CHAPTER 2

EXPERIMENTAL

2.1 Instrument and apparatus

Instruments and Apparatus	Model	Company	Country
1. Analytical Balances	GR-200	A&D	Japan
2. Deionized water purification unit	-	Millipore	United States
3. A Ten-port selection valve	01V-0201L	Valco Instruments	United States
4. Bench drill (KANTO)	KT-4106	-	Taiwan
5. CO_2 Laser	SF960	Jinan Senfeng Technology	China
6. Destop computer	Pentium D	Intel Corporation	United States
7. Flow-through cell	-	Hellma	Germany
8. Fiber optic cable	733-2447	Ocean Optics Incorporation	United States
9. Hot plate stirrer	-	Fisher Scientific	United States
10. Light-emitting diode lamp	CL-RW200	Shenzhen Caijing Electronics	China
11. Oven	ON-22G	GMI Incorporation	United States
12. PEEK tubing	0.01 mm i.d.	Upchurch Scientific	United States
13. Peristaltic pump	REGLO-D	ISMATEC	Germany
14. Poly methyl methacrylate	-	PAN ASIA Industrial	Thailand

Instrument and apparatus (Continued)

Instruments and Apparatus	Model	Company	Country
15. pH meter	pHTestr10	Eutech Instrument	Switzerland
16. Sonicator	S-30S	Elma Hans Schmidbauer GmbH	Germany
17. Spectrophotometer	USB4000	Ocean Optics Incorporation	United States
18. Teflon tubing	-	Cole-parmer	United States
19. Tygon tubing	-	Cole-parmer	United States
20. UV/VIS spectrophotometer	6305	Jenway	England
21. UV/VIS spectrophotometer	6400	Jenway	England

2.2 Chemicals

All chemicals were of analytical reagent grade unless otherwise specified and used without any additional purification. Deionized water was employed throughout the experiments. All chemicals used in this work are recorded as below:

Chemical	Molecular formula	Company	Country
1. Ammonia	NH_3	Fisher Scientific	England
2. Ammonium acetate	CH_3COONH_4	Merck	Germany
3. Aluminium nitrate	$Al(NO_3)_2$	Merck	Germany
4. Barium chloride	$BaCl_2$	Merck	Germany
5. Cadmium nitrate	$Cd(NO_3)_2$	BDH	England
6. Cetylpyridinium chloride	$C_{21}H_{38}ClN$	Merck	Germany

Chemical (Continued)

Chemical	Molecular Formula	Company	Country
7. Chromium nitrate	$Cr(NO_3)_3$	Merck	Germany
8. Copper sulfate	$CuSO_4$	BDH	England
9. EDTA di-sodium salt	$C_{10}H_{16}N_2O_8$	Merck	Germany
10. Ethanol	C_2H_6O	Merck	Germany
11. Glacial acetic acid	$C_2H_4O_2$	Merck	Germany
12. Hydrochoric acid, 37%	HCl	Carlo Erba	Italy
13. Iron standard solution 1000 ppm	Fe(III)	Fisher Scientific	England
14. Lead nitrate	$Pb(NO_3)_2$	Carlo Erba	Italy
15. Magnesium nitrate	$Mg(NO_3)_2$	Merck	Germany
16. Nickel standard solution 1000 ppm	Ni(II)	Fisher Scientific	England
17. Nitric acid	HNO_3	Merck	Germany
18. Nitroso-R salt	$C_{10}H_5NNa_2O_8S_2$	Merck	Germany
19. Potassium thiocyanate	KSCN	Fisher Scientific	England
20. Quinine hydrochloride	$C_{20}H_{24}N_2O_2$	Merck	Germany
21. Sodium acetate trihydrate	$CH_3COONa.3H_2O$	Merck	Germany
22. Sodium hydroxide	NaOH	Carlo Erba	Italy
23. Xylenol orange	$C_{31}H_{32}N_2O_{13}S$	Fluka	Switzerland
24. Zinc standard solution 1000 ppm	Zn(II)	Fisher Scientific	England

2.3 Preparation of standard solutions and reagents

All chemicals employed in this research are of analytical reagent grade. Working solutions were prepared with deionized water. Laboratory glassware were cleaned prior to use with 10% (v/v) nitric acid, left overnight and rinsed with deionized water.

2.3.1 Preparation of standard solutions and reagents for nickel determination with FIA

2.3.1.1 Stock nickel solution

A standard stock solution of Ni(II) 20 mg L^{-1} was prepared from a standard nickel solution (1000 mg L^{-1}). Working standard solutions were freshly prepared from stock solutions of Ni(II) and diluted with the 0.1 mol L^{-1} of ammonium acetate buffer.

2.3.1.2 Stock reagent of nitroso-R salt

A stock nitroso-R salt 0.08 mol L^{-1} solution was prepared by dissolving 3.0010 g of nitroso-R salt in deionized water and adjusting the volume to 100 mL. The working solution of nitroso-R salt was daily prepared by appropriate dilution of stock nitroso-R salt solution in suitable buffer solution.

2.3.1.3 Ammonium acetate buffer solution

Ammonium acetate buffer solution of pH 6.5-9.5 were prepared by mixing an suitable ratio of 0.5 mol L^{-1} ammonium hydroxide with 0.5 mol L^{-1} ammonium acetate (38.50 g NH_4OAc in 1000 mL deionized water).

2.3.2 Preparation of standard solutions and reagents for zinc determination

2.3.2.1 Stock zinc solution

A standard stock solution of Zn(II) 20 mg L^{-1} was prepared from a standard zinc solution (1000 mg L^{-1}). Working standard solutions were freshly prepared from stock solutions of Zn(II) and diluted with the 0.1 mol L^{-1} of acetate buffer pH 5.5.

2.3.2.2 Stock reagent of xylenol orange

The stock xylenol orange 2.0 x 10^{-3} mol L^{-1} was prepared by dissolving 0.1562 g of xylenol orange in 100 mL acetate buffer solution. Working standard solutions were freshly prepared from stock solutions and diluted with the 0.1 mol L^{-1} of acetate buffer pH 5.5.

2.3.2.3 Acetate buffer pH 5.5

A 0.1 mol L^{-1} of acetate buffer pH 5.5 was prepared by dissolving 0.7381 g of sodium acetate in 50 mL of deionized water followed by addition of 0.057 mL of glacial acetic acid and adjusted to volume in a 100 mL volumetric flask with deionized water.

2.3.2.4 Quinine hydrochloride solution

Enhancer stock solution 0.01 mol L^{-1} was prepared by dissolving 0.3970 g of quinine hydrochloride in 5 mL ethanol and diluted with deionized water in 100 mL volumetric flash.

