APPENDICES

APPENDIX A

SPECIATION OF Fe(II)/Fe(III) USING FLOW INJECTION SPECTROPHOTOMETRY WITH 1,10-PHENANTHROLINE

Iron is one of major metal species of concern in many samples such as natural waters, food and serum. The speciation of Fe(II)/Fe(III) is very important for environmental and biological studies because its chemical forms influence the chemical and toxicological properties of iron and other trace elements .The most popular methods for speciation of Fe(II)/Fe(III) based on the measurement of the absorbance of the Fe(II)-1,10 phenanthroline complex.

In this work, it was focused on speciation of Fe(II)/Fe(III) using FI-spectrophotometry with 1,10- phenanthroline. The attempt to adopt LabVIEW program as recorder has been made.

The reverse-FIA (r-FIA) is another type of FIA. Unlike normal FIA (n-FIA), the role of sample and reagent are reversed. The method is based on the injection of reagent into carrier stream of sample while n-FIA is based on the injection of sample into carrier stream of reagent. The r-FIA offers advantages of minimise reagent consumption while sample amount is less limited.

LabVIEW program is a graphical programming language G that relies on graphical symbols rather than textual language to describe programming actions. This program was written for using as recorder instead of chart recorder. It is more convenient for data record and procession comparing with paper chart recorder. Moreover, it offers economic benefit. Paper of chart recorder is quite expensive. Therefore, LabVIEW is very useful tool to record data via PC.

A.1 Principle

The Fe(II)/Fe(III) speciation by using r-FIA system (as shown in **Figure A.1**) was performed by injecting of 1,10-phenanthroline into a flowing stream of standard /sample containing Fe(II) and /or Fe(III), Fe(II) forms a red complex which can be continuously monitored for the absorbance. Fe(III) in the stream is reduced by ascorbic acid to Fe(II). The total Fe(II) then reacts with 1,10-phenanthroline. The difference in amounts obtained with and without using the reducing agent is the Fe(III) contents. The reactions of the method can be described in **equation A.1 and A.2.**

$$Fe^{3+} \xrightarrow{\text{Ascorbic acid}} Fe^{2+} \quad \text{...... equation A.1}$$

$$Fe^{2+} + \text{1,10-phenanthroline} \longrightarrow \text{red complex} \quad \text{...... equation A.2}$$

(measured at 515 nm)

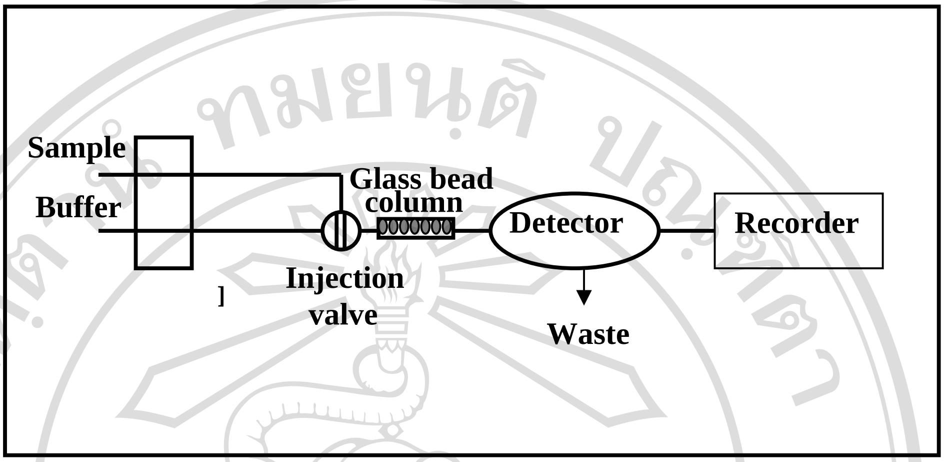

Figure A.1 r-FIA system for Fe(II)/Fe(III) speciation

A.2 Conditions

The conditions for this method [114] are shown in **Table A.1**

Table A.1 Conditions for Fe(II)/Fe(III) speciation

Parameters	**Conditions**
Chemical	0.6% (w/v) 1,10-phenanthroline, 1.0% (w/v) ascorbic acid (as reducing agent) and 2.0 M acetate buffer pH 4.5
Linear range, mg l^{-1}	0.05-2.00
Glass bead length, cm	20
Flow rate, ml min^{-1}	2.6 for acetate buffer line 5.8 for standard/sample line

A.3 Calibration

Fe(II) determination was carried out in the concentration range of 0.05-2.0 mg l^{-1}.The calibration for Fe(II) determination is depicted in **Figure A.2.** The signal from both chart recorder and LabVIEW program are illustrated in **Figure A.3 a)** and **b),** respectively.

