CHAPTER 2

EXPERIMENTAL

2.1 Instruments and Apparatus

Instruments and Apparatus	Model	Company	Country
1. Analytical Balance	AB 304-s	Mettler Todedo	Switzerland
2. Digital multimeters	UT6o Series	Uni-Trend	Hong kong
3. Flat spiral glass flow cell: glass tubing, i.d. 1.5 mm, spiral coil diameter 25 mm	-	-	Australia
4. Flow rate tygon pump tubing	-	TACS	Australia
5. Hot plate stirrer	-	Fisher Scientific	USA
6. Peristaltic pump	-	Gilson Miniplus 3	France
7. pH meter	827 pH lab	Metrohm	Switzerland
8. Six port selection valve	V 450	Upohurch	USA
9. Photomultiplier tube	9828SB	Electron Tubes	UK
10. Power supply	PM20D	Electron Tubes	UK
11. PTFE membrane 25 mm ×12 m × 0.076 mm	-	Pro- tech group	Australia
12. Three-way Connector (T-Shaped)	6365-90	Cole-Parmer	USA

Instruments and Apparatus (Continued)

Instruments and Apparatus	Model	Company	Country
13. Ultrapure water purification system	-	Millipore	France
14. Ultrasonicator	136H	NDI	Germany
15. UV/VIS spectrophotometer	U-2001	Hitachi	Japan

2.2 Chemicals

Chemical	Molecular Formula	Company	Country
1. Aluminium nitrate	$Al(NO_3)_3$	Merck	Germany
2. Ammonium nitrate	NH_4NO_3	BDH	England
3. Barium nitrate	$Ba(NO_3)_2$	Ajax	Australia
4. Cadmium nitrate	$Cd(NO_3)_2$	Fluka	Switzerland
5. Calcium nitrate	$Ca(NO_3)_2$	BDH	England
6. Chromium nitrate	$Cr(NO_3)_3$	Merck	Germany
7. Cobalt nitrate.	$Co(NO_3)_2$	BDH	England
8. Copper nitrate	$Cu(NO_3)_2$	BDH	England
9. Disodium hydrogen phosphate	Na_2HPO_4	BDH	England
10. EDTA	$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$	BDH	England
11. Ethanol	C_2H_6O	Merck	Germany
12. Ferric nitrate	$Fe(NO)_3.9\ H_2O$	Carlo Erba	Italy
13. Ferrous sulphate	$FeSO_4$	Carlo Erba	Italy
14. Lead nitrate	$Pb(NO_3)_2$	Carlo Erba	Italy
15. Luminol	$C_8H_7N_3O_2$	Fluka	Swizerland
16. Magnesium nitrate	$Mg(NO_3)_2$	Merck	Germany
17. Manganese sulphate	$MnSO_4$	Merck	Germany
18. Nitric acid	HNO_3	Merck	Germany
19. Nickel nitrate	$Ni(NO_3)_2$	Merck	Germany
20. Phosphoric acid	H_3PO_4	Merck	Germany

Chemicals (Continued)

Chemical	Molecular Formula	Company	Country
21. Potassium chloride	KCl	BDH	England
22. Potassium cyanide	KSCN	Merck	Germany
23. Potassium dichromate	$K_2Cr_2O_7$	Fisher Scientific	USA
24. Potassium iodide	KI	Sigma	Germany
25. Sodium acetate trihydrate	$CH_3.COONa.3H_2O$	Merck	Germany
26. Sodium bromide	NaBr	BDH	England
27. Sodium chloride	NaCl	Fluka	Switzerland
28. Sodium fluoride	NaF	Merck	Germany
29. Sodium hydrogen carbonate	$NaHCO_3$	Merck	Germany
30. Sodium hydrogen phosphate	NaH_2PO_4	BDH	England
31. Sodium hydroxide	NaOH	Merck	Germany
32. Sodium iodide	NaI	Fluka	Switzerland
33. Sodium nitrate	$NaNO_3$	Merck	Germany
34. Sodium nitrite	$NaNO_2$	Merck	Germany
35. Sodium sulphate	Na_2SO_4	BDH	England
36. Sulfuric acid	H_2SO_4	Merck	Germany
37. Zinc nitrate	$Zn(NO_3)_2$	Ajax	Australia
38. Zinc oxide (nanoparticles)	ZnO	-	Thailand

2.3 Preparation of Standard and Reagents Solutions

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

2.3.1 Nitrate stock solution (0.1000 mol L^{-1})

Nitrate stock standard solution was prepared by dissolving 0.8499 g of sodium nitrate in ultrapure water in a 100 mL volumetric flask, which was then transferred into polyethylene (PE) bottle and kept in 4 °C refrigerator for further use. Working standard nitrate solutions were prepared from stock solution of nitrate by diluting with phosphate buffer solution pH 8.0.

