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Deferiprone a Non-toxic Reagent for Determination of Iron in Samples via Sequential Injection Analysis

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Abstract

We present for the first time the use of deferiprone as a non-toxic complexing agent for the determination of iron by sequential injection analysis in pharmaceuticals and food samples. The method was based on the reaction of Fe(III) and deferiprone in phosphate buffer at pH 7.5 to give a Fe(III)-deferiprone complex, which showed a maximum absorption at 460 nm. Under the optimum conditions, the linearity range for iron determination was found over the range of 0.05-3.0 μg mL^{-1} with a correlation coefficient (r^2) of 0.9993. The limit of detection and limit of quantitation were 0.032 μg mL^{-1} and 0.055 μg mL^{-1}, respectively. The relative standard deviation (%RSD) of the method was less than 6.0% (n=11), and the percentage recovery was found in the range of 96.0 to 104.0%. The proposed method was satisfactorily applied for the determination of Fe(III) in pharmaceuticals and food samples with a sampling rate of 60 h^{-1}.

Keyword: Deferiprone, Iron, Sequential Injection

Introduction

Deferiprone (1,2-dimethyl-3-hydroxy-4-pyridinone) or L1 which is depicted in Fig. 1a is a white crystalline solid with a molecular weight of 139.15 g·mol^{-1}. It is an iron chelater indicated for the treatment of patients with transfusional iron overload due to thalassemia syndromes when current chelation therapy is inadequate. It has a high efficiency for binding iron to form Fe(III)-deferiprone complex with a mole ratio (Fe(III) : deferiprone) of 1:3 (Fig. 1b). This complex is stable in an aqueous solution with the maximum absorption wavelength at 450 nm [1, 2, 3].

Fig. 1

Iron is an essential mineral for health and a component of hemoglobin in the red blood cells which carry oxygen from the lungs to the cells of the body. In addition, it is involved in reactions within the body that produces energy [4]. Iron is generally found in water and food such as liver, beef, pork, tofu, soybean, cereals, spinach, watercress, etc. In pharmaceutical it can be found in the iron form such as ferrous fumarate. A lack of iron affects the development of the red blood cells and causes iron deficiency anemia[5]. In order to avoid such deficiencies, an adequate supply of iron is needed. Ferrous fumarate [6], is a drug formula that provides the body with the extra amounts of iron. It is used to treat or prevent iron deficiency anemia, a condition that occurs when the body has fewer red blood cells than it needs owing to a poor diet, excess bleeding, or as the result of other medical problem.

Sequential Injection Analysis (SIA) is one of the flow-based analytical techniques that is used for the determination of iron in various samples with emphatic advantages such as simplicity, rapidity, sensitivity, reproducibility, flexibility and low chemical consumption [4]. However, the drawback has emerged because most of the reagents employed such as tiron [5],

1,10 phenantroline [6], 2-(5-bromo-2-pyridylazo)-5-[*N*-*n*-propyl-*N*-(3-sulfopropyl) amino] aniline [7] and Ferrozine[8] are toxic and have health impacts on humans. The developing of a non-toxic reagent which is sensitive, less toxic chemical in waste production, cost effective and human friendly is needed. In drug formulations norflocaxin [9] which is a less toxic reagent for iron determination has been used in batch-wise method. Liawrungrath *et. al* employed Flow Injection analysis along with norflocaxin [10] for the determinarion of Fe(III). Ruengsitagoon [11] used FIA with chlortetracycline to detect Fe(III). Grudpan *et.al*[12] also utilized FIA with aspirin to determine the amount of Fe (III) in the solution. Although those methods use drugs which are less toxic, the drawbacks of theses technique are higher amounts of chemical consumption and waste production when compared to the SIA technique. Therefore, to solve this problem the use of a non-toxic reagent along with the SIA for the determination of iron is an attractive alternative technique.