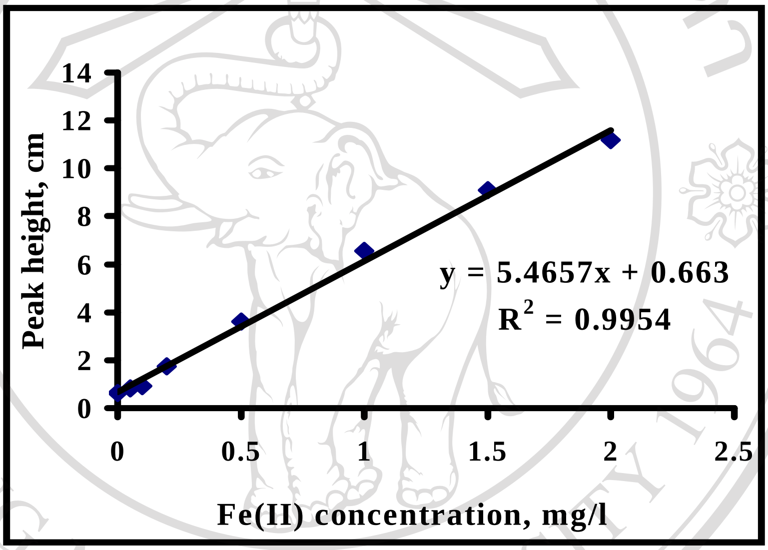

Figure A.2 Calibration for Fe(II) determination using FI-spectrophotometry with 1,10- phenanthroline

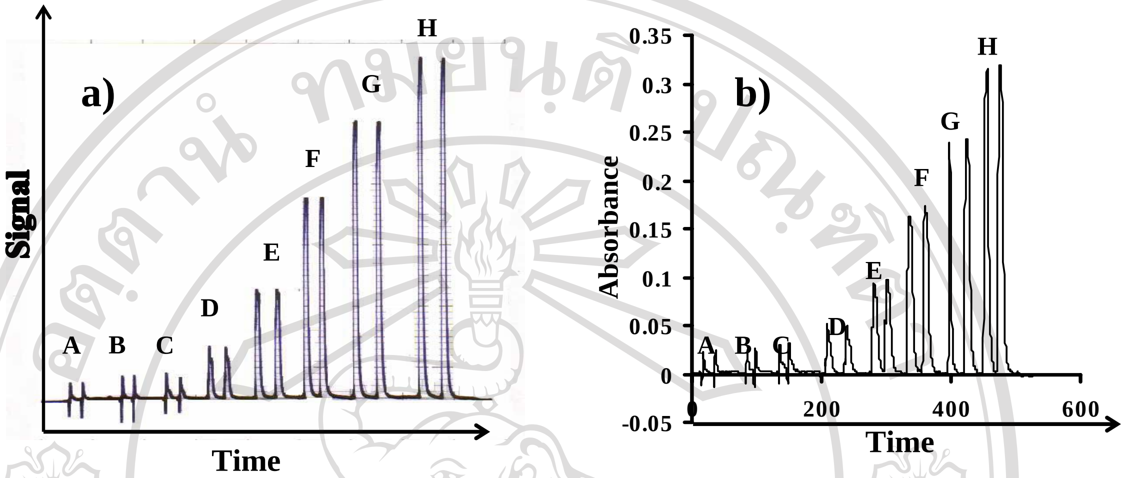

Figure A.3 FIgrams of Fe(II) standards using (a) a chart recorder and (b) a LabVIEW program; concentration: A= 0 (blank), B= 0.05, C= 0.10, D= 0.20, E= 0.50, F= 1.00, G= 1.50, H= 2.00 mg l^{-1}

A.4 Fe(II)/Fe(III) speciation

Fe(II)/Fe(III) speciation was priliminary performed by using standard of various concentrations of Fe(II) and Fe(III). The results are shown in **Table A.2.** The FIgrams are depicted in **Figure A.4.** It was found that the speciation of Fe(II)/Fe(III) can be achieved. This can be observed as following:

1. The signal of the mixture of Fe(II)/Fe(III) concentration of 0.10, 0.25, 0.50 mg l^{-1} with reducing agent are higher approximately for 2 times of the signal of each mixture without reducing agent.
2. The signal of the mixture of Fe(II) 0.25 mg l^{-1} and Fe(III) 0. 25 mg l^{-1} with reducing agent is almost equal to the signal of the mixture of Fe(II) 0.5 mg l^{-1} and Fe(III) 0.5 mg l^{-1} without reducing agent.

Table A.2 Fe(II)/Fe(III) speciation

Concentration, mg l^{-1}		**Signal (Abs), n=2**	
Fe(II)	Fe(III)	without reducing agent	with reducing agent
0	0	0.009 ±0.001(A)	0.014 ± 0.001 (B)
0.10	0.10	0.028 ±0.004 (C)	0.052 ±0.004 (D)
0.25	0.25	0.068 ±0.004 (E)	0.120 ±0.000 (F)
0.50	0.50	0.125 ±0.007 (G)	0.205 ±0.005 (H)