2.3.2 Nitrite stock solution (0.1000 mol L^{-1})

Nitrite stock standard solution was prepared by dissolving 0.6900 g of sodium nitrite in ultrapure water in a 100 mL volumetric flask, which was then transferred into polyethylene (PE) bottle and kept in 4 °C refrigerator for further use. Working standard nitrite solutions were prepared from stock solution of nitrite by diluting with ultrapure water.

2.3.3 Luminol stock solution (2.0 x10^{-3} mol L^{-1})

The stock CL reagent solution was prepared by dissolving 0.4430 g of luminol in 2.50 mL of 2.0 mol L^{-1} NaOH and diluted to 250 mL with ultrapure water in a 250 mL volumetric flask. Working luminol solutions as reagent solution were prepared from stock solution of luminol by diluting with sodium hydroxide solution.

2.3.4 Sulfuric acid solution (0.15 mol L^{-1})

The solution of sulfuric acid was prepared by dissolving 4.15 mL of concentrated sulfuric acid in ultrapure water in a 500 mL volumetric flask.

2.3.5 Hydrogen peroxide stock solution (100 mmol L^{-1})

The hydrogen peroxide solution was prepared by dissolving 1.02 mL of 30% hydrogen peroxide solution in ultrapure water in a 100 mL volumetric flask. Working hydrogen peroxide solutions were prepared from stock solution of hydrogen peroxide by diluting with ultrapure water.

2.3.6 EDTA stock solution (10 mmol L^{-1})

The EDTA stock solution was prepared by dissolving 0.3722 g of EDTA in ultrapure water in a 100 mL volumetric flask. Working EDTA solution were prepared from stock solution of EDTA by diluting with ultrapure water

2.3.7 Sodium hydroxide stock solution (2.0 mol L^{-1})

The sodium hydroxide stock solution was prepared by dissolving 8.00 g of sodium hydroxide in ultrapure water in a 100 mL volumetric flask. Working sodium hydroxide solution were prepared from stock solution of sodium hydroxide and diluted with ultrapure water. The concentration of sodium hydroxide by hydrochloric acid solution 2.0 mol L^{-1}.

2.3.8 Phosphate buffer solution (pH 8.0)

The phosphate buffer solution pH 8.0 was prepared by mixing 47.35 mL of 0.2000 mol L^{-1} Na_2HPO_4 solution and 2.65 ml of 0.2000 mol L^{-1} NaH_2PO_4 solution in ultrapure water in a 50 mL volumetric flask, then transferred into polyethylene (PE) bottle. And balanced pH by 0.1 mol L^{-1} phosphoric acid solution.

2.4 Flow Injection Chemiluminescence Flow Design

2.4.1 Preliminary investigation of the flow injection chemiluminescence for determination of nitrite

Figure 2.1 showed the experimental set up for the flow injection chemiluminescence (FI-CL) manifold for the determination of nitrite, which consisted of the three channels peristaltic pump to propel a sulfuric acid solution steam (C), hydrogen peroxide solution steam (R1) and luminol solution steam (R2). The total flow rate was set at 3.0 mL min^{-1} with equal flow rate for all reagent and carrier streams (1 mL for each line). A 50 µL standard nitrite solution was injected via an injection valve into the carrier stream of 0.3 mol L^{-1} sulfuric acid solution (C). It was merged with the stream of 4 mmol L^{-1} hydrogen peroxide and EDTA in sulfuric acid solution (R1) in the reaction coil (1.07 mm inner diameter, 50 cm in length). The mixed-reagent solution was merged with the steam of luminol solution (R2) in a custom-build luminomiter to give chemiliminescent light.

