

# A Simple Flow Injection Spectrophotometric Determination of Iron Using Nitroso-R salt as complexing agent

Senee Kruanetr<sup>1</sup>, Wish Thanasarakhan<sup>1</sup>, Urai Tengjaroenkul<sup>1</sup>, Boonsom Liawruangrath<sup>2</sup> and Saisunee Liawruangrath<sup>1,\*</sup>

<sup>1</sup>Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand

<sup>2</sup>Department of Pharmaceutical Chemical, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand 50200, Thailand

## Abstract

Flow injection (FI) spectrophotometric method for iron (III) determination using Disodium-1-nitroso-2-naphthol-3,6-disulphonate (nitroso-R salt) is performed. It is based on the measurement of Fe(III)-nitroso-R salt complex at 720 nm formed by the reaction between Fe(III) and nitroso-R salt in an acetate buffer solution pH 5. The FI parameters that affect the signal response have been optimized in order to get the better sensitivity and low standard deviation. The linear range for determination of iron in water samples was over the range of 0.05- 4.0  $\mu\text{g mL}^{-1}$  with a correlation coefficient ( $r^2$ ) of 0.9997. The limit of detection ( $3\sigma$ ) was 0.011  $\mu\text{g mL}^{-1}$  with sample throughput of 110 samples  $\text{h}^{-1}$ . The repeatability measured from three standard Fe (III) (0.1, 2.0 and 4.0  $\mu\text{g mL}^{-1}$ ) were 1.42, 1.29 and 1.01% ( $n = 11$ ) respectively. The proposed method was successfully applied to determination of Fe (III) in water samples and found to be in good agreement with those obtained by flame atomic absorption spectrophotometric (FAAS) method.

**Keywords:** Flow injection spectrophotometric; Determination; Iron

## 1. Introductions

Iron is one of the most important elements involved in the industrial and in the living process, being indispensable to all members of the plants and animal kingdoms. Several analytical techniques have been used for determining iron in various samples including ion chromatography [1, 2], UV-Vis spectrophotometry [3-11] atomic absorption spectrometry [12-15], ICP-AES [16, 17], chemiluminescence [18], capillary electrophoresis (CE) [19] and voltammetry [20]. Various flow-based methods for iron determination in water have been reported based on FIA [11, 18, 21-27], stop flow analysis [28] and SIA [29-31] based on spectrometric detection. There are a wide choice of complexing agents for determining iron in various samples spectrophotometrically, for examples, ferrozine [11], N,N-dimethylformamide (DMF) [21], 1,10 phenanthroline [19] and nitroso-R salt [32]. Disodium-1-nitroso-2-naphthol-3,6-disulphonate (nitroso-R salt) was introduced in 1921 by Van Klooster for the detection of cobalt [33] and subsequently used for determination of small quantities of this metal in various samples. Other metals such as iron [32, 34-36], copper [34, 37] and nickel [38-40] were reported based on complexation reaction between each metal ions with nitroso-R salt under suitable experimental conditions. Selectivity for determining these metal ions were achieved by adjustment of pH values with suitable buffer solution and measurement of the absorbance of each metal complex at its  $\lambda_{\text{max}}$  which was characteristic of each metal ions. For example,  $\text{Fe}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Ni}^{2+}$  complexes gave the maximum absorption wavelengths at 710, 425, 480 and 480 nm respectively [34, 38]. Among the cited spectrophotometric methods, many papers used nitroso-R salt as complexing agent for iron determination only in batch wise process [15, 32,

34-36] which consumed rather large amounts of reagents and samples (>1000 ml per day) leading to generated a large amount of chemical waste and also low samples throughput. Flow injection spectrophotometric methods consume less reagents, less waste generation and higher samples throughput than those by the batch-wise ones. This analytical method seems promising to satisfy with these purposes. In this paper a simple, rapid and reproducible flow injection spectrophotometric method for determining iron using nitroso-R salt as complexing agent was performed. It is based on the complexation reaction between Fe(III) with nitroso-R salt in an acetate buffer solution. The optimum conditions for iron determination were investigated.