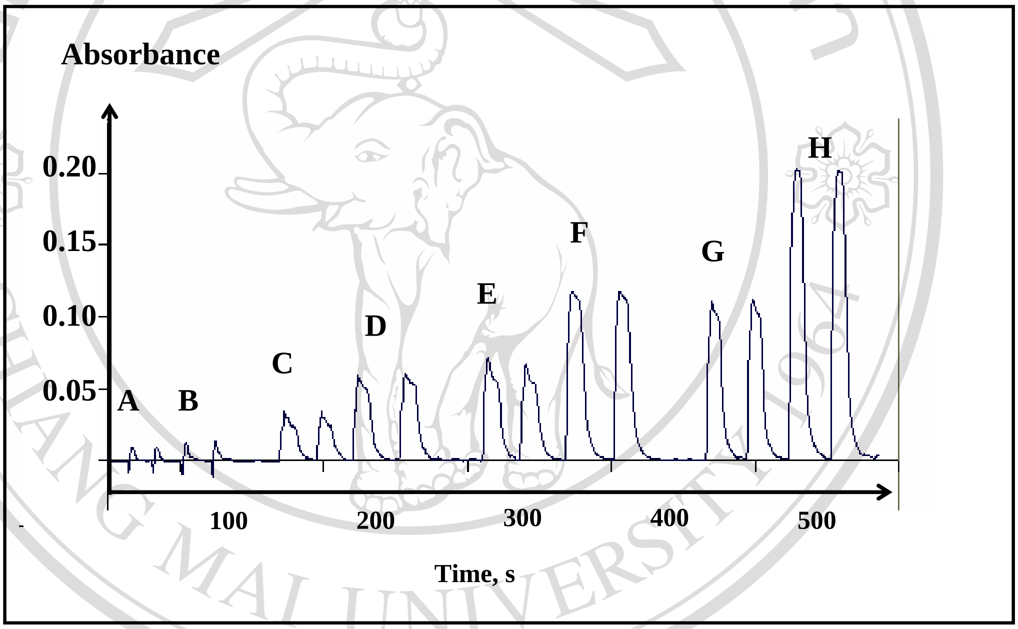

Figure A.4 FIgrams of Fe(II)/Fe(III) speciation using LabVIEW program as recorder;

A&B- blank without and with reducing agent, respectively,

C&D- standard mixture of Fe(II) 0.10 mg l^{-1} and Fe(III) 0.10 mg l^{-1} without and with reducing agent, respectively,

E&F- standard mixture of Fe(II) 0.25 mg l^{-1} and Fe(III) 0.25 mg l^{-1} without and with reducing agent, respectively,

G&H- standard mixture of Fe(II) 0.50 mg l^{-1} and Fe(III) 0.50 mg l^{-1} without and with reducing agent, respectively.

A.5 Conclusion

From the studies, it can be demonstrated that the r-FIA for speciation of Fe(II)/Fe(III) with 1,10-phenanthroline offers possibility for rapid, reproducible and simple procedure.

APPENDIX B

SEQUENTIAL INJECTION ANALYSIS FOR PARACETAMOL DETERMINATION

Paracetamol is widely used for analgesic. In order to control quality, various methods have been utilized for paracetamol determination such as spectrophotometry [115], electrochemical method [116] and HPLC [117]. However these methods are time and reagent consuming and lack of simplicity, thus SIA could be an alternative.

In this work, it was focused on paracetamol determination using SIA coupling with spectrophotometry. The method is based on nitrosation reaction of paracetamol with sodium nitrite in an acidic medium to yield an unstable nitroso derivative, which could be converted to a more stable compound by addition of sodium hydroxide. The product yellow compound can be monitored at 430 nm. The diagram of the nitrosation reaction of paracetamol is illustrated in **Figure B.1** and the system is shown in **Figure B.2**.

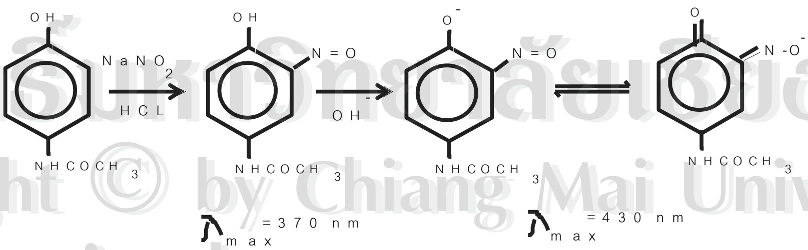

Figure B.1 Schematic diagram of the nitrosation reaction of paracetamol [118]

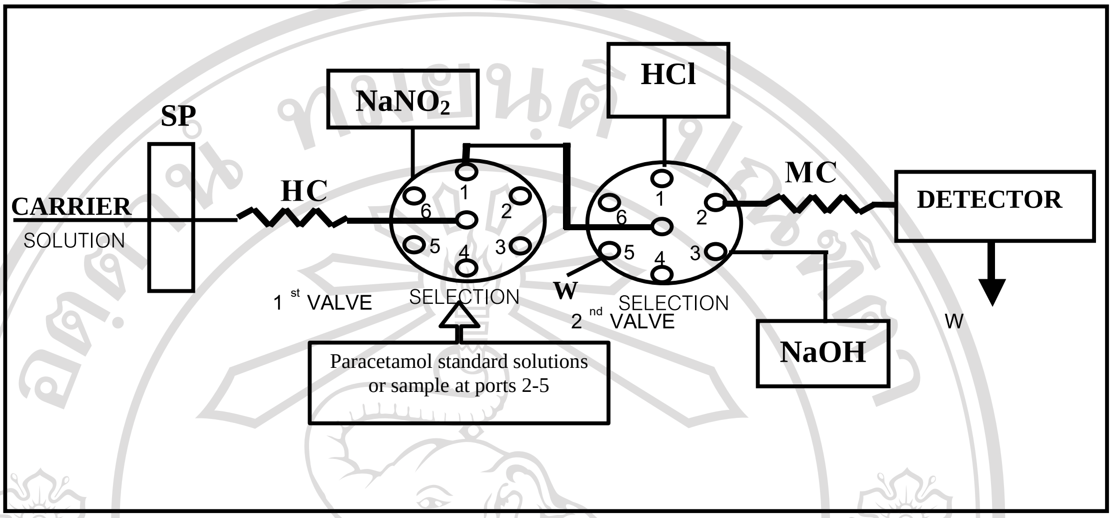

Figure B.2 The SI manifold for paracetamol determination: SP= syringe pump, HC= holding coil, W= waste and MC= mixing coil

B.1 Operation procedure

Sequences of the SI-system have been investigated in order to provide an optimum performance. The best sequence for the paracetamol determination is presented in Table B.1.