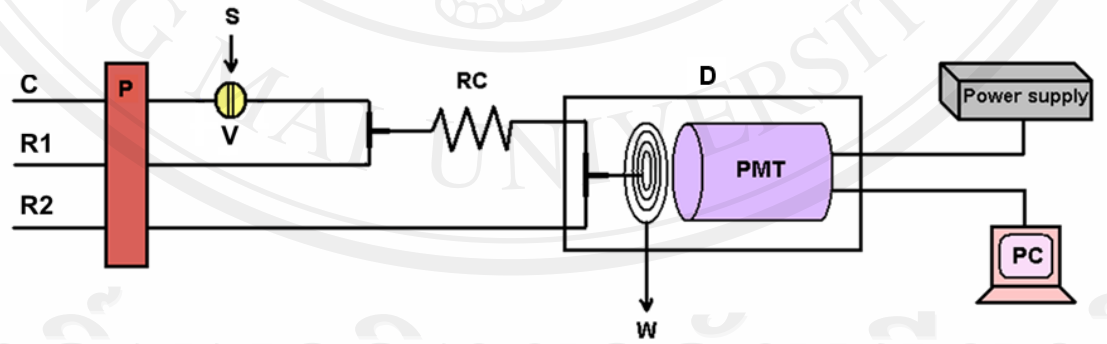

Figure 2.1 The FI-CL manifold for nitrite determination. C: H_2SO_4 solution, R1: H_2O_2 and EDTA in H_2SO_4 solution, R2: Luminol and EDTA in NaOH solution, S: Sample/standard solution, P: Pump, V: Injection valve, RC: Reaction coil, D: Chemiluminescence detecter, PMT: Photomultiplier tube, W: Waste, PC: Personal computer

2.4.2 A gas diffusion flow injection chemiluminescence (GDFI-CL) for determination of nitrate and nitrite

A schematic diagram of the GDFI-CL system used for the determination of nitrate and nitrite shown in Figure 2.2 and Figure 2.3.

The optimization of experimental conditions such as the reagent conditions and some physical variables, including the flow rate and the sample volume injection was carried out by means of a univariation method. For this feature, a variable was modified maintaining the other variables at their constant values. The optimum conditions were investigated by injecting 50 µL of $1x10^{-5}$ mol L^{-1} nitrite solution into the carrier stream of 0.15 mol L^{-1} H_2SO_4 with a flow rate of 3.0 mL min^{-1}. The aceeptor stream reagent of 3.0 mmol L^{-1} H_2O_2 in the 2.50 mmol L^{-1} EDTA solution and 0.15 mol L^{-1} H_2SO_4 solution, the chemiluminescence reagent consists of 2.50 mmol L^{-1} luminol in the 0.60 mol L^{-1} NaOH and 2.50 mmol L^{-1} EDTA solution. The appropriate conditions were obtained by judging from the highest CL intensity of each parameter using the FI manifold with CL detection as shown in Figure 2.2.

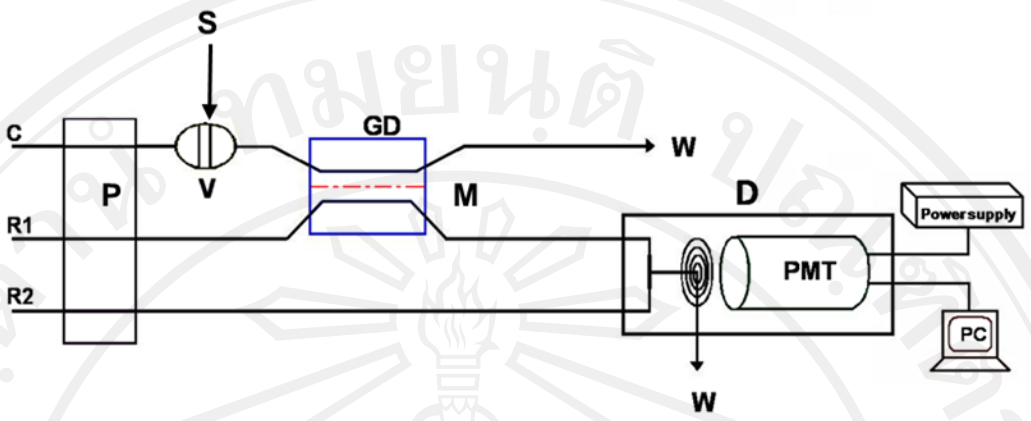

Figure 2.2 The GDFI-CL manifold for nitrite determination. C: H_2SO_4 solution, R1: H_2O_2 and EDTA in H_2SO_4 solution, R2: Luminol and EDTA in NaOH solution, S: Sample/standard solution, P: Pump, V: Injection valve, GD: Gas diffusion unit, M: PTFE membrane, D: Chemiluminescence detecter, W: Waste, PC: Personal computer