In this issue we present the use of deferiprone, a non-toxic complexing reagent for the determination of iron in samples (ferrous fumarate tablets, water sample and food sample) by using the SIA system. The method is based on the measurement of the absorbance of Fe(III)-deferiprone complex which is formed between iron(III) and deferiprone when the complex is dissolved in buffer solution. The optimum conditions for determining iron content were also investigated.

2. Experimental

2.1. Chemicals

The chemicals used were of analytical reagent grade and employed without any further purification. Deionized water was used for preparation and/or diluted solutions throughout the experiments.

Working stock standard solution of Fe(III) (10.0 μg mL^{-1}) was prepared by diluting 5.00 mL of 1000 μg mL^{-1} stock standard Fe(III) solution (AAS standard, Merck, Germany)

into a 500 mL volumetric flask and adjusting the volume with 1.0% nitric acid. Working solutions were prepared by appropriate dilution of the working stock solution.

Buffer solutions ranging from pH 3 to pH 6 and from pH 6 to pH 9 were prepared by mixing an appropriate ratio of 0.1 mol L^{-1} acetic acid with 0.1 mol L^{-1} sodium acetate ($C_2H_3O_2Na$: 8.204 g L^{-1}), and 0.1 mol L^{-1} disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$: 11.87 g L^{-1}) with 0.1 mol L^{-1} potassium dihydrogen phosphate (KH_2PO_4: 9.073 g L^{-1}), respectively. The required pH was achieved by adjusting with 1.00 mol L^{-1} sodium hydroxide and/or 1.0 %v/v hydrochloric acid.

A 1.0 mmol L^{-1} deferiprone stock solution was prepared by dissolving 0.0070 g of fine powder deferiprone (20 capsules, each capsule containing 500 mg of deferiprone) in deionized water and adjusted to volume in a 50 mL volumetric flask. The working solution of deferiprone was prepared by appropriate dilution of the stock solution with phosphate buffer pH 7.5.

2.2. Apparatus

The assembled SIA system used in this work was depicted in Fig. 2. It consisted of a computer controlled peristaltic pump (Reglo digital ISM 834, IMATEC Co., Inc.) (P) with Tygon pump tubing (1.02 mm i.d. and 2.25 mm o.d.) which was connected to PTFE tubing that was immerged into the deionization water reservoir as a carrier (C). A ten-port selection valve (VICI, Valco Instrument, USA) (SV) controlled by a computer software was used for the aspiration of all solutions into the system. PTFE tubing 1.02 mm i.d. and 150 cm long was used as the holding coil (HC) that was placed between the pump and valve. Deferiprone solution (R), sample or standard Fe(III) solution (S) and phosphate buffer solution pH 7.5 reservoirs (B) were introduced into the system via the selection valve through PTFE tubing. A UV-VIS spectrophotometer (Perkin Elmer lambda 25) with a flow through cell (10 mm path length) was used as the detector (D). A personal computer with special software was used for collection of signals and control of all devices in the system.

Fig. 2

2.3 Sample preparation

Ferrous fumarate tablets were purchased from drug store and prepared according to the standard USP method [13]. About 0.1 g of the drug powder (20 tablets) was accurately weighed and transferred into a 250 mL beaker containing 25 mL of water, 2.5 mL of nitric acid and 7.5 mL of perchloric acid. The solution was heated and evaporated to a volume of approximately 1 mL. It was then filtrated and washed with 2.0 mL hydrochloric acid. The filtrate solution containing Fe(III) was transferred into a 100 mL volumetric flask and made up to volume with deionized water.

Water samples were collected from rainwater, tap water, drinking water, Mahasarakham University canal water and Chee River in Mahasarakham province, Thailand. The samples were filtered through a 0.45 μm filter at the sampling sites and then acidified with concentrated nitric acid. After that, they were stored in polyethylene bottle that had been washed previously with nitric acid and deionized water for several times. Water sample (100 mL) was treated with 2.0 mol L^{-1} nitric acid and heated to a small volume. It was then cooled to room temperature, filtered and diluted to appropriate concentration.