Table B.1 Sequence of SI operation

Sequence	Volume (μl)
1. Filling up line with standard/sample	
1.1 Aspirate standard/sample to HC	500
1.2 Aspirate water from reservoir to SP	1,500
1.3 Send sample/standard to W	1,500
2. Determination step	
2.1 Aspirate HCl to HC	300
2.2 Aspirate $NaNO_2$ to HC	75
2.3 Aspirate sample/standard to HC	10
2.4 Aspirate NaOH to HC	250
2.5 Aspirate water from reservoir to SP	2,500
2.6 Flow all reagents and sample in HC to detector	2,500
2.7 Repeat 2.1-2.6 for another injection	

B.2 Optimisation

The parameters including reagent concentrations and volumes of all reagents, mixing coil length and flow rate of the reagents have been optimised. The optimum conditions are presented in **Table B.2.**

Table B.2 Optimum conditions for paracetamol determination

Parameters	Value
Concentration of HCl, M	0.05
Concentration of $NaNO_2$, M	0.10
Concentration of NaOH, M	0.15
Volume of HCl, μl	300
Volume of $NaNO_2$, μl	75
Volume of NaOH, μl	250
Volume of Paracetamol, μl	10
Holding coil length, cm	50
Mixing coil length, cm	50
Flow rate, ml min^{-1}	6

B.3 Calibration

Paracetamol determination was performed. The calibration was linear in the range of 400-1,000 mg l^{-1} and 1,000-2,500 mg l^{-1} as depicted in **Figure B.3.** The SIgrams of standard paracetamol are illustrated in **Figure B.4**.

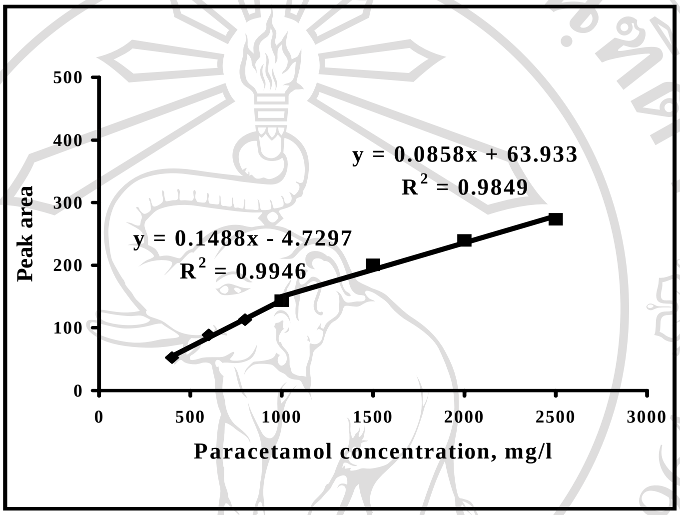

Figure B.3 Calibration of paracetamol determination using SIA

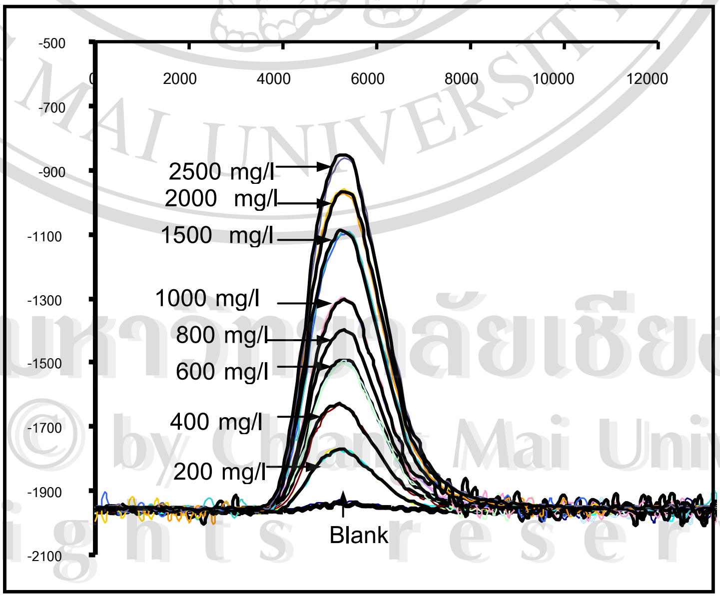

Figure B.4 SIgrams of paracetamol standard solutions

B.4 Sample preparations

B.4.1 SIA method

B.4.1.1 For paracetamol tablet

1. 20 tablets of samples were accurately weighed and finely powdered.
2. Approximately 500 mg of finely powder (1) was weighed and then 60 ml DI-water was added and followed by standing it on magnetic stirrer hot plate for 15 min. Temperature must be kept constant in the range 60-70 ^{0}C.
3. The suspension in (2) then was filtered and made up to 250 ml with DI-water. The further appropriate dilutions were made using DI-water.

B.4.1.2 For paracetamol syrup

1. Density of syrup was determined by weighing syrup for 25 ml at room temperature.
2. Appropriate amount of sample was weighed in 100 ml volumetric flask, the volume was made to 100 ml with DI-water. The sample was shaked and then filtered. Finally, the portion was taken for paracetamol determination.

B.4.2 Standard method (HPLC method) [119]

Samples were prepared as similar as the sample preparation for SIA method. However, for HPLC method, 25% methanol was used as solvent instead of DI-water and samples must be put in an ultrasonicate bath for 5 min after stirring by magnetic stirrer.