2.4 Water samples collection and pretreatment for metal determination

2.4.1 Water samples collection and pretreatment for nickel determination

Water samples were collected with different time from electroplating industry and electronics industry in Northern Region Industrial Estate, Lam Phun Provinces, Thailand. The water samples were filtered over No.41 Whatman filter paper to remove suspended particulate matter at the selected sampling sites and stored at 4°C in the dark in polyethylene bottles that had been previously washed with 15% nitric acid and rinsed with deionized water. After filtration, the water samples were acidified by adding 2 mL of concentrated nitric acid in each liter of water to preserve the water samples before for nickel determination.

A 100 mL of water sample was transferred into 200 mL beaker using pipetted and then added 5.0 mL concentrated nitric acid to each sample and heated the samples on a hotplate until the volume has been reduced to the lowest volumes as possible (about 10 mL). Then set it to cool down at room temperature and transfer into 50 mL volumetric flasks, 5 mL deionized water was increased (filtered if necessary) and 5% NaF were added for removed of Fe(III). Finally its volume was adjusted to 50 mL volumetric flask with deionized water and subsequently analyzed by the proposed FIA method.

2.4.2 Water samples pretreatment for zinc determination

Natural water samples were collected from Kuang River in Lam Phun Province, Thailand, where the Northern Industrial Estate is located. Sampling sites were presented in appendix B (W1-W7). The sample were collected in polyethylene

bottles with addition of concentrated nitric acid (2 mL per a liter of water sample) to preserve the water sample for zinc determination and stored at 4°C in the dark.

The water samples preparation prior to analysis was performed. A 100 mL of water sample was transferred into a 250 mL beaker and then concentrated nitric acid (5 mL) was added to 100 mL of sample contained in a beaker, which was covered with a watch glass and it heated on a hotplate until its volume was reduced to 10 mL. The mixture of sample water was cooled at room temperature. After that, the watch glass and the beaker were rinsed with deionized water. Both solution and rinsed water were transferred to a 100 mL volumetric flask. Then, the pH of the sample solution was adjusted to 5.5 with 1.0 mol L^{-1} of sodium hydroxide, diluted into a 100 mL volumetric flask with deionized water and subsequently analyzed by the proposed μFA method.

2.5 Methodology for the FIA system for determination of nickel with nitroso-R salt as complexing agent

2.5.1 Preliminary studies of spectrophotometric of Ni-nitroso-R salt complex for selecting the maximum absorption wavelength

The solutions of 2.0 x 10^{-4} mol L^{-1} nitroso-R, with and without of each 2.0 and 3.0 mg L^{-1} of Ni(II) was prepared. Each concentration of Ni(II) solution was added 0.10 mL of 0.02 mol L^{-1} nitroso-R salt solution, diluted with ammonium acetate buffer pH 8 in 10 mL volumetric flask, mixed well and then transferred into the sample cell. Then absorption spectrum of each nickel-nitroso-R salt complexes and

the reagent blank solutions were scanned over a range of 380-700 nm using JENWAY 6400 spectrophotometer and the signals were recorded with Jenway Scan Software.

2.5.2 Study of the composition of the Ni(II)-nitroso-R salt complex with mole-ratio method

The mole-ratio method of Ni(II)-nitroso-R salt complex was definite in which nickel(II) ions concentrations were fixed (1.0×10^{-5} mol L^{-1}) while the nitroso-R salt concentration was varied between 1.0×10^{-5} mol L^{-1} and 6.0×10^{-5} mol L^{-1}.

2.5.3 The FIA system for determination of nickel

2.5.3.1 Instrument setup

The proposed two lines FI manifold for nickel determination is shown in Figure 2.1. The system consisted of a ten-port selection valve (V) controlled by a motor driver circuit board and a peristaltic pump (P) with Tygon tubing (0.84 mm i.d. and 1.24 mm o.d.) which were controlled by computer software programmed in our laboratory named "SIA" for injecting accurate volume of samples (S) and transporting appropriate flow rate of reagent (R), a PTFE tubing (0.84 mm i.d., 30 cm long) mixing coil was used as reactor, a 10 mm path length with 120 μL flow through cell in the cell compartment of the UV-Vis spectrophotometer (Jenway 6305) as detector (D). A home-made computer software named "SIA" was used for collecting the absorption signal and controlling the entire system.

2.5.3.2 The method for determination of nickel using FIA

The FIA system (Figure 2.1) is designed and connected with a spectrometer (Jenway 6305) to obtain a flow manifold for determining Ni(II). The method involved the injection of 80 μL standard or sample solution containing Ni(II) by switching the

selection valve into a reagent stream of 0.104 mol L^{-1} nitroso-R salt buffered pH 8.5 with 0.5 mol L^{-1} ammonium acetate buffer with an appropriate flow rate of 2.5 mL min^{-1} using peristaltic pump with the specially designed software to control the flow system (sample injection volume calculated from aspirating time and flow rate). Nitroso-R salt and Ni(II) solutions were reacted completely on the 30 cm mixing coil (MC) resulting in a yellow Ni(II)–nitoso-R complex then passed through the flow through cell of the spectrophotometer at 490 nm. The amount of Ni(II) content in water samples were calculated by reference to the calibration graph prepared under identical conditions. A comparative determination of the Ni(II) in the sample solutions was carried out by FAAS method.

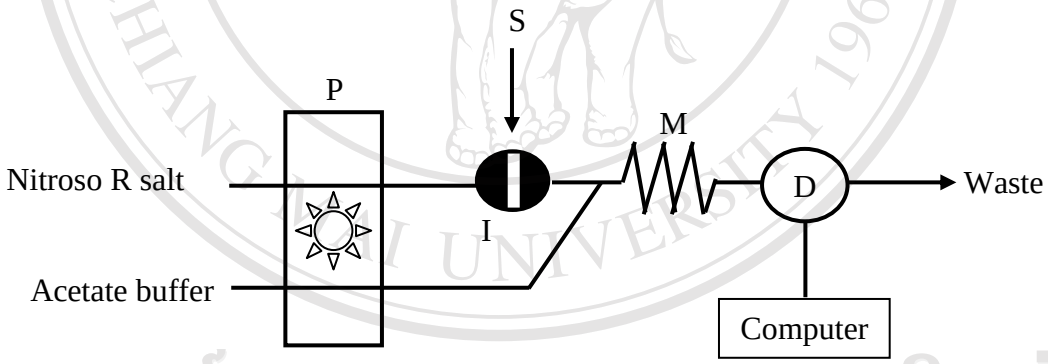

Figure 2.1 The design of the FIA system for the determination of Ni(II): S, sample; M, mixing coil; I, injection valve; P, pump; D, detector

2.5.3.3 Optimization of the experimental conditions by univariate method

The proposed FIA system was optimized by a univariate method, to select the optimum conditions for the highest sensitivity with low background and standard deviation of Ni(II)-nitroso-R complex. The value of one variable was changed while keeping the other variables at their constant values. By maintaining that variable at its optimum value, another was studied. The optimization of the experimental conditions was carried out by using standard Ni(II) solution. In all experiments, five replicate measurements were performed for each studied parameter. The studied ranges for the optimization of all parameters were investigated as shown in Table 2.1.