The simultaneous determination of nitrite and nitrate was made possible by the on-line photoreduction of nitrate to nitrite using a low pressure UV lamp irradiation set at the wavelength of 254 nm. Photoreduction of UV irradiation performance was improved by using zinc oxide nanoparticles as catalyst. The sample stream flowing through by pump1 to photoreaction column (A), which retain for photoreduction at 8 min. Then the sample was injected to flow injection system via injection valve after reduction. The appropriate conditions were obtained by judging from the highest CL intensity of each parameter using the FI manifold with CL detection as shown in Figure 2.3.

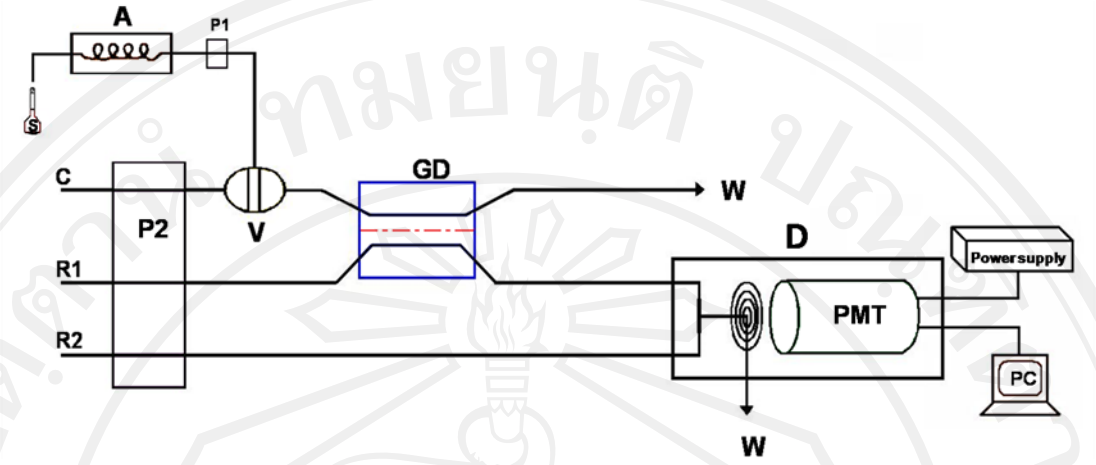

Figure 2.3 The GDFI-CL manifold for nitrate determination. C: H_2SO_4 solution, R1: H_2O_2 and EDTA in H_2SO_4 solution, R2: Luminol and EDTA in NaOH solution, S: Sample/standard solution (ZnO nanoparticles added), A: Photochemical reactor, P: Pump, V: Injection valve, GD: Gas diffusion unit, M: PTFE membrane, D: Chemiluminescence detecter, W: Waste, PC: Personal computer

2.5 Procedure

2.5.1 Flow injection chemiluminescence (FI-CL) system

The 50 µL aliquot of standard or sample solution containing nitrite was injected manually into a 0.15 mol L^{-1} H_2SO_4 carrier stream (C) , flow rate 1 mL min^{-1}, which then merged with the reagent stream (R1) of 3.00 mmol L^{-1} H_2O_2 in acidic solution. After mixing well at the reaction coil, the combined reaction mixture was mixed with the luminol reagent (R2), flowing at 1 mL min^{-1}, and then passed through a flat spiral coil flow cell where the CL intensity was detected by a PMT operated at a voltage of 825 V. The chemiluminescence intensity was increased with corresponding to nitrite concentration.

2.5.1.1 Optimization of the FI-CL system by univariate method

The studied range for the developed flow injection procedure for determination of nitrite in FI-CL was shown in Table 2.1. The univariation optimization was started with investigation of suitable PMT voltage by keeping other conditions at constant values while varying the PMT voltage over the range of 725-1000 V. When the studied parameter was undergone changing to the optimized value, the next parameter was varied (see Table 2.2). The other parameters were performed in the same manner throughout the optimization process. To optimize the conditions of the FI-CL manifold (Figure 2.1), the preliminary experimental conditions (Table 2.2) were investigated.