Food samples: chicken liver, beef liver, pork liver, tofu and soybean were purchased from Sermthai supermarket, Mahasarakham province. About 1.0 g of homogenized sample was accurately weighed in a porcelain crucible and was subjected to dry ashing in a muffle furnace at ~400-500 ^{0}C, until white ash was obtained. The ash was dissolved in 2.0 mL of concentrated nitric acid and warm water. Then the sample solution was diluted to 100 mL with deionized water.

2.4. Procedure

The procedure for the determination of iron using deferiprone as reagent by the assembled SIA system is illustrated in Fig. 2. Firstly, the system was cleaned thoroughly by switching a ten-port selection valve (SV) to the detector position and propelling the carrier solution (DI water) for 3 minutes. The empty PTFE tubing connections between the selection valve and three solution reservoirs, phosphate buffer solution (B), sample solution (S) and deferiprone solution (R) were filled by switching a ten-port selection valve to their positions and drawing each solution using a computer controlled peristaltic pump (P). Secondly the solution of deferiprone was aspirated into a holding coil (HC), in order to aspirate the standard Fe(III) or sample solution and phosphate buffer solution pH 7.5 by switching a ten-port selection valve (SV) to the positions R, S and B. The aspiration volume was calculated from the velocity of pump roller and the holding time of the selection valve at each position. All solutions were then mixed together forming Fe(III)-deferiprone complex at the holding coil (HC). The absorbance of the complex was then measured at 460 nm by switching the selection valve to the detector position and delivering the mixed solution to a flow through cell (1.0 cm path length), which was placed into the cell compartment of UV-Vis spectrophotometer (D) (Perkin Elmer Lambda 25).

3. Results and Discussion

3.1 Preliminary study

3.1.1 Absorption spectrum of Fe(III)-deferiprone complex

The Fe(III)–deferiprone complex was prepared using 1.0 $\mu g \cdot mL^{-1}$ of Fe(III) and 0.10 $mmol \cdot L^{-1}$ of deferiprone in buffer solution. The maximum absorption of this complex was investigated over the range of 300–600 nm via UV-Vis spectrophotometer (Perkin Elmer Lambda 25). Figure 3 revealed that the stable complex showed maximum absorption at 460 nm while the absorption signals of both pure Fe(III) and deferiprone solution did not interfere with the complex. Generally, the complexation reactions between metal ions and ligands are pH dependent. Selectivity for spectrophotometric determination of each metal ion can be achieved by adjusting the pH level of the reaction medium. This is not only affecting on the selectivity but also influenced on the stoichiometry of the complexes resulting in hypsochromic shift and/or bathochromic shift of the maximum absorption wavelength depending on the reaction concerns. Therefore, the influence of pH from pH 3.0 to 9.0 on the absorbance of the complex was also investigated. The result showed that, the absorbance of the Fe(III)-deferiprone complex increased while the pH of the solution was increasing up to 7.5. Above of this pH, the absorbance was decreased significantly. It can be explained that, pH 7.5 was increased the ionic strength of the solution and stabilized the anionic forms of deferiprone resulting to form the Fe(III)–deferiprone complex efficiently [2]. Thus the maximum absorption wavelength of the complex at 460 nm at pH 7.5 was used for further studies.

3.1.2 The stoichiometry

The stoichiometry of Fe(III) and deferiprone to form a stable Fe(III)-deferiprone complex was explored using the mole-ratio method [14]. A series of solutions were prepared in which the concentration of Fe(III) was kept constant while the concentration of deferiprone varied. The absorbance of each solution was then measured and plotted against the mole-ratio

of deferiprone. Fig. 3 showed a break in the slope of the curve that occurred at the mole-ratio (Fe(III) : deferiprone) of 1:3 which was in accordance to the previous study [2].

Fig. 3

3.2. Optimization

The parameters including aspiration sequence, flow rate, aspiration volume and the concentration of reagent were examined using the univariate method. This was accomplished by varying the investigated parameter while the others were fixed. For each parameter explored five replicates were performed using 1.0 μg mL^{-1} of standard Fe(III) solution. The optimum values were selected at the highest absorbance and low background.