Table 2.1 The studied range for the optimization of all parameters of FIA

Variable	Studied range
pH	6.5-9.5
Concentration of nitroso-R salt (x 10^{-2} mol L^{-1})	0.26-2.08
Reaction coil length (cm)	10-50
Flow rate (mL min^{-1})	1.0-5.0
Sample volume (μL)	50-100

2.5.4 Analytical characteristics study

2.5.4.1 Linear range

Working standard solutions of Ni(II) ion over the ranges of 0.1 – 3.7 mg L^{-1} were prepared from the intermediate Ni(II) stock solution (20 mg L^{-1}). The series of

Ni (II) standard solutions with different concentrations were flowed into the FIA system by means five replicate results. Concentrations of Ni(II) were measured by FIA method and recorded as peak heights. The investigation of linear range was obtained by plotting the peak heights against various concentrations of Ni(II).

2.5.4.2 Precision of the proposed method

The precision of the proposed method was verified by injecting 11 replicates of 1 mg L^{-1} standard Ni(II) solution and calculated % RSD from the equation 2.1.

$$\%RSD = \frac{SD \times 100}{\overline{X}} \qquad (2.1)$$

When %RSD = percentage relative standard

SD = standard deviation

$\overline{X}$ = mean

2.5.4.3 Accuracy of the proposed method

The accuracy of the proposed method were verified by spiking the water samples with 0.5 mg L^{-1} Ni(II) standard solution using the recommended procedure. Then, Ni(II) concentrations were calculated from linear regression equation obtained from the calibration graph. Finally, the percentage recovery was calculated from the equation 2.2 as follows;

$$\%Recovery = \frac{(\text{total metal concentration-metal concentration in sample}) \times 100}{\text{Spiked metal concentration}} \qquad (2.2)$$

2.5.4.4 Detection limit [259]

Using the FIA manifold (Figure 2.1) and the optimum conditions, 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. Clearly, detection limit is depends on baseline and the analyte signal.

$$C_L = 3 \times \frac{S_{y/x}}{b} \quad (2.3)$$

$$Sy/x = \left\{ \frac{\Sigma(Y_i - \hat{Y}_i)^2}{n-2} \right\}^{\frac{1}{2}} \quad (2.4)$$

When Y_i = response value from the instrument corresponding to the individual x-values

$\hat{Y}_i$ = value of y on the calculated regression line corresponding to the individual x-values

n = number of points on the calibration line

b = slope of the straight line

2.5.4.5 Effects of interfering ions

The effects of some possible interferences as Al^{3+}, Na^{+}, K^{+}, Ca^{2+}, Ba^{2+}, NO_3^{-}, NO_2^{-}, Cl^{-}, SO_4^{2-}, Pb^{2+}, Cr^{3+}, Mg^{2+}, Cd^{2+}, Cl^{-}, PO_4^{3-}, Mn^{2+}, Zn^{2+}, Cu^{2+} and Fe^{3+} on the determination of Ni(II) in water samples were assumed for the maximum concentration ratio of interference species ($\mu g\ mL^{-1}$) to Ni(II) up to 200:1. The

tolerance is defined as the foreign species concentration causing error smaller than ±10% for determining the analyte of interest [50].

2.5.4.6 Validation method

In order to validate the FIA method for Ni determination, a comparative determination of nickel (II) by the FAAS method was carried out. Results obtained by both methods were verified by using student t-test. The calculated t_{cal} value was obtained from the equation as follows [259];

$$t = \frac{\bar{x}_d \sqrt{n}}{S_d} \quad (2.5)$$

$$S_d = \sqrt{\frac{\sum(x_d - \bar{x}_d)^2}{n-1}} \quad (2.6)$$

$$\bar{x}_d = \frac{\sum x_d}{n} \quad (2.7)$$

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

2.6 Mini-CNC machine modification with diode laser for fabrication of PMMA chips

2.6.1 Home-made mini-CNC machine

The home-made mini CNC system was fabricated as shown in Figure 2.2. This machine consist of three motors (1.8 degree per step, 3V, 1.75A, SANYO DENKI

103771) using the resolution of 0.001 mm for movement with the maximum speed of 10 mm·s^{-1} by the combined X-Y stages. The platform (P) is used to setup a material.

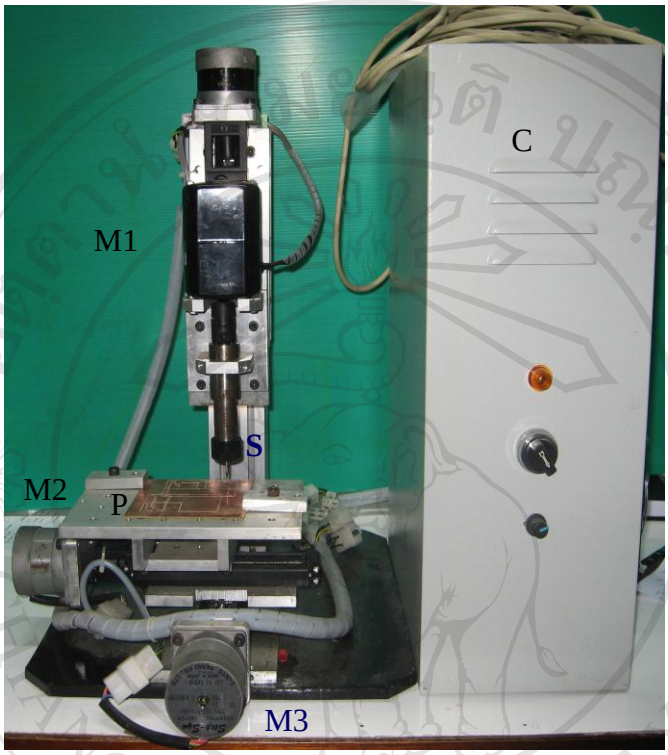

Figure 2.2 A home-made mini-CNC machine; M1, M2, M3 : motors, C**:**control cage , P: platform and S: spindle

The mini-CNC system is connected to the computer with a parallel port directly. KCAM program is applied to control the operation of the mini-CNC machine. Generally, this machine is used for engraving the material such as circuit board.