B.5 Application

The method was applied for paracetamol determination in pharmaceutical preparations. Samples are classified into 3 types as shown in **Table B.3**. The results for paracetamol determination obtained from proposed method and standard method were compared in **Table B.4.** The performance of the system including linear range, precision and detection limit was presented in **Table B.5.**

Table B.3 List of paracetamol samples in each type

Paracetamol tablet	**Formula paracetamol tablet**	**Paracetamol syrup**
CEMOL	DECOLGEN	BERAMOL SYRUP
BERAMOL	NUTA	TEMPRA SYRUP
TYLENOL	PARAFON	CALPOL SUSPENSION
PARACET	TIFFY	TYLENOL SUSPENSION
PARACAP		

Table B.4 Paracetamol determination by using SIA and HPLC method

Samples	**Paracetamol amount**						
	Label (mg)	**SIA**				**HPLC**	
		Range A^1		**Range B^2**			
		Found (mg/tablet)	**% label**	**Found (mg/tablet)**	**% label**	**Found (mg/tablet)**	**% label**
TIFFY	500	481	96	420	84	492	98
CEMOL	500	506	101	461	92	462	92
BERAMOL	500	506	101	500	100	472	94
TYLENOL	500	505	101	475	95	459	92
PARACET	500	555	111	495	99	478	96
PARACAP	500	501	100	498	100	464	93
DECOLGEN	500	487	97	488	98	454	91
NUTA	500	523	105	467	93	469	94
PARAFON	300	308	103	279	93	276	92
BERAMOL SYRUP	120	130	108	117	97	109	91
TEMPRA SYRUP	120	126	105	118	98	111	92
CALPOL SUSPENSION	120	127	106	111	93	110	92
TYLENOL SUSPENSION	160	164	103	162	101	147	92

[1] Range A= range 400-1,000 mg l^{-1}

[2] Range B= range 1,000 –2,500 mg l^{-1}

Table B.5 Performance of the proposed method

Analytical characteristics	Value
Linear range, mg l^{-1}	400-1,000 and 1,000-2,500
Precision, %RSD, n=11	
- range 400-1,000 mg l^{-1}	4.6
- range 1,000-2,500 mg l^{-1}	2.9
Detection limit[1], mg l^{-1}	
- range 400-1,000 mg l^{-1}	70
- range 1,000-2,500 mg l^{-1}	294
Sample frequency (f), h^{-1}	60

[1] Detection limit defined as 3 times of blank deviation plus blank signal [105]

B.6 Conclusion

SIA can be achieved for paracetamol determination. The calibrations were linear in the ranges of 400-1,000 mg l^{-1} and 1,000–2,500 mg l^{-1}. The detection limits were 70 mg $l^{-1}$and 294 mg l^{-1}, respectively. This method was applied for paracetamol determination in pharmaceutical preparations. The results were agreed well with HPLC method (t-test evaluation at 95 % confidence level). The results were precise with 1.9-4.6 %RSD for standard and sample of paracetamol. The proposed system handles 60 injections per hour. Moreover, this method requires small volume of reagents and sample (μl level).

APPENDIX C

FLOW INJECTION SPECTROMETRIC DETERMINATION OF NITRITE

Nitrite ions are widely used as preservatives in meat products. It is known that nitrite causes methaemoglobinaemia and, with secondary and tertiary amines, yields the cancerogen nitrosoamines. Due to this toxic, it is important to develop new analysis methods for determination of nitrite in real samples. The methods, for example, spectrophotometry [120] and capillary electrophoretic [121] are employed for nitrite determination. However, these methods are labour intensive methods that are demanding in time and materials. Moreover, the reagents used are toxic to human health. Therefore, the rapid method and less toxic reagents used is considered. In this work, flow injection spectrometric determination of nitrite, which could offers those benefits, was investigated.

The method is based on nitrosation reaction of paracetamol with sodium nitrite in an acidic medium. The reaction and chemicals are as same as the reaction for paracetamol determination in **Appendix B (Figure B.1)**. However, in this case, nitrite is analyte while paracetamol is reagent. The FI system is shown in **Figure C.1**.