## 2. Experimental

### 2.1. Reagents

All reagents used were of analytical reagent grade and used without any further purification. All solutions were prepared and/or diluted with deionized reverse osmosis water.

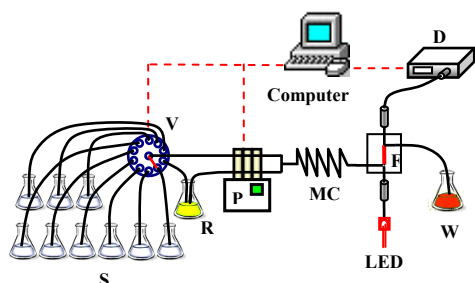
A standard stock solution 10  $\mu\text{g mL}^{-1}$  iron was prepared from a standard iron solution (AAS standard, 1000  $\mu\text{g mL}^{-1}$  Merck, Germany). Working standard solutions were prepared by appropriate dilution of this stock standard solution.

A stock reagent 2.0 %w/v nitroso-R salt (Merck, Germany) solution was prepared by dissolving 2.0 g nitroso-R salt in deionized water and adjusting volume to 100 mL.

Buffer solutions of pH 3-7 and pH 7-10 were prepared by mixing an appropriate ratio of 0.5 mol  $\text{L}^{-1}$  acetic acid with 0.5 mol  $\text{L}^{-1}$  sodium acetate, and 0.5 mol  $\text{L}^{-1}$  ammonia with 0.5 mol  $\text{L}^{-1}$  ammonium acetate, respectively.

## 2.2. Apparatus

The two channels FI manifold for iron determination is shown in Fig. 1. The system consisted of a ten-port selection valve (VICI, Valco Instruments, USA) (V) and a four-channel peristaltic pump (ISMATEC) (P) with Tygon tubing (0.84 mm i.d. and 1.24 mm o.d.) which were controlled by computer software (visual basic, Microsoft, USA) programmed in our laboratory name "A-Flow" for injecting accurate volume of samples (S) and delivering appropriate flow rate of reagent (R), a PTFE (0.84 mm i.d., 30 cm long) mixing coil was used as reactor, a 10 mm path length with 120  $\mu\text{L}$  flow through cell (F) in the cell compartment of the with UV-Vis spectrophotometer (USB2000, Ocean optics) using two fiber optics as detector (D). The first probe was connected to flow through cell and the white light emitting diode (LED) (super bright) as light source. The second one was connected to flow through cell and spectrophotometer as detector and a computer was used for collecting the absorption signal and controlling the entire system.



**Fig. 1** Schematic diagram of flow injection system for iron determination (R) reagent, (S) sample, (V) selection valve, (P) peristaltic pump, (MC) mixing coil, (F) flow cell, (LED) light source, (D) detector, (W) waste

## 2.3. Sample collection and pretreatment

Water samples were collected from different seven selected sites at Chiang Mai and Lumpun Provinces in Thailand where the Northern Industrial Estate was located. The water samples were filtered through a 0.45  $\mu\text{m}$  membrane filter at the sampling sites and stored in polyethylene containers that had been previously washed with 10% nitric acid and rinsed with deionized water for several times. After filtration, a 5 mL of concentrated hydrochloric acid was added in each liter of sample. Water sample (100 mL) was treated with 2.0 mol  $\text{L}^{-1}$  hydrochloric acid followed by addition of 2.0 mL 35% v/v  $\text{H}_2\text{O}_2$  and heated to 200  $^\circ\text{C}$ . It was allowed to cool to room temperature, filtered and diluted to appropriate concentration. The treated water samples were used for analysis of iron.