2.6.2 Mini-CNC machine modification with diode laser

The diode laser (light amplification by stimulated emission of radiation) is a solid state laser. The characteristics of laser are to produce a monochromatic (single color) light, coherence, directionally and high intensity. The wavelength of diode laser is 808 nm with maximum power of 5 W (model T808-5) as shown in Figure 2.3. It is the Diode-Pumped Solid-State (DPSS) laser that based on AlGaAs-GaAs system with operation current: 4.5~5A and operation voltage: <2.2V. It is mounted at z-direction of mini-CNC that consists of the focusing lens, the black PMMA block and aluminium heatsink.

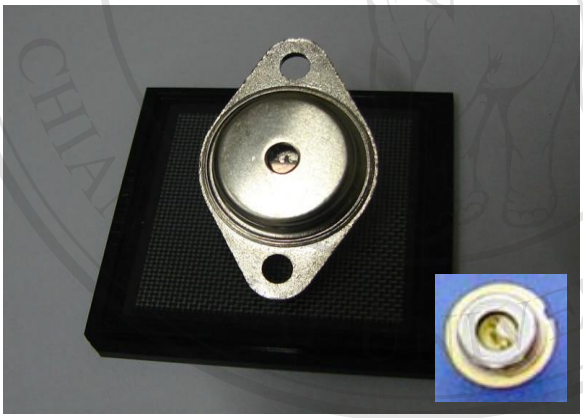

Figure 2.3 Diode laser of 5 W (808 nm) and focusing lens (New Diode Laser Company, Xibeiwang, Haidian, Beijing 100094, China)

2.6.3 The design of PMMA chip

Various patterns of PMMA microchannels were designed with Drawing Software as shown in Figure 2.4. Each pattern of microchannels had two inlet and one outlet holes for solution in and out. All lines of each pattern must be completely

connected that it makes the smooth moving of the mini-CNC and lead to a perfect microchannel.

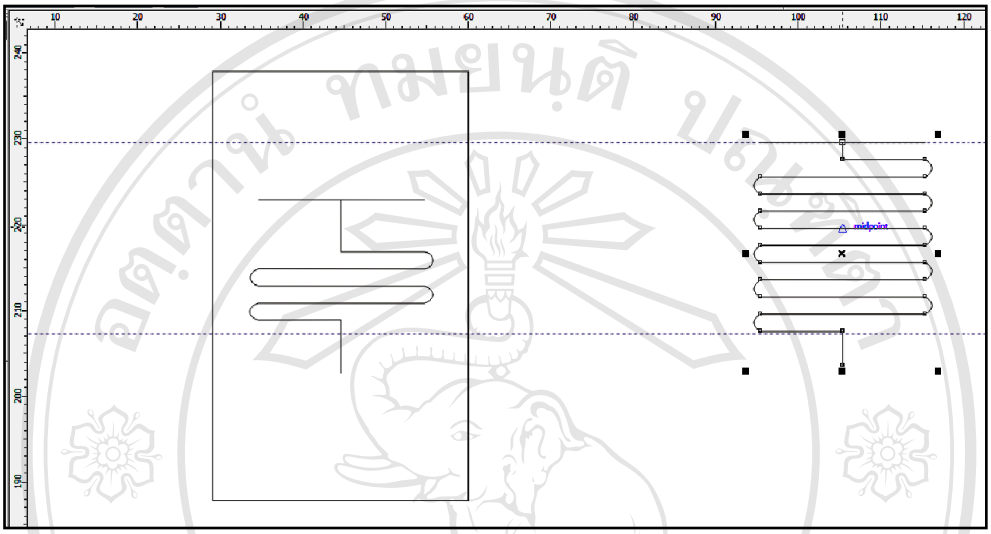

Figure 2.4 The design of the PMMA microchannels patterns

2.6.4 Fabrication of PMMA chips

The diode laser mini-CNC machine (section 2.6.2) was operated for fabrication of a PMMA chip. The PMMA (PAN ASIA Industrial CO., LTD.) plates were used as substrate that each plate was 40 mm x 60 mm rectangle PMMA with a thickness of 1.0 mm that was set up on the platform. Next, the pattern of microchannel was sent to KCAM program (Figure 2.5) that was used to control the moving of platform and the power of diode. After that, the operation of this mini-CNC system as shown in Figure 2.6 is provided by the combined motion of X and Y movements for direct writing of laser beam on the PMMA sheet to produce microchannel based on the desired channel dimension or patterns.