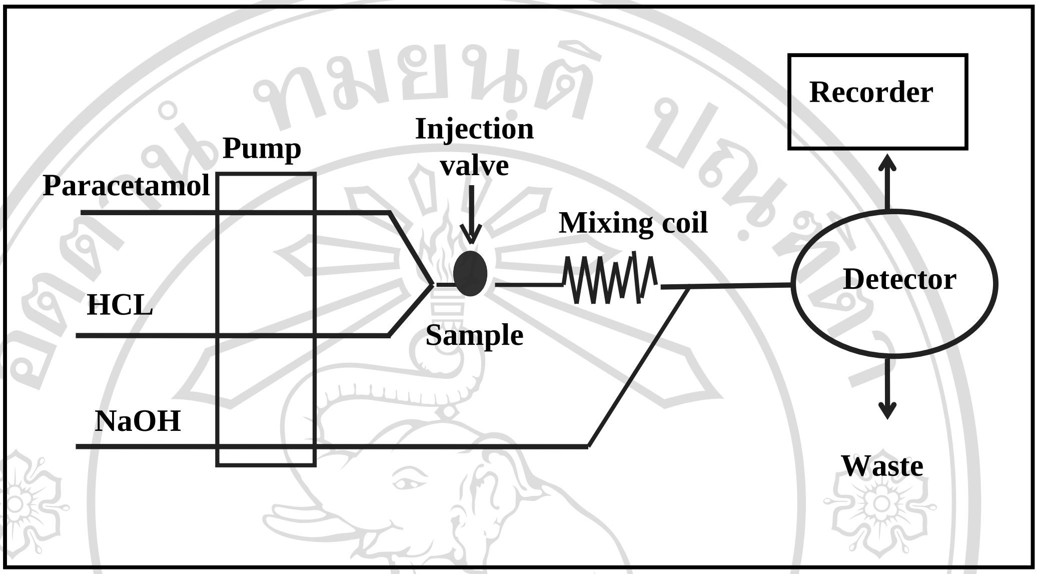

Figure C.1 The FI manifold for nitrite determination

C.1 Optimisation

The parameters including concentration and volume of all reagents, sample volume, mixing coil length and flow rate of the reagents have been optimised. The optimum conditions are presented in **Table C.1.**

Table C.1 Optimum conditions for nitrite determination.

Parameters	Value
Concentration of HCl, M	0.07
Concentration of paracetamol, M	0.10
Concentration of NaOH, M	0.2
Sample volume, µl	220
Mixing coil length, m	1
Total flow rate, ml min^{-1}	5

C.2 Calibration

Standard nitrite determination was performed. The calibration was linear in the range of 50-300 mg l^{-1} as depicted in **Figure C.2.** The FIgrams of standard nitrite are illustrated in **Figure C.3**.

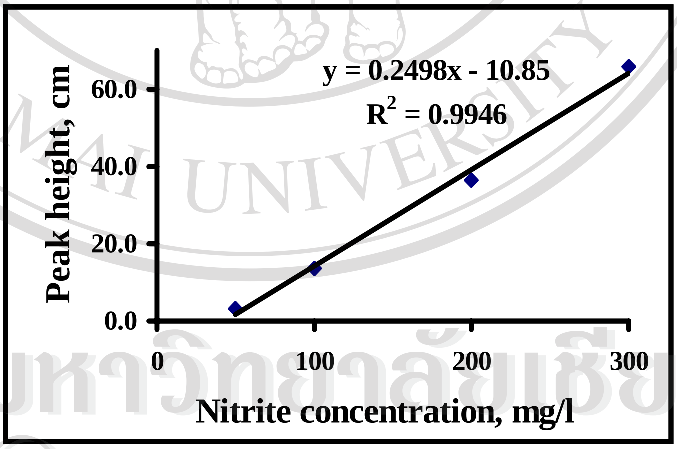

Figure C.2 Calibration of nitrite determination using FIA

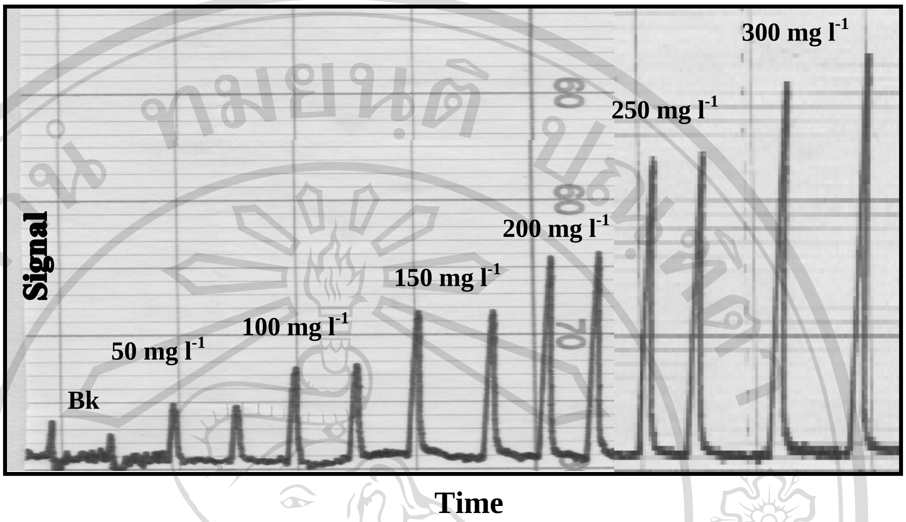

Figure C.3 FIgrams of nitrite standard solutions

C.3 Performance of the system

The performance of FI spectrometric determination for nitrite is summarised in **Table C.2.**

Table C.2 Performance of the proposed method for nitrite determination

Analytical characteristics	Value
Linear range, mg l^{-1}	50-300
Precision, %RSD, n=11 (For 100 mg l^{-1}standard of nitrite)	3.5
Detection limit[1], mg l^{-1}	40
Sample frequency (f), h^{-1}	60

[1] Detection limit defined as 3 times of blank deviation plus blank signal [105]

C.4 Conclusion

The FI spectrometric system for nitrite determination offers possibility of simple and rapid method. However, as it could be seen from **Table C.2**, detection limit obtained by using the proposed system is quite high. The improvement of detection limit should be done further in order to analyse nitrite in cured meat product (normally, approximately 10-50 mg l^{-1} of nitrite presented in cured meat samples).

APPENDIX D

THE EXTRACTION OF NITRITE FROM SAUSAGE SAMPLE BY SONICATION

The extraction of nitrite from a solid sample by sonication was investigated aiming for rapid and high efficiency extraction. Nitrite was determined spectrophotometrically. It is based on Griess reaction [63]. Nitrite reacts with sulfanilamide in acidic medium to form a diazonium salt, which couples with N-(1-napthyl)ethylenediamine dihydrochloride to form a reddish purple azo dye. Absorbance of the dye was measured at 540 nm. The reaction of the method is expressed in **Figure D.1**. This method was applied to the determination of nitrite in a sausage sample.