## 2.4. Procedure

The FI system (Fig. 1) was assembled with a fiber optic spectrometer to obtain a flow manifold for determining Fe(III). The method involved the injection 70  $\mu\text{L}$  of standard or sample solution containing Fe(III) by switching the selection valve into a reagent stream of 0.3% w/v nitroso-R salt adjusted to pH 5 with 0.5 mol  $\text{L}^{-1}$  acetate buffer with an appropriate flow rate of 2.5  $\text{mL min}^{-1}$  using peristaltic pump with the specially desired

software to control flow system (injection volume calculated from aspiration time and flow rate). Nitroso-R salt and Fe(III) were reacted completely on 30 cm mixing coil (MC) resulting in a green Fe(III)-nitroso-R complex and then passed through the flow through cell (F) using fiber optic probe to measure the absorbance at 720 nm.

## 3. Results and discussion

### 3.1. Selectivity of nitroso-R salt

Preliminary investigation indicated that the FI system for iron determination using nitroso-R salts had been found to give a selective means compared with copper, cobalt and nickel because they showed different absorption wavelengths (720, 495, 485 and 520 nm for Fe(III), Cu(II), Co(II) and Ni(II)-complexes, respectively). In addition, selectivity of the nitroso-R salt for these metal determinations could be achieved by adjustment of pH of the reaction medium using appropriate buffer solution. Therefore, the effects of pH on the absorbance of each metal-complex at its  $\lambda_{\text{max}}$  were examined. It was clear that for Fe(III), Cu(II), Co(II) and Ni(II) could be selectively determined at pH 5, 7, 6 and 8 respectively. In order to investigate the selectivity of the reagent for iron over copper, cobalt and nickel, effects of pH (3.0 to 10.0) on the absorbances of Fe(III), Cu(II), Co(II) and Ni(II)-nitroso-R complexes at 720 nm were examined. Results are plotted in Fig. 2. It was seen that Fe(III)-nitroso-R complex exhibited the greater sensitivity at 720 nm (pH 5) than those obtained with Cu(II), Co(II) and Ni(II) complexes.

### 3.2. Optimization of the experimental conditions

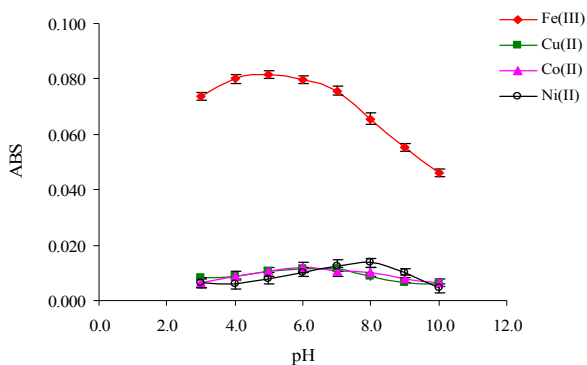
The optimum experimental conditions were investigated by univariate method. The parameters, range studied and the optimum conditions were shown in Table 1.

**Table 1** Optimum conditions for iron determination by the proposed FIA using univariate method.

Parameter	Studied range	Optimum conditions
Wavelength ( $\lambda$ )	600-800 nm	720 nm
pH	3-10	5
Mixing coil length	10-50 cm	30 cm
Injection volume	50-100 $\mu\text{L}$	70 $\mu\text{L}$
Flow rate	1-5 $\text{mL min}^{-1}$	2.5 $\text{mL min}^{-1}$
Reagent concentration	0.1-1.0 % (w/v)	0.3 % (w/v)

#### 3.2.1. Effect of pH

The absorbance of Fe(III)-nitroso-R complex was studied by changing pH in the range of 3.0 to 10.0. The pH values were adjusted with the buffer solutions shown in 2.1. As a consequence, the absorbance of Fe(III)-nitroso-R complex was increased while increasing pH up to about 4.0-6.0 (Fig 2), above this the absorbance decreased significantly. Hence, pH 5.0 was chosen as the optimum pH value and was used for further investigations to obtain the highest sensitivity.