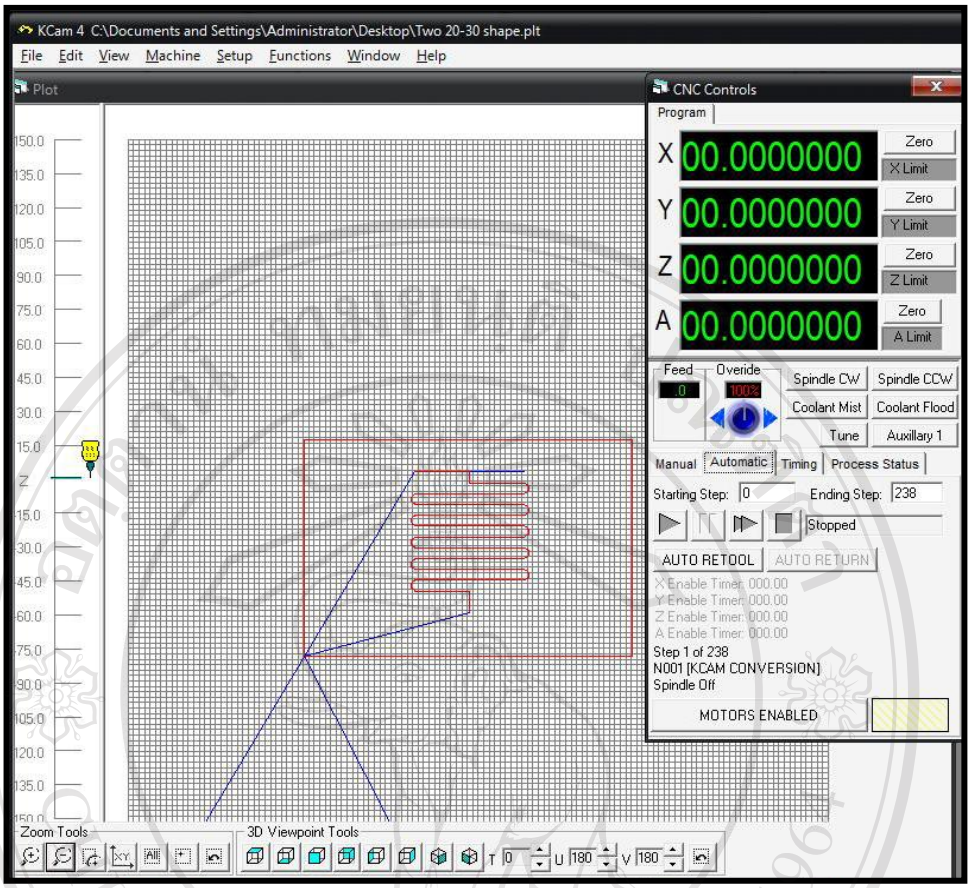

Figure 2.5 KCAM program for control diode laser mini-CNC machine

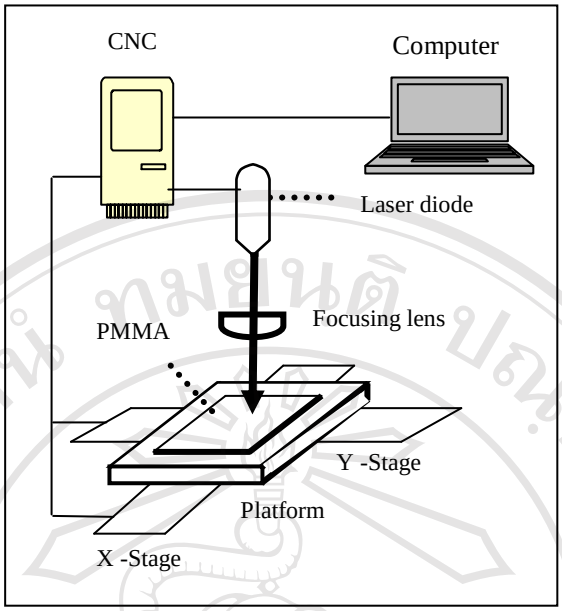

Figure 2.6 Schematic view of diode laser mini-CNC machine system with X-Y stages

2.6.5 Investigation of size of microchannel

The characteristics of the diode laser and its performance were investigated. The dimension of microchannels was obtained by controlling laser power and scanning speed of X-Y stages. The parameters that affect on the channels depth, width and smooth were examined as laser power and scanning speed. The laser power and the scanning velocity were varied from 2.5 to 5.0 W and 1.0 to 10.0 mm/min, respectively.

The PMMA microchannels were cut a cross section with the size of 0.5 x 0.5 cm and then the depth and the width of microchannels were measured using a scanning electron microscope (SEM). In addition, the smooth of microchannels surface are studied using close up SEM images.

2.6.6 Surface modification and thermal bonding

After patterning, the PMMA chips were washed with soapy water, sonicate in distilled water for 5 min and finally rinsed with distilled water for eliminated the dust particle and passively dried at room temperature. After that, dichloromethane 100 mL is added to 500 mL Erlenmeyer flask that was put on hotplate which was pre-heated to 65ºC. Next, the PMMA chip was exposed to the vapor of dichloromethane for 10 min, then passively cooled to room temperature. The smooth of PMMA microchannel before and after treated was examined with SEM.

The open PMMA microchannel was sealed with a blank PMMA plate using thermal method. Before thermal bonding between PMMA-microchannel and PMMA blank plate, conventional mechanical drilling was performed to produce holes that are conical in shape on blank PMMA plate. The blank plate with the size of 4.0 x 6.0 cm and 1.5 cm of thickness was drilled two through holes (i.d. 1.0 mm) for create reservoir ports and another through hole (i.d. 0.60 mm, part length 1.0 cm) for detection cell including channel outlet. All holes relate to pattern of PMMA tiny channel. The volume of detection cell was 3 µL.

For bonding process, all PMMA substrates were washed with soap water, rinsed with deionized water and dried at room temperature to eliminate the particles. Both the PMMA microchannel and cover plates contained alignment holes which fit into a block to align the two plates prior to sealing (Figure 2.7). A conventional oven is employed for the heating control that it is equipped with an electronic thermostat. Two PMMA substrates were placed between two glass plates (3.0 mm of thickness) (Figure 2.8) and the top and bottom covered with two aluminum plates (3.0 mm of

thickness) that permitted the transfer of heat by conduction and generated a uniform spreading of heat on the contact surface. Figure 2.7 displayed the bonding block that stick together with nuts and screws for low bonding pressure control. The PMMA substrates are heated to 140ºC in 20 min and then they were bonded at 140ºC for 15 min. Subsequently, the bonded block was cooled down to 70ºC within 20 min. Finally, the bonded PMMA chip is cooled down to room temperature and it was removed from the block.

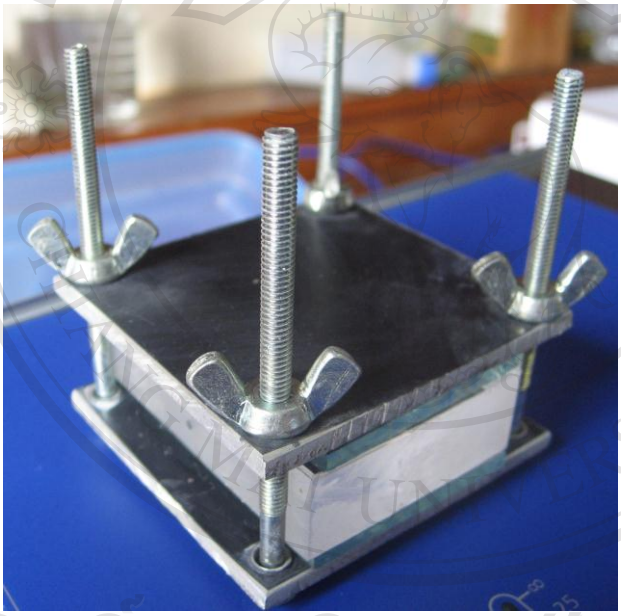

Figure 2.7 The bonding block for two PMMA substrates

2.6.7 A home-made PMMA microflow analyser

The microflow analyzer was produced with the U-turns pattern of T-junction microchannel (PMMA chip) (Figure 2.8). The dimension of PMMA chip was 206 μm wide, 202 μm deep and 30 mm long channel. This system consists of two flow lines.