3.2.1 Aspiration sequence

The aspiration sequence of the solution was carried out by aspirating equal volumes (10 μL) of 1.0 μg·mL^{-1} of standard Fe(III) (S), 0.10 mmol·L^{-1} of deferiprone (R) and phosphate buffer solution at pH 7.5 (B) to a holding coil using six different series, S-R-B, S-B-R, R-S-B, R-B-S, B-S-R and B-R-S. The absorption signals of each series were then measured and evaluated. The results found that the series R-S-B provided the highest absorbance. Therefore, the suitable aspiration sequence of the solution was R-S-B (deferiprone, standard Fe(III) and buffer).

3.2.2. Effect of flow rate

The effect of flow rates on the absorption signal of Fe(III)–deferiprone complex was investigated over the range of 0.5–5.0 mL min^{-1} under the conditions of 1.0 lgmL^{-1} Fe(III),0.10 mmol L^{-1} deferiprone and phosphate buffer pH 7.5. Fig. 5showed that, the absorbance of the complex was not significant difference at any studied range of flow rate, but with the better precision for flow rate was 2.5 mL min^{-1} to lower. Indicating that, flow rate of the solution was negligible effect on the absorbance of the complex due to a small internal diameter of tubing was used in the SIA system, leading to a lower dispersion of the solution during its

movement from the holding coil to the detector. Hence, a flowrate of 2.5 mL min^{-1} was selected.

Fig. 4

3.2.3. Aspiration volume of the solution

The aspiration volumes of the solutions (standard Fe(III), reagent and buffer) were affected by the reaction time of Fe(III) solution and deferiprone to form the Fe(III)-deferiprone complex efficiently. Higher aspiration volumes of the solution lead to longer mixing times. A suitable volume leads to mixing and forming the complex efficiently resulting in higher absorbance. The aspiration volume of the solution including 1.0 $\mu g \cdot mL^{-1}$ standard Fe(III) (S), buffer pH 7.5 (B) and 0.10 $mmol \cdot L^{-1}$ deferiprone (R) were investigated by controlling pump flow rate and maintaining time of the selection valve at each position of the solution (R, S and B) to obtain a volume in the range of 5.0-50.0 μL. Fig 5 showed the initial absorbance of Fe(III)-deferiprone complex increasing rapidly as the aspiration volume of the standard Fe(III) and depferiprone solution increases in the ranges from 5.0-40.0 μL and 5.0-35.0 μL, respectively. Over these volumes the absorbance decreased gradually and a broad peak was observed. In contrast, the absorbance of the complex remained unchanged while the aspiration volume of the buffer solution under the studied range increases. Hence, the suitable aspiration volumes of the standard Fe(III), deferiprone and buffer solution were 40.0 μL, 35.0 μL and 15.0 μL, respectively.

Fig. 5

3.2.4. Effect of deferiprone concentration

Normally in flow analysis techniques, the amount of reagent is greater than the required stoichiometry of the complex which is needed to complete the color development.

Therefore, the effect of various concentrations of deferiprone solutions in the range of 0.01–0.65 mmol L^{-1} on the absorption signal of Fe(III)–deferiprone complex were examined. Fig. 7 showed that the absorbance increased with the concentrations of deferiprone from 0.01 to0.45 mmol L^{-1}. At higher concentrations the absorbance decreased slightly due to that, at the concentration of 0.45 mmol L^{-1}, the concentration of deferiprone was 25 times more than the concentration of Fe(III) used (0.0175 mmol L^{-1}) which was excess to complete the color development. Over this concentration, the increment in concentration of deferiprone did not form any more Fe(III)–deferiprone complex. As a result, 0.45 mmol L^{-1} was selected as the optimum concentration of deferiprone

Fig. 6

3.3. Analytical figures of merit

The validity of the proposed method including the linearity ranges, the detection limits (LOD), the quantitation limits (LOQ), the precision and the accuracy were examined under the optimum conditions as shown in Table 1.

