2003, San Antonio, USA.
2. **Voltammetic Determination of Silver (I) Using Carbon Paste Electrode Modified with 1, 8-dihydroxyanthraquinone.** Naraphat (Jaray) Rannurak, Piyarat Sukhotu, Pipat Chooto. Poster presentation at **The Postgraduate Education and Research Program in Chemistry, Congress III**, 9-12 May 2004, Jomtian Palm Beach Resort, Pattaya, Cholburi.

3. **Flow injection Determination of Zinc Using Xylenol Orange.** Naraphat Rannurags, Saisunee Liawruangrath, Poster presentation at

PERCH-CIC Conference V, 6-9 May 2007, Jomtian Palm Beach Resort, Pattaya, Cholburi.

4. **Simple Flow Injection Spectrophotometric Procedure for Determination of Zinc(II).** Naraphat Rannurags, Saisunee Liawruangrath, Poster presentation at The 18-20 October 2007 at **The 33rd Congress on Science and Technology of Thailand,** Walailak University, Nakhon Si Thammarat, Thailand.
5. **Reverse Flow Injection Spectrophotometric Procedure for the Determination of Iron(III) Using Doxycycline**. Naraphat Rannurags, Saisunee Liawruangrath, Boonsom Liawruangrath, Poster presentation at **PERCH-CIC Conference VI**, 3-6 May 2009, Jomtian Palm Beach Resort, Pattaya, Cholburi, Thailand.

THE RELEVANCY OF THE RESEARCH WORK IN THAILAND

In recent years, many scientists have tried to design and fabricate miniaturized instrumentations for chemical analysis and use as a basis for the development of greener analytical methods that are environmentally friendly. The increasing demand for small instruments and potential analysis system are essential. In particular, the application of the measurement related to the trace analysis in the environment. In the analytical processes usually take up a lot of time for a large number of samples analyses and manual systems. For these reasons, analytical techniques using automatic system, high sample throughput and minimum consumption of reagent/sample are required. Miniaturization all functions of analytical instrumentation and methods would assist these purposes. This research focuses recent advances, trends and application of miniaturized analytical systems for natural water analyses in Chiang Mai and Lam Phun Province (Thailand).

The aims of this research are development of LOC techniques and home-made laser instrument with diode laser for fabrication of PMMA chips for analyzing trace metals in water samples. This work shows that notable advances have been made in mini-CNC machine modified with diode laser and development of LOC methods for trace metals analyses. These machine and systems have been perfect for creating PMMA chips. In term of economic point of vision, the use of the modified instrument for fabrication of chips is to reduce the cost for the imported instrument. The reagent consumption in this research is very small leading to minimum waste generation and reduce the charge for the chemical waste management. This would be able to help the Thai Government to save the budget and reduce environmental problem of Thailand in the near further.