The PMMA cover plate (15 mm thickness) obtained was clear color with three small channels. The first and the second channel were used for delivering sample or standard and reagent to the μFA system. PTFE inlet tubing of 0.2 mm i.d. (Cole Parmer) was inserted to the holes at channel one and channel two of PMMA chip prior to filling a droplet with a mixer of two component of Epoxy Glue (E-POX-E 5 GLUE, Cognis Chemicals (M) Sdn Bhd, Malaysia) and the time taken about six minute for solidify of Epoxy Glue with crystal clear epoxy. The third one was used for detection cell (3 μL) where the detection absorbance of the complex product and the solution passed out. The μFA detection cell was combined with two fiber optic probes (Figure 2.8). The first fiber optic probe locating at the vertical axis at the end of the detection cell inside the blank PMMA plate is connected to detector as USB4000 spectrophotometer (Ocean Optics, USA) and the second one locating at the vertical axis under the bottom PMMA plate was coupled to LED as light source. Both fiber optic probes was covered using PEEK flangeless nuts (Cheminert) fitting to a 1/16" i.d., 4 mm o.d. Spectrasuit software is employed to control fiber optic spectrometer and also used for data record.

The microflow analyzer system was displayed as schematic view in Figure 2.8. It consists of a PMMA chip, a ten-port with zero dead volume selection valves (VICI, Valco Instruments, USA) and a peristaltic pump (ISMATEC peristaltic pump 0.001–68 mL min^{-1} per channel with Tygon pump tubing 0.19 mm i.d.) organized by using computer program which were employed for injection of small volume samples and transporting appropriate flow rate of reagent. PTFE tubings (0.19 mm i.d.) were employed as flow lines linked with the μFA.

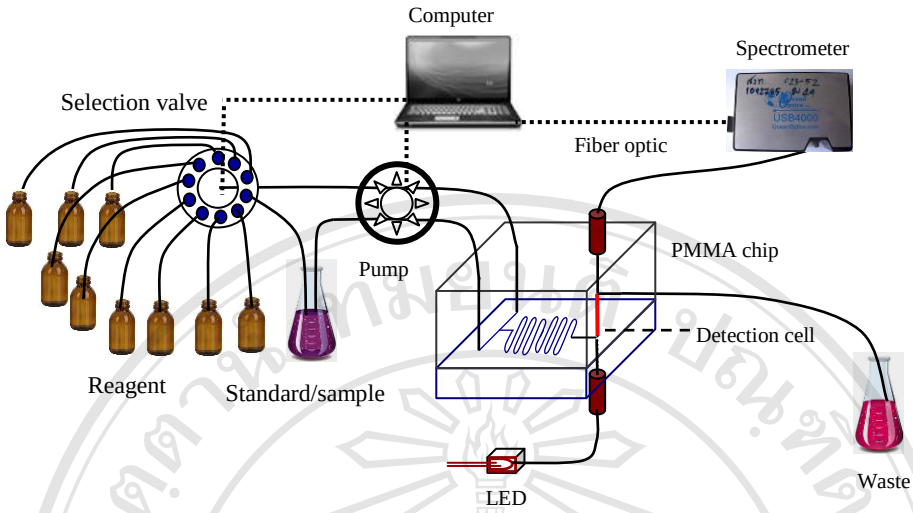

Figure 2.8 The microflow analyzer with the T-junction PMMA chip

2.6.8 Validation of the proposed PMMA chip

The proposed µFA instrumentation (Figure 2.8) has been tested to the determination of iron (III) based on the complex product between iron (III) and chlortetracycline (7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride) resulting in an intense yellow water soluble complex using micro reverse flow analysis (µrFA).

2.7 Fabrication of a PMMA chip with CO_2 laser for determination of Zn(II)

2.7.1 The design of PMMA microchannels

The Y-junction patterns of PMMA microchannels were designed with Drawing program. Each pattern was then sent to a laser control program for direct writing of laser beam on 1 mm thickness PMMA sheet with the size of 4.0 x 4.0 cm^2.

The commercial CO_2 laser machine was used to engrave the PMMA substrate with various patterns according to the design. Moreover, the blank plate of PMMA with the size of 4.0 x 4.0 cm^2 (thickness 1.5 cm) was used for seal the open mocrochannel of PMMA.

2.7.2 CO_2 laser machine setup

The CO_2 laser machine (Figure 2.9) is used for fabrication of a PMMA chip. This machine had a maximum beam power of 80 W and its wavelength is 10.6 μm. A focused laser beam (2 cm) can scan over a two-dimensional region by moving the laser beam. The maximum velocity of the CO_2 laser is 400 mm s^{-1} in the cut mode. The PMMA sheet is set on the fixed working area and the machine operation was completely by the moving of the laser beam. The scanning speed and the output of the CO_2 laser were all decided by the computer program.

Laser cutting program in laser output mode is employed for operation of the CO_2 laser machine (Figure 2.10). For fabrication of PMMA microchannel, this machine is used in cut mode. The parameters in cut mode are beam speed, power and corner power that influent the dimensions of microchannel.

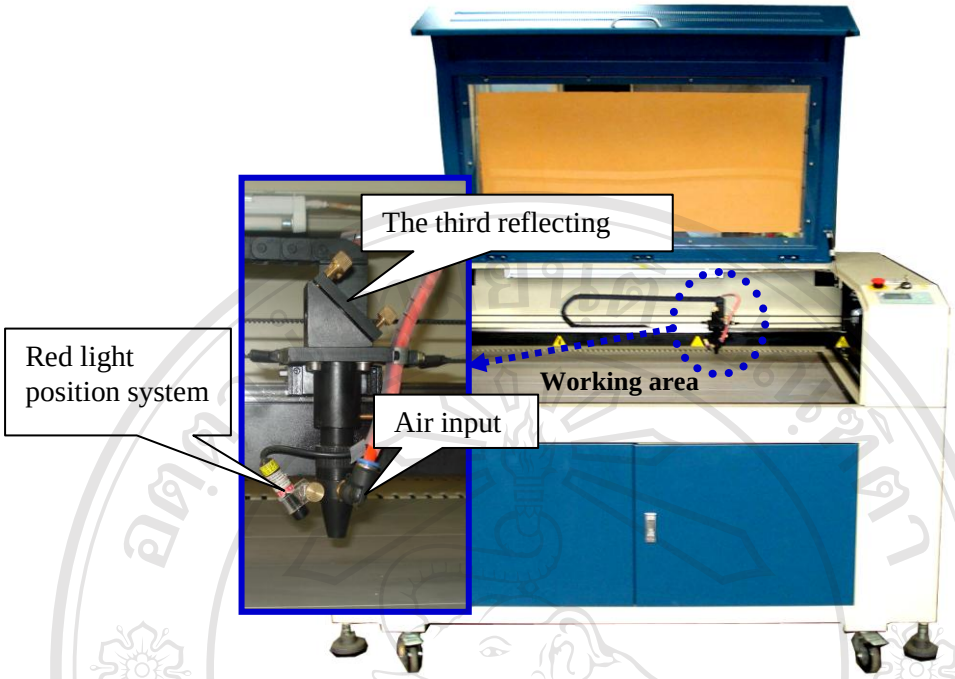

Figure 2.9 CO_2 laser machine (SF960 Jinan Senfeng Technology Co., LTD, China)