The linearity range for the calibration graph was investigated by varying the concentration of standard iron(III) over the range 0.01 μg mL^{-1} to 10.0 μg mL^{-1}. The linearity range for iron determination was investigated over the range 0.5 μg mL^{-1} to 3.0 μg mL^{-1}, which was expressed by the equation $y = 0.0822x + 0.0012$ ($r^2 = 0.9993$), where y is the absorbance of Fe(III)-deferiprone complex and x is the concentration of iron (μg mL^{-1}). Fig 8 showed the SIA-gram of iron over the concentration range 0.05 μg mL^{-1} to 3.0 μg mL^{-1}

The detection limit (LOD) and the quantitation limits (LOQ)were determined according to the concentration of the analyte leading to a signal that was three times (3r) and ten times (10r)of the blank standard deviation. It was found that the LOD andLOQ were 0.032 lgmL^{-1} and 0.055 lgmL^{-1}, respectively. The precision of the proposed method in terms of repeatability and

reproducibility was performed by measuring 11 replicates of three standard iron(III) solutions covering different concentration levels: low, medium and high (0.10, 1.0 and 3.0 lgmL^{-1}), where the peak high as absorbance was measured. Statistical evaluation revealed that the percentage relatively standard deviation of three studied concentration levels for repeatability were 4.3%,2.4% and 1.7%, respectively, and the reproducibility were 4.8%,3.3% and 2.2%, respectively. The accuracy as the percentage recovery was investigated by adding the standard iron(III) solutions into the samples which were determined by the proposed method. Table 2 showed the percentage recoveries of iron were over the range from 96% to104%.

3.4. Investigation of interfering ions

The effect of some interference species on the determination of iron was investigated. The maximum weight ratio of the species explore to Fe(III) was up to 500:1. The tolerance is defined as the interference species concentration causing an error smaller than ±5 % for determination of the analyte of interest. The tolerance values of the investigated species using 1.0 µg mL^{-1} of Fe(III) as an standard were > 500 µg mL^{-1} for Cr^{3+}, Ni^{2+}, Ba^{2+}, SO_4^{2-}, PO_4^{3-}, NO_3^-, I^-, Cl^-, CO_3^{2-}, Co^{2+}, Mn^{2+}, Pb^{2+}, Mg^{2+}, Cu^{2+} and Cd^{2+}; 200 µg mL^{-1} for Zn^{2+}, Cd^{2+} and Ca^{2+}; and 3 µg mL^{-1} for Al^{3+}. The most serious interferences were caused by Al^{3+} due to the contestable formation of the complexes with deferiprone leading to low absorption signal. This interfering ion may well lead to the development of a new method for determining aluminium with an appropriate improvement of the selectivity. However Al(III) is absent in the studied samples. Therefore, it can be considered to have no interference in this case.

3.5. Sample analysis

The proposed reagent was applied to determine the amount of iron in Ferrous furmarate tablets, water samples and food samples by comparison to the standard FAAS method. The

samples were prepared prior to analysis, which were described previously in section 2.3. Table 2 showed the content of iron which ranges from 45 to 65.7 mg per tablet found in ferrous furmarate tablets, and the range from 0.06 to 16.50 μg mL^{-1} found in water and food samples. The sampling rate of this method was found to be 60 h^{-1}. In addition, the results obtained from the proposed method were not significant different with those calculations from the labeled amounts. The analysis by the standard FAAS method, which was evaluated by the student t-test with a confidence value of 95% gave us the following values (t_{cal} = 0.4446, t_{table} = 2.1009). These values indicate that using deferiprone for the determination of iron can be used as an alternative human friendly choice .

Table 3 showed the analytical characteristics of the proposed method for determining iron compared to some previous publications based on flow analysis using various reagents. This method provides a wide linearity range, rapid, sensitive and low chemical consumption. Moreover, the main benefit of this approach is that it uses a non-toxic reagent unlike the other methods.