Table 2.1 Preliminary experimental conditions of FI-CL for studying optimum PMT voltage

Variable	Fixed Value
Concentration of Sufuric acid (mol L^{-1})	0.3
Concentration of EDTA (mmol L^{-1})	3
Concentration of hydrogen preoxide (mmol L^{-1})	4
Concentration of luminol (mmol L^{-1})	2
Concentration of sodium hydroxide (mmol L^{-1})	0.5
Sample volume (μL)	50
Reaction coil length (cm)	50
Flow rate (mL min^{-1})	1

The studied ranges for the FI-CL parameters were shown in Table 2.2.

Table 2.2 The studied range for the FIA parameters

Variable	Studied range
PMT voltage (V)	725-1000
Concentration of Sufuric acid (mol L^{-1})	0.1-0.35
Concentration of EDTA (mmol L^{-1})	0.5-3.5
Concentration of hydrogen preoxide (mmol L^{-1})	1.0-5.0
Concentration of luminol (mmol L^{-1})	0.1-5.0
Concentration of sodium hydroxide (mmol L^{-1})	0.1-0.9
Sample volume (μL)	0.4 – 2.3
Reaction coil length (cm)	25 – 150
Flow rate (mL min^{-1})	50 - 400

2.5.2 Gas diffusion flow injection chemiluminescence (GDFI-CL) system for determination of nitrite

Aqueous standard sample of 50 μL volume were injected by polyethylene syringe into the acidic donor steam (0.15 mol L^{-1} H_2SO_4) of the GDFI-CL system (Figure 2.2). In the case of nitrite standard, nitrous acid was generated as a result of the reaction between nitrite in the standard and the sulfuric acid in the donor steam. The nitrous acid gaseous was generated and transported through the donor channel and diffused through the hydrophobic PTFE membrane into the mixed reagent accepter steam (3.00 mmol L^{-1} H_2O_2 in acid medium). After that, nitrous acid was generated to peroxynitrous acid with mixed reagent in the accepter steam, which then merged with the luminol reagent solution, and passed through a flat spiral coil flow

cell where the CL intensity was detected by a PMT operated at a voltage of 825 V. The output of the PMT, which is proportional to the CL intensity, was monitored continuously. The GDFI-CL signals corresponding to peak heights were plotted versus various concentrations of nitrite.

2.5.2.1 Optimization of the GDFI-CL system by univariate method

The studied ranges for the GDFI-CL parameters were developed from previous flow injection chemiluminescence study for determination of nitrite.

2.5.2.2 Linearity of calibration graph

Using the GDFI-CL manifold (Figure 2.2) under the optimum conditions, linear range of calibration graph was studied from the relationship return the intensity and several nitrite standards in the concentration ranging from $1x\ 10^{-8}$ – $1x10^{-1}$ mol L^{-1}. Intensity corresponding to various concentrations of nitrite were measured by GDFI-CL procedure and recorded as peak heights as function of time. A typical calibration graph was obtained by plotting the peak heights against various concentrations of nitrite.

2.5.2.3 Precision

The precision of the proposed method was verified by injecting 12 replicates of $1x\ 10^{-5}$ mol L^{-1} standard nitrite solution, and the % RSD was calculated from the equations as follows;

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

Where $\%RSD$ = percentage relative standard

SD = standard deviation

$\overline{X}$ = mean

2.5.2.4 Detection limit

The detection limit is defined as the minimum concentration of analyte that can be detected and can be calculated from following equation (2.2).

$$S_m = S_{bl} + kSD_{bl} \quad (2.2)$$

Where Sm is the minimum analytical signal

S_{bl} is the mean blank signal

SD_{bl} is the standard deviation of blank

k is the confidence level of detection : 3

The detection limit is the analyte concentration providing the minimum analytical signal.