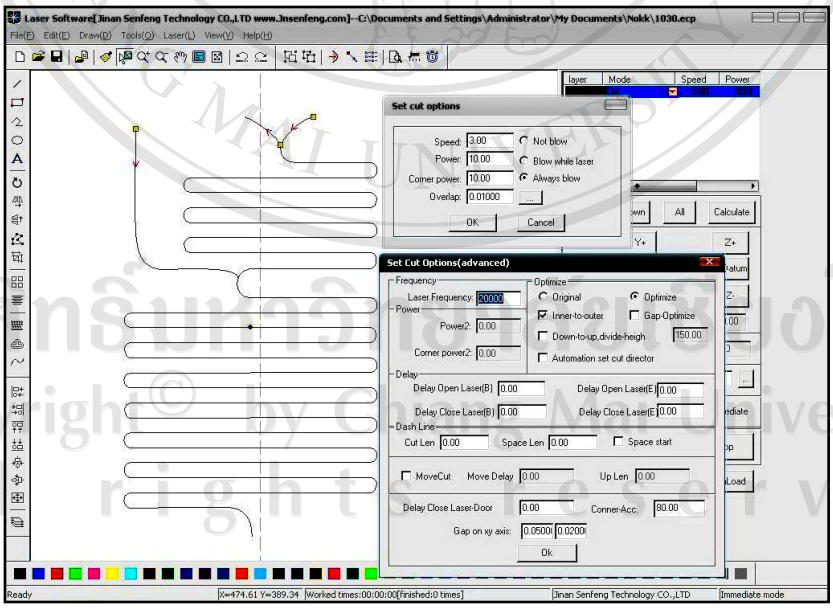

Figure 2.10 Laser operation and setup parameters program for fabrication of PMMA chip

2.7.3 Dimensions of PMMA microchannels

Dimensions of the Y-junction PMMA microchannels from CO_2 laser machine were investigated. The dimension of microchannels was obtained by controlling laser power and scanning speed of laser beam. The parameters that affect on the channels depth, width and smooth were examined by changing laser power and scanning speed. The laser power and the scanning velocity were varied from 10 to 20 W and 1.0 to 20.0 mm s^{-1}, respectively.

The PMMA microchannels were cut a cross section with the size of 0.5 x 0.5 cm and then the depth and the width of microchannels were measured using a scanning electron microscope (SEM). In addition, the smooth of microchannels surface are studied using close up SEM images from top view.

2.7.4 Surface modification and thermal bonding

Surface modification with solvent and thermal bonding of PMMA microchannel process was done described earlier in section 2.6.5, but the size of PMMA sheet was 4.0 x 4.0 cm^2 (1.0 mm thickness) and the covered plate with the size of 4.0 x 4.0 cm^2 (thickness 1.5 cm) is drilled two through holes (i.d. 1.0 mm) for creating reservoir ports and another through hole (i.d. 0.50 mm, part length 1.0 cm) for detection cell including channel outlet. All holes relate to the pattern of PMMA microchannel. The volume of detection cell was 2 µL.

2.7.5 Fabrication of microflow analyzer

The microflow analyzer system used is described schematically in Figure 2.11. It consisted of the PMMA chip, a ten-port with zero dead volume selection valves (VICI, Valco Instruments, USA) and a peristaltic pump (ISMATEC peristaltic pump

0.001–68 mL min^{-1} per channel with Tygon pump tubing 0.19 mm i.d.), controlled by using computer software which were used for injecting accurate volume of samples and transporting appropriate flow rate of reagent. PTFE tubing (0.19 mm i.d.) were used as flow lines connected with the μFA.

The PMMA chip system for determining zinc (Figure 2.11) consisted of the Y-junction with U-Turns microchannels. The PMMA μFA was fabricated by using laser ablation (CO_2 laser machine); the channel dimensions were 195 μm wide, 194 μm deep and 20 mm long. The PMMA cover plate obtained was clear color with three small channels. The PMMA cover plate and PMMA microchannels were bonded at 140^{o}C in an oven. The first and the second channel are used for transporting sample and reagent to the μFA. PTFE inlet tubing of 0.2 mm i.d. (Cole Parmer) was inserted into the holes at channel one and channel two of PMMA chip prior to filling a droplet of a mixer two component of Epoxy Glue (E-POX-E 5 GLUE, Cognis Chemicals(M) Sdn Bhd, Malaysia) and the time taken about six minute for solidify of Epoxy Glue with crystal clear epoxy. The third one is used as detection cell (2 μL) where the absorbance of the complex was detected, and the solution was passed out. The μFA detection cell was integrated with two fiber optic probes. The first fiber optic probe is connected to the USB4000 spectrophotometer as detector and the second one is connected to the LED light source. Spectrasuit software is used to control the fiber optic spectrometer and also used for data collection.

2.7.5.1 Preliminary studies on the spectrophotometric of Zn(II)-xylenol orange complex using quinine enhancer

The solutions of 3.0 x 10^{-5} mol L^{-1} XO, with and without of each 1.0, 2.0 and 3.0 of Zn(II) was prepared. Each concentration of Zn(II) solution was added 0.15 mL of 0.002 mol L^{-1} XO solution, diluted with acetate buffer pH 5 in a 10 mL volumetric flask, mixed well and then transferred into the sample cell. Then absorption spectra of each Zn(II)-XO complexes and the XO blank solutions were scanned over a range of 380-700 nm using JENWAY 6400 and the signals were recorded with the Jenway Scan software.

The solutions of XO 3.0 x 10^{-5} mol L^{-1} mixed with quinine 2 x 10^{-3} mol L^{-1}, with and without of each 1.0, 2.0 and 3.0 of Zn(II) was prepared. Each concentration of Zn(II) solution were added 0.15 mL of 0.002 mol L^{-1} XO solution, 0.020 mL of 0.01 mol L^{-1} quinine, diluted with acetate buffer pH 5 in 10 mL volumetric flask, mixed well and then transferred into the sample cell. Then absorption spectra of each Zn(II)-XO complexes in quinine solution and the XO in quinine blank solutions were scanned over a range from 380-700 nm using JENWAY 6400 and the signals were recorded with Jenway Scan software.