**Fig. 2** Effect of pH on the absorbance of the metal-nitroso-R complexes at 720 nm. ( $1.0 \mu\text{g mL}^{-1}$  of Fe(III), Cu(II), Co(II) and Ni(II))

### 3.2.2. Effect of nitroso-R salt concentrations

The stoichiometry of the complex is Fe(III) : nitroso-R salt = 1:3. The effects of nitroso-R salt concentrations (0.1 to 1.0 % w/v) on the absorbance of Fe(III)-nitroso-R complex were studied. The absorbance increased with increasing concentrations of nitroso-R from 0.1 to 0.3 %w/v, above which the absorbance slightly decreased. As a result, the optimum concentration of nitroso-R salt was 0.3 %w/v.

### 3.2.3. Effect of Flow rate

Reagent and/or carrier flow rates can affect on the FI signals. Therefore the effects of total flow rates on absorption signal of Fe(III)-nitroso-R complex were investigated. The total flow rate was varied over the range of 1.0 to 5.0  $\text{mL min}^{-1}$ . It was found that, the absorbance increased with increasing flow rate up to 2.5  $\text{mL min}^{-1}$ , above this flow rate the absorbance decreased. Therefore, a flow rate of 2.5  $\text{mL min}^{-1}$  was chosen.

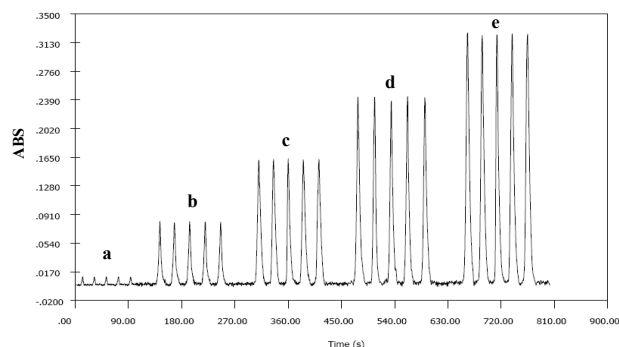
### 3.2.4. Effect of sample volume

The influence of sample volume on Fe(III) determination was studied by controlling pump flow rate and changing the switching time of selection valve at sample line over the range between 50  $\mu\text{L}$  and 100  $\mu\text{L}$ . Initially, the absorbance increased rapidly with increasing sample volume up to 70  $\mu\text{L}$ , above which the absorbance slightly decreased with peak boardening probably owing to the effect of dispersion. Hence, the injection volume of 70  $\mu\text{L}$  was considered to be optimum sample introduction volume, which was used throughout the experiments.

### 3.3. Analytical figures of merit

Regarding to the proposed FIA system for determination of iron (III) in water samples illustrated in Fig. 1, the linear calibration ranges and the detection limits (LOD) for iron were examined. The LOD was determined as the concentration of the analyte leading to a signal that was three times of the blank standard deviation ( $3\sigma$ ) as reported by Miller and Miller [41]. The calibration graph was linear over the range 0.05–4.0  $\mu\text{g mL}^{-1}$  (Fig. 3), which was expressed by the regression equation  $Y = 0.0803X + 0.0021$  ( $r^2 = 0.9997$ ). Where Y is the absorbance of Fe(III)-nitroso-R complex and X is iron concentration ( $\mu\text{g mL}^{-1}$ ).

The LOD was  $0.011 \mu\text{g mL}^{-1}$ . The precision of the method based on repeatability was performed, by 11-replicates of three standard solutions, 0.1, 1.0 and 4.0  $\mu\text{g mL}^{-1}$ , and the peak heights (as absorbance) of which were measured. Statistical evaluation revealed that the relative standard deviations of the three concentrations of iron (III) solutions were 1.42, 1.29 and 1.01%, respectively. The percentage recovery was studied by spiking 1.0  $\mu\text{g mL}^{-1}$  of iron (III) standard solution into water samples. It was shown that the percentage recovery was  $97.7 \pm 1.16 \%$  ( $n=5$ ).