2.5.2.5 Accuracy of the proposed method

The accuracy of the proposed method were verified by spiking the food samples with various concentrations of nitrite standard solution using the recommended procedure. Then, nitrite concentrations were calculated from linear regression equation obtained from the calibration graph. Finally, the percentage recovery was calculated from the equation as follows;

$$\%\text{Recovery} = \frac{(\text{total nitrite concentration} - \text{nitrite concentration in sample}) \times 100}{\text{Spiked nitrite concentration}} \quad (2.3)$$

2.5.2.6 Interference studies

The interference effects of some possible foreign ions in FIA system for nitrite determination were studied by the proposed FIA procedure under the optimum conditions. A systematic study to check for the effects of some possible foreign ions

(Al^{3+}, Fe^{2+}, Cu^{2+}, Ba^{2+}, Co^{2+}, Cr^{3+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Na^{+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, K^{+}, I^{-}, Br^{-}, Cl^{-}, F^{-}, HCO_3^{-}, SO_4^{2-}, NO_3^{-}, PO_4^{3}, IO_3^{--}, $Cr_2O_7^{2-}$) on nitrite determination was performed by adding known amounts of each interference to 1x 10^{-5} mol L^{-1} of nitrite standard solution.

2.5.3 Gas diffusion flow injection chemiluminescence (GDFI-CL) system with on-line photoreduction for determination of nitrate using zinc oxide nanoparticles as catalyst

Innitailly photoreaduction (10 min) of nitrate to nitrite using a low pressure UV lamp set the wavelength at 254 nm was investigated. Its performance was improved by using zinc oxide nanoparticles as catalyst. The zinc oxide nanoparticles suspension prepared by diluting the zinc oxide stock suspension with ultrapure water and was added to nitrate standard solution. Time taken for photoreduction reaction of nitrate to nitrite with zinc oxide nanoparticles was less than those without it. In this case, nitrate standard solution was reduced to nitrite by on-line photoreduction. The photoreduction of nitrate to nitrite was taken place after nitrate solution tream was passed through the photoreactor column and was remained for 10 min to complete reduction. The resulting product as nitrite was then injected into the acidic donor steam (0.15 mol L^{-1} H_2SO_4) to form nitrous acid and diffused through the hydrophobic membrane into the mixed reagent accepter steam (3.00 mmol L^{-1} H_2O_2 in an acid medium). After that, nitrous acid was generated as a result of the reaction between nitrite in the standard and the sulfuric acid in the donor steam. Then, nitrous acid was converted to peroxynitrous acid by the mixed reagent in the accepter steam, which was then merged with the luminol reagent, and passed through a flat spiral coil

flow cell where the CL intensity was detected by a PMT operated at a voltage of 825 V. The output of the PMT, which is proportional to the CL intensity, was monitored continuously. The GDFI-CL signals corresponding to peak heights were plotted versus various concentrations of nitrate.

2.5.3.1 Linearity of calibration graph

Using the GDFI-CL manifold (Figure 2.3) under the optimum conditions, linear range of calibration graph was studied from the relationship return the intensity and several nitrate standards in the concentration ranging from 1×10^{-3} – 1×10^{-1} mol L^{-1}. Intensity corresponding to various concentrations of nitrite were measured by GDFI-CL procedure and recorded as peak heights as function of time. A typical calibration graph was obtained by plotting the peak heights against various concentrations of nitrate.

2.5.3.2 Precision

The precision of the proposed method was verified by injecting 12 replicates of 1×10^{-2} mol L^{-1} standard nitrite solution, and the % RSD was calculated from the equations (2.1).

2.5.3.3 Detection limit

The detection limit is defined as the minimum concentration of analyte that can be detected and can be calculated from equation (2.2).

2.5.3.4 Accuracy of the proposed method

The accuracy of the proposed method were verified by spiking the food samples with various concentrations of nitrate standard solution using the recommended procedure. Then, nitrate concentrations were calculated from linear

regression equation obtained from the calibration graph. Finally, the percentage recovery was calculated from the equation (2.3).

2.6 Validation Method

In order to determine the accuracy of the method, nitrite and nitrate in food samples were determined by using the validation method (the colorimetric and xylenol method) versus the proposed GDFI-CL method. The nitrite and nitrate concentrations obtained by both methods were compared and verified by using student *t*-test at 95% confident level.