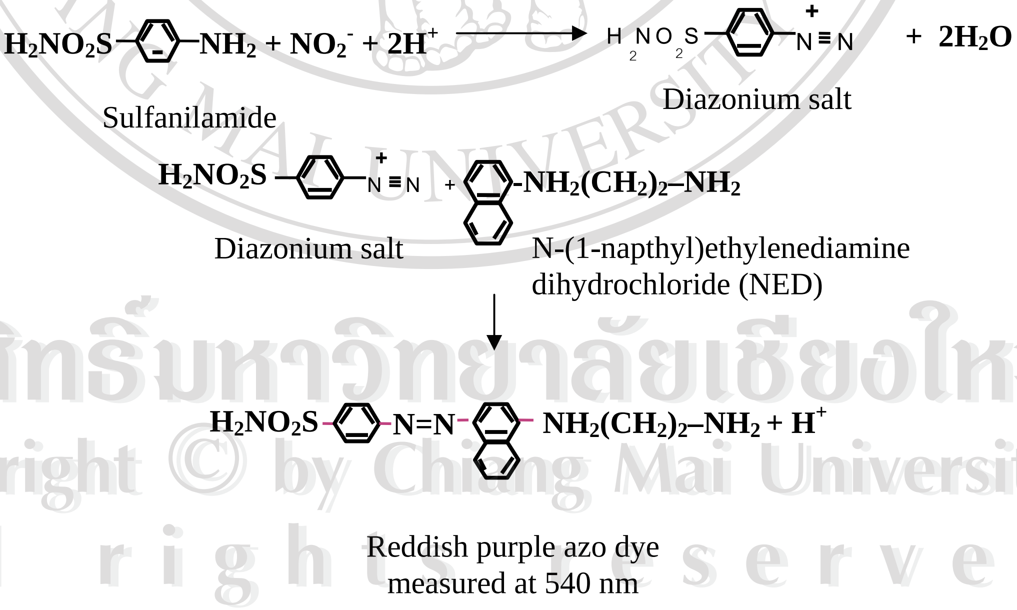

Figure D.1 Schematic diagram of Griess reaction

D.1 Nitrite extraction by sonicattion procedure

Nitrite presented in meat sample, for example sausage, can be extracted efficiently by sonication procedure as following:

1. Sample approximately 25 g was weighed, then 50 ml-hot water was added into the sample.
2. The slurry (1) was stirred and put into sonicate bath for 30 min.
3. The sample was then centrifuged at 4,000 rpm for 20 min, the supernatant was collected.
4. The decant from (3) was extracted again by adding 30 ml-hot water, and sonicated for 5 min. Then it was centrifuged at 4,000 rpm for 10 min, the supernatant was collected with supernatant (3).
5. The decant from (4) was extracted again by adding 30 ml-hot water and sonicated for 5 min. Then it was centrifuged at 4,000 rpm for 10 min, the supernatant was collected with supernatant (3).
6. All supernatant (3+4+5) was adjusted volume to 250 mL with DI-water.
7. 25 ml of solution (6) was taken into beaker and ammonium sulfate 15 g was added to coagulate protein, which is main substance in the samples, and then it was centrifuged at 4,000 rpm for 20 min.
8. The solution (7) was filtered and the volume was made to 50 ml with DI-water. This solution will be taken for nitrite determination by spectrometric method.

D.2 Calibration

Standard nitrite determination was performed by using spectrometric determination. The calibration was linear in the range of 100-800 μg l^{-1} as depicted in **Figure D.1.**

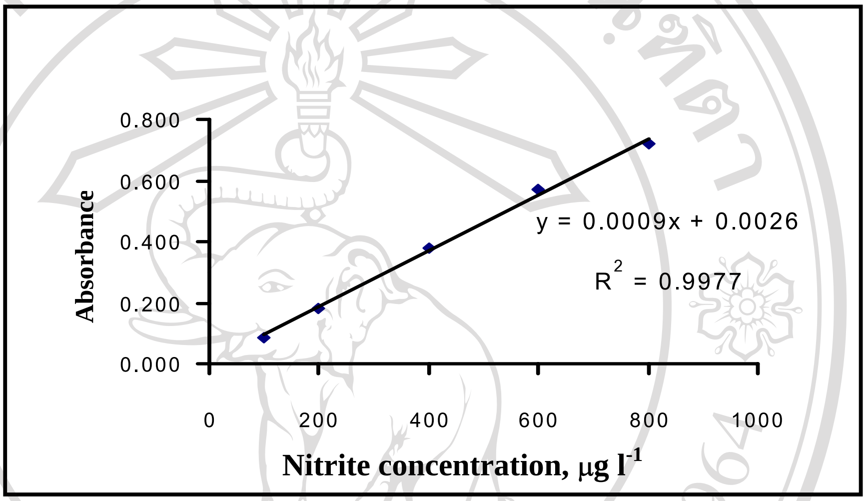

Figure D.2 Calibration for standard nitrite determination by spectrometric method

D.3 Recovery of the nitrite standard

The efficiency of the extraction method was observed from recoveries of the standard of nitrite solution. Recovery study using a sausage sample was made by adding nitrite standard solution (4, 8, and 16 ml of 20 mg l^{-1}) into a 25 g portion of the sausage sample yielding added nitrite of 3.2, 6.4 and 12.8 μg g^{-1}, respectively. Then %recoveries were calculated as shown in **Table D.1.**

Table D.1 Recoveries of nitrite added into digested sausage sample

Nitrite added to sample µg l^{-1}	**Nitrite found µg l^{-1}**	**% Recovery**
0	8.6$^{(A)}$	-
3.2	11.8	100
6.4	15.6	109
12.8	21.0	97

$$\% \text{ Recovery} = \frac{\text{Nitrite found} - 8.6^{(A)}}{\text{Nitrite added to sample}} \times 100$$

D.4 Conclusion

The extraction of nitrite in a sausage sample by sonication was a rapid and efficient method. %Recoveries of standard nitrite solution added in sausage samples in the range of 97-109 were established.