2.7.5.2 The method for Zn(II) determination using μFA

The μFA system (Figure 2.11) integrated with a fiber optic spectrometer to obtain a microflow manifold for determination of Zn(II). The method involved the injection 5 μL of standard or sample solution containing Zn(II) by switching the selection valve into a XO stream of 1.0 x 10^{-4} mol L^{-1} mixed with quinine hydrochloride 1.0 x 10^{-3} mol L^{-1} in acetate buffer pH 5.5 with an appropriate flow rate

of 40 μL min^{-1} using peristaltic pump with the specially desired software to control the flow system (sample injection volume calculated from aspiration time and flow rate). XO and Zn(II) were reacted on Y-junction with U-turns microchannel of the PMMA chip producing a red-violet colored complex arising from xylenol orange-Zn(II) complex and then passed through the detection cell (2 μL) fixed in the PMMA cover plate, where the fiber optic probe was placed to measure the absorbance at 590 nm. The concentration of Zn(II) in each water sample was calculated by reference to the calibration graph prepared under optimum conditions. A comparative determination of the Zn(II) in the water samples was carried out by ICP-OES method.

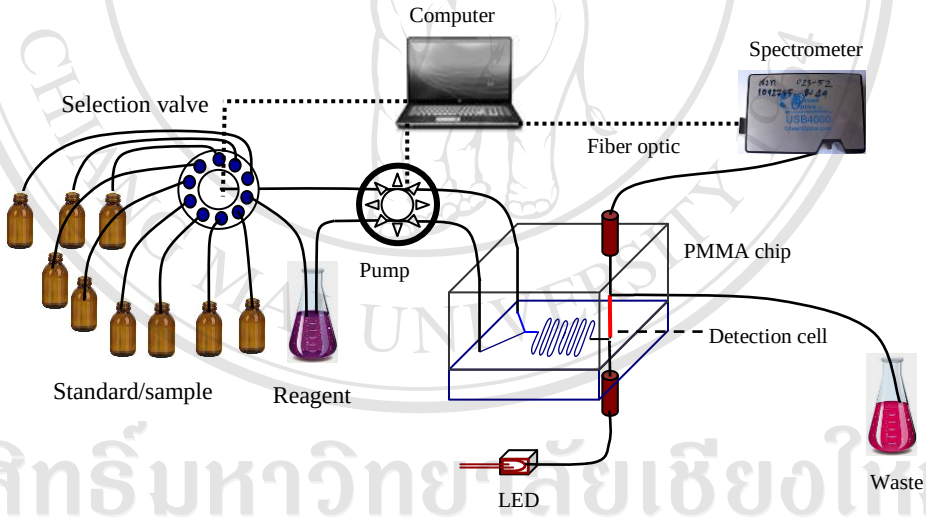

Figure 2.11 The μFA system with Y-junction pattern for Zn(II) determination: Standard, Zn(II)/water sample; reagent, XO mixed quinine photosensitizer

2.7.5.3 Optimization of the experimental conditions

The proposed μFA system was optimized by a univariate method, to select the optimum conditions for the highest sensitivity with low background and standard deviation of Zn(II)-XO complex. The value of one variable was changed while keeping the other variables at their constant values. By maintaining that variables at its optimum value, another was studied. The optimization of the experimental conditions was carried out by using standard Zn(II) solution. In all experiments, five replicate measurements were made for each studied parameter. The studied range for the optimization of all parameters of the μFA for determination of Zn(II) was shown in Table 2.2.

Table 2.2 The studied ranges for the optimization of all parameters of μFA

Variable	Studied range
Wavelength (nm)	575 - 600
pH	4.0 – 6.5
Concentration of xylenol orange (x 10^{-4} mol L^{-1})	0.25 – 2.5
Concentration of quinine (x 10^{-3} mol L^{-1})	0.05-2.5
Flow rate (μL min^{-1})	20 - 70
Sample volume (μL)	2 - 7

2.7.6 Analytical characteristics study

2.7.6.1 Linear range

Working standard solutions of Zn(II) ion over the ranges of 0.01 – 1.8 mg L^{-1} were prepared from the intermediate Zn(II) stock solution (20 mg L^{-1}). The series of Zn(II) standard solutions with different concentrations were flowed through the PMMA chip of the μFA system by means five replicate results. Concentrations of Zn(II) were measured by USB4000 spectrometer and recorded as peak heights with Spectrasuite Software. The investigation of linear range was obtained by plotting the peak heights against various concentrations of Zn(II).

2.7.6.2 Precision of the proposed method

The precision of the proposed method was verified by injecting 11 replicates of 0.5 and 1.0 mg L^{-1} standard Zn(II) solution and calculated % RSD from the equation 2.1.

2.7.6.3 Accuracy of the proposed method

The accuracy of the proposed method was verified by spiking the water samples with various concentrations of Zn(II) standard solution (0.5 mg L^{-1}) respectively using the recommended procedure. Then, Zn(II) concentrations were calculated from linear regression equation obtained from the calibration graph. Finally, the percentage recovery was calculated from the equation 2.2.

2.7.6.4 Detection limit [259]

Using the μFA system (Figure 2.11) and the optimum conditions, the detection limit of Zn(II) 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. Clearly, detection limit is depends on baseline and the analyte signal. It is calculated from equations 2.3 and 2.4.

2.7.6.5 Interference studies

The interference effects of some possible foreign ions in μFA system for zinc determination were examined by the proposed μFA procedure under the optimum conditions. A systematic study to check for the effects of some possible foreign ions (Na^+, K^+, Cu^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Cr^{3+}, Cd^{2+}, Al^{3+}, Mn^{2+}, Mg^{2+}, Na^+, Ca^{2+}, NO_3^-, SO_4^{2-}, HCO_3^-, Br^-, I^-, Cl^-,) on the determination of Zn(II) in water samples by adding known amounts of each interference to 0.5 mg L^{-1} of Zn(II) standard solution. The tolerable concentration of each interfering ion for determination of Zn(II) was taken as a highest concentration causing error smaller than ±10% [263].

2.7.6.6 Validation method

In order to validate the μFA method for Zn determination, a comparative determination of Zn(II) by the FAAS method was carried out. Results obtained by both methods were verified by using student t-test. The calculated t_{cal} value was obtained from the equations (2.5, 2.6, 2.7) as follows [259].