2.6.1 AOAC Colorimetric method for determination of nitrite in food samples

A sample 5.00 g finely comminuted and thoroughly mixed sample into a 50 mL beaker. Forty mL H_2O was added heated to 80^0 C and mixed thoroughly with a glass rod. Care should be taken to break up or lumps, which was transfer to a 500 mL volumetric flask. The beaker and the rod were thoroughly washed with successive portions of the hot H_2O and adding all washings to flask. After that enough hot H_2O was added to bring volume to 300 mL. The sample solution was put into a steam bath heated and stood for 2 hours with shaking occasionally. The sample solution cool was to room temperature, diluted to the volume with H_2O, remixed and filtered. A 2.50 mL sulfanilamide reagent was added to aliquot of standard solution containing 5-50 □g $NaNO_2$ in a 50 mL volumetric flask and mixed. After 5 min, the 2.50 mL NED reagent was added, diluted to 50 mL with water and let the color to be developed for about 15 min. A portion of the solution was then transferred to photometer cell and the nitrite concentration determinated at 540 nm against the reagent blank containing

45 mL H_2O, 2.5 mL sulfanilamide reagent, and 2.5 mL NED reagent. The content of nitrite in the sample solution was calculated by reference to the calibration curve.

2.6.2 AOAC Xylenol method for determination of nitrate in food samples

A 5.00 g of finely comminuted sample was thoroughly mixed with 80 mL warm H_2O. Lumps were broken up and heat on steam bath for 1 hour with stirring occasionally. After the cooling the sample to room temperature, the sample solution was transferred to a 100 mL volumetric flask, diluted to the volume with water and mixed well. The solution was filtered, or let settle, and a 40 mL filtrate, or supernate was pipetted into a 50 mL volumetric flask. (No correction for volume occupied by meat is necessary.) Then 3 drops of bromocesol green indicator were added followed by adding H_2SO_4 (1+10) dropwise until the color changed to yellow. Nitrites were oxidized to nitrates by adding 0.2000 N $KMnO_4$ solution dropwise with shaking about 1 min until the color was changed to faint pink. Then 1 mL of H_2SO_4 (1+10) and 1 mL of 200 g L^{-1} phosphotungstic acid solution were added to dilute the volume to 100 mL, mixed well and filtered.

Into a 500 mL flask an aliquot ($\leq$ 20 mL) of standard solution containing of 0.025 mg nitrate was measured. Enough among of Ag-NH_4OH solution was added to ppt all chlorides and most of excess phosphotungstic acid without decanting or filtering and make up to the volume with water. About 3 times volume liquid in flask of H_2SO_4 (3+1) solution was added, mixed the solution and cooled down to about 35 ^oC and 0.05 mL of *m*-xylenol was then added with shaking and hold for 30 min at 30-40 ^oC. After nitration was complete, a 150 mL of H_2O was added, care should be

takes to wash off stopper, and distillating about 40-50 mL into receiver containing 5 mL of 10 g L^{-1}NaOH solution. In order to quickly remove any nitroxylenol solidifying in condenser, this can be done by stopping H_2O flow and letting condenser become warm. The distillate solution was transferred to a 100 mL volumetric flask and diluted to the volume with H_2O. Nitrate in sample solution was determined by comparing the reading of color of suitable aliquot of sample solution with standard curve prepared at 450 nm. The color standard prepared from 10 mL of nitrate standard solution, which was using 0.05 mL of *m*-xylenol and 30 mL of H_2SO_4 (3+1), and diluting the distillate to 500 mL.

2.6.3 Student T- test

In order to validate the FIA method for nitrite determination, a comparative determination of nitrite by the UV/VIs 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 ;

$$t_{calculated} = \frac{\overline{x}_1 - \overline{x}_2}{S_{pooled}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \qquad (2.4)$$

$$S_{pooled} = \sqrt{\frac{\sum_{set1}(x_i - \overline{x}_1)^2 + \sum_{set2}(x_j - \overline{x}_2)^2}{n_1 + n_2 - 2}} \qquad (2.5)$$

Where; S_{pooled} the pooled standard deviation

$n_1 + n_2 - 2$ number of degree of freedom

2.7 Analytical recovery

The recoveries of nitrite/nitrate by the proposed method were verified by spiking two known amounts of nitrite/nitrate standard solution in food samples. After analysis of the sample solution by the proposed method, the nitrite/nitrate concentrations were calculated from linear regression equation obtained from the calibration graph. Finally, the percentage recovery was calculated from the equation as follows;

$$\%\ Recovery = \frac{(total\ \ nitrite/nitrate\ \ conc. - nitrite/nitrate\ \ conc.\ in\ sample) \times 100}{spiked\ nitrite/nitrate\ \ conc.} \qquad (2.6)$$