APPENDIX E

CONDITIONS FOR ANODIC STRIPPING VOLTAMMETRY FOR ARSENIC SPECIATION (BATCH PROCEDURE)

E.1 Gold –film electrode preparation [90]

Before each deposition of a gold film, polish the electrode with alumina powder and wash it successively with nitric acid and double distilled water. Submerge the electrode in 40 mg l^{-1} gold(III) solution and leave in open circuit during a 5 min purge with nitrogen. After ensuring that gas bubbles are not lodged on the surface of the glassy carbon electrode (GCE), apply potential of –0.2 V vs. Ag/AgCl to the electrode for 4 min. Since the gold deposited on GCE as a brown powder from stirred solution and as a lustrous plate from quiescent solution, the plating was done in a quiescent solution. For the optimum reproducibility, the electrode was re-plated at 0.5 V for 10 s between each measurement.

E.2 As(III) determination [90]

The standard/sample was deaerated with pure nitrogen for 5 min. The potential of –0.3 V was applied for the sample cell with the rotating rate of 5,000 rpm for 4 min. After deposition, the rotation of the electrode was turned off and left for 30 s at –0.3 V that the solution became quiescent. Stripping was carried out in the differential pulse mode starting at –0.3 V with a scan rate of 30 mV s^{-1} in positive direction. Other operation parameters of the polarographic analyser included a pulse width of 50 ms and a pulse height of 50 mV.

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September, 2003- January, 2004	Granted by the Development and Promotion of Science and Technology Talent Project (DPST), Thailand and Technical University of Denmark, Denmark

Doctoral research

Development of Flow-Based Techniques for the Determination of Some Heavy Metals in Environmental Samples

Practical experiences

May–September, 2002	Ph.D. research, the Institute of Environment and Resources (E&R) of Technical University of Denmark (DTU), Lyngby, Denmark

September, 2003- January, 2004	Ph.D. research, the Institute of Environment and Resources (E&R) of Technical University of Denmark (DTU), Lyngby, Denmark

International journals

1. R. Burakham, S. Duangthong, L. Patimapornlert, N. Lenghor, S. Kasiwad, L. Srivichai, S. Lapanantnoppakhun, J. Jakmunee and K. Grudpan, *Anal. Sci.*, **20**(2004)841.
2. S. Duangthong, H. Mosbaek and K. Grudpan, *An Automated On-line Solvent Extraction and Flame Atomic Absorption Spectrometric Determination of Heavy Metals in Soil Samples.* Manuscript in preparation.
3. S. Duangthong, H. Mosbaek and K. Grudpan, *Chromium Speciation Analysis by Using an Automated On-Line Solvent Extraction Coupling with Flame Atomic Absorption Spectrometry.* Manuscript in preparation.

International conferences

1. K. Grudpan, S. Lapanantnoppakhun, S. Duangthong, L. Srivichai, S. Kasiwad and J. Jakmunee. *Flow Injection and Sequential Injection Determination of Paracetamol in Pharmaceutical Preparations Based on Nitroso Derivatives with Nitrite*, Poster Presentation, 11th International Conference on Flow Injection Analysis, Including Related Techniques, 16-20 December 2001, Chiang Mai, Thailand.

2. R. Burakham, S. Duangthong, L. Patimapornlert, N. Lenghor, S Lapanantnoppakhun, J. Jakmunee and K. Grudpan. *Sequential Injection Analysis for Determination of Paracetamol in Pharmaceutical Preparations Using Nitrosation Reaction.* Poster Presentation, 9th International Conference on Flow Analysis, 17-21 February 2003, Geelong, Australia.
3. S. Duangthong, H. Mosbaek and K. Grudpan. *On-line Solvent Extraction and Flame Atomic Absorption Spectrometric Determination of Heavy Metals in Soil Samples.* Oral Presentation, 6th International Conference on Environmental Management and Technology, 4-6 August 2003, Renaissance Palm Garden Hotel, Putrajaya, Malaysia.
4. S. Duangthong, H. Mosbaek and K. Grudpan. *Chromium Speciation Analysis by Using an Automated On-line Solvent Extraction Coupling with Flame Atomic Absorption Spectrometry.* Oral Presentation, 13th European Conference on Analytical Chemistry, 5-10 September 2004, Salamanca University, Salamanca, Spain.
5. S. Duangthong, H. Mosbaek and K. Grudpan. *Cr(III)/Cr(VI) Speciation by Using an Automated On-line Solvent Extraction Coupling with Flame Atomic Absorption Spectrometry*. Poster Presentation, 2nd Asian International Conference on Ecotoxicology and Environmental Safety, 26-29 September 2004, Songkhla, Thailand.

National Conferences

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