**Fig. 3** The signals for calibration graph. Fe(III) concentrations; (a)  $0.1 \mu\text{g mL}^{-1}$ , (b)  $1.0 \mu\text{g mL}^{-1}$ , (c)  $2.0 \mu\text{g mL}^{-1}$ , (d)  $3.0 \mu\text{g mL}^{-1}$  and (e)  $4.0 \mu\text{g mL}^{-1}$

### 3.4. Effects of Interfering ions

The effect of some possible interferences (foreign species) on the determination of Fe(III) in water sample was undertaken for the maximum concentration ratio of foreign species ( $\mu\text{g mL}^{-1}$ ) to Fe(III) up to 200:1. The tolerance concentration is defined as the foreign species concentration causing error smaller than  $\pm 10 \%$  for determining the analyte of interest. The tolerance concentrations of the studied species to  $1.0 \mu\text{g mL}^{-1}$  Fe(III) under the optimum conditions were  $>200 \mu\text{g mL}^{-1}$  for  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{SO}_4^{2-}$ ;  $100 \mu\text{g mL}^{-1}$  for  $\text{Pb}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Cl}^-$  and  $\text{PO}_4^{3-}$ ;  $80 \mu\text{g mL}^{-1}$  for  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$ ;  $25 \mu\text{g mL}^{-1}$  for  $\text{Co}^{2+}$  and  $\text{Cu}^{2+}$  and  $20 \mu\text{g mL}^{-1}$  for  $\text{Ni}^{2+}$ .

### 3.5. Sample analysis

The proposed method was applied to the determination of Fe(III) in natural water samples. They were collected from different site at Chiang Mai and Lumpun Provinces in Thailand where the Northern Industrial Estate was located. The results are given in Table 2 compared with those obtained by FAAS. It is shown that the results obtained by the proposed FIA agreed well with those obtained by FAAS, because the calculated student t-value (0.46,  $n=11$ ) was less than the theoretical value (2.23) at confident level of 95%. The amounts of Fe(III) in water samples analyzed by the proposed method were in the range of 0.2-20  $\mu\text{g mL}^{-1}$ .

**Table 2** Comparative determination of iron in water samples ( $\mu\text{g mL}^{-1}$ ) by using the proposed FIA and FAAS methods

Sample	Iron contents ( $\mu\text{g mL}^{-1}$ ), n = 5			
	The proposed FIA		FAAS	
	a	b	a	b
S1	0.26 $\pm$ 0.03	0.28 $\pm$ 0.03	0.27 $\pm$ 0.06	0.30 $\pm$ 0.05
S2	1.50 $\pm$ 0.18	1.60 $\pm$ 0.20	1.50 $\pm$ 0.23	1.73 $\pm$ 0.11
S3	3.40 $\pm$ 0.20	3.61 $\pm$ 0.30	3.80 $\pm$ 0.20	4.09 $\pm$ 0.21
S4	3.83 $\pm$ 0.30	4.90 $\pm$ 0.20	4.57 $\pm$ 0.25	5.00 $\pm$ 0.33
S5	9.90 $\pm$ 0.30	9.70 $\pm$ 0.12	11.01 $\pm$ 0.30	12.00 $\pm$ 0.27
S6	8.30 $\pm$ 0.22	8.90 $\pm$ 0.22	9.30 $\pm$ 0.30	8.30 $\pm$ 0.20
S7	17.71 $\pm$ 1.70	19.00 $\pm$ 1.15	19.20 $\pm$ 1.50	18.50 $\pm$ 1.00

a = calibration method, b = standard addition method

#### 4. Conclusion

The proposed method has been satisfactorily applied to the determination of Fe(III) in water samples with the relative standard deviation (%RSD) in the ranges of 1.01 to 1.42% and the percentage recovery of  $97.7 \pm 1.16$  (n=5). In addition, the use of nitroso-R salt as complexing agent for iron determination using the proposed FIA device is simple and inexpensive which has been proven to be highly precise (RSD of less than 2%) sensitive, (LOD =  $0.011 \mu\text{g mL}^{-1}$ ), accurate (%recovery of  $97.7 \pm 1.16$ , n = 5 ) and rapid with a sample throughput of  $110 \text{ h}^{-1}$ . The proposed FIA system also consumed small amount of chemicals and reagents (less than  $200 \text{ mL h}^{-1}$ ), with lower waste production than the batch-wise method.

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