4. Conclusion

The proposed protocol demonstrates for the first time the use of deferiprone a non-toxic complexing agent for the determination of iron in real samples (ferrous fumarate tablets, water samples and food samples) with a relative standard deviation (%RSD) of less than 6%, the percentage recovery over the range from 96.0 to 104.0% and a sampling of 60 h^{-1}. A linear response for iron determination was observed over the range from 0.05 to 3.0 μg mL^{-1} with a correlation coefficient (r^2) of 0.9993. The limit of detection and limit of quantitation were 0.032 μg mL^{-1} and 0.055 μg mL^{-1}, respectively. We have successfully demonstrated that this method presents high levels of precision, sensitivity, reproducibility and accuracy compared to other methods. The analytical protocol indicates that this method is proven to be a green analytical technique and environmentally friendly.

Acknowledgements

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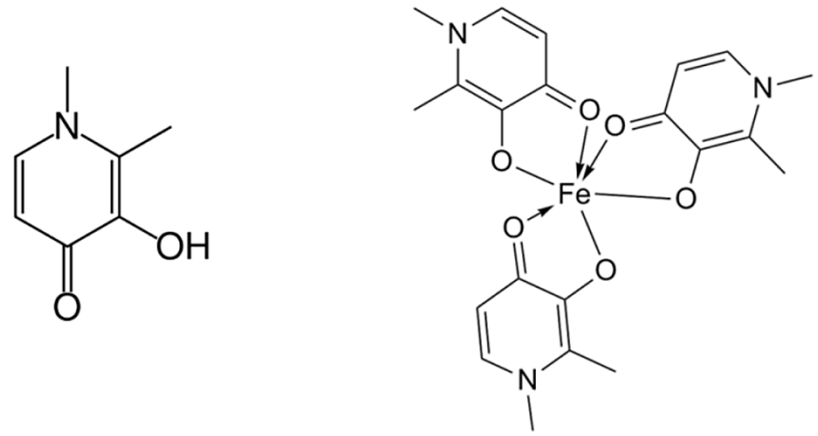

a **b**

Fig. 1 a) Chemical structure of deferiprone b) Fe(III)-deferiprone complex.

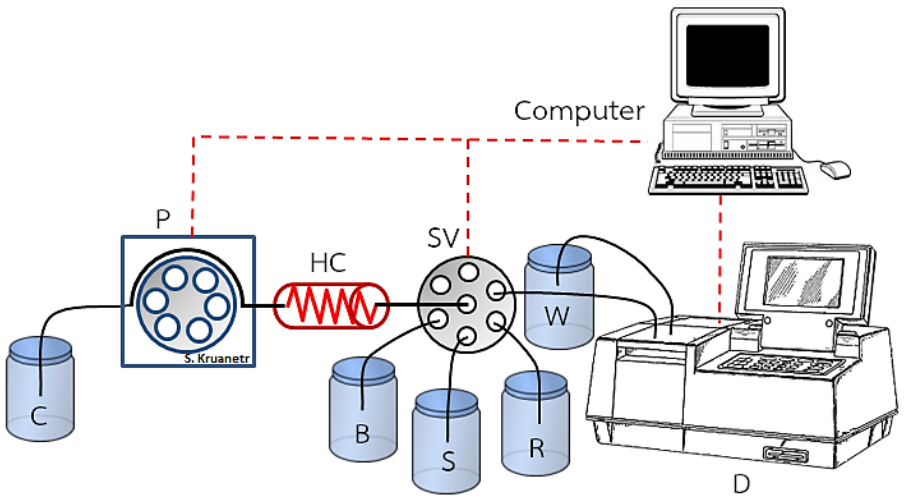

Fig.2 Assembled SIA system for determining Fe(III): C, carrier (deionization water); P, peristaltic pump (Reglo digital ISM 834, IMATEC Co., Inc.); HC, holding coil (PTFE tubing 1.02 mm i.d., 150 cm long); SV, ten-port selection valve (VICI, Valco Instrument, USA); R, deferiprone; S, sample or standard Fe(III); B, phosphate buffer solution pH 7.5; D, UV-VIS spectrophotometer (Perkin Elmer lambda 25); W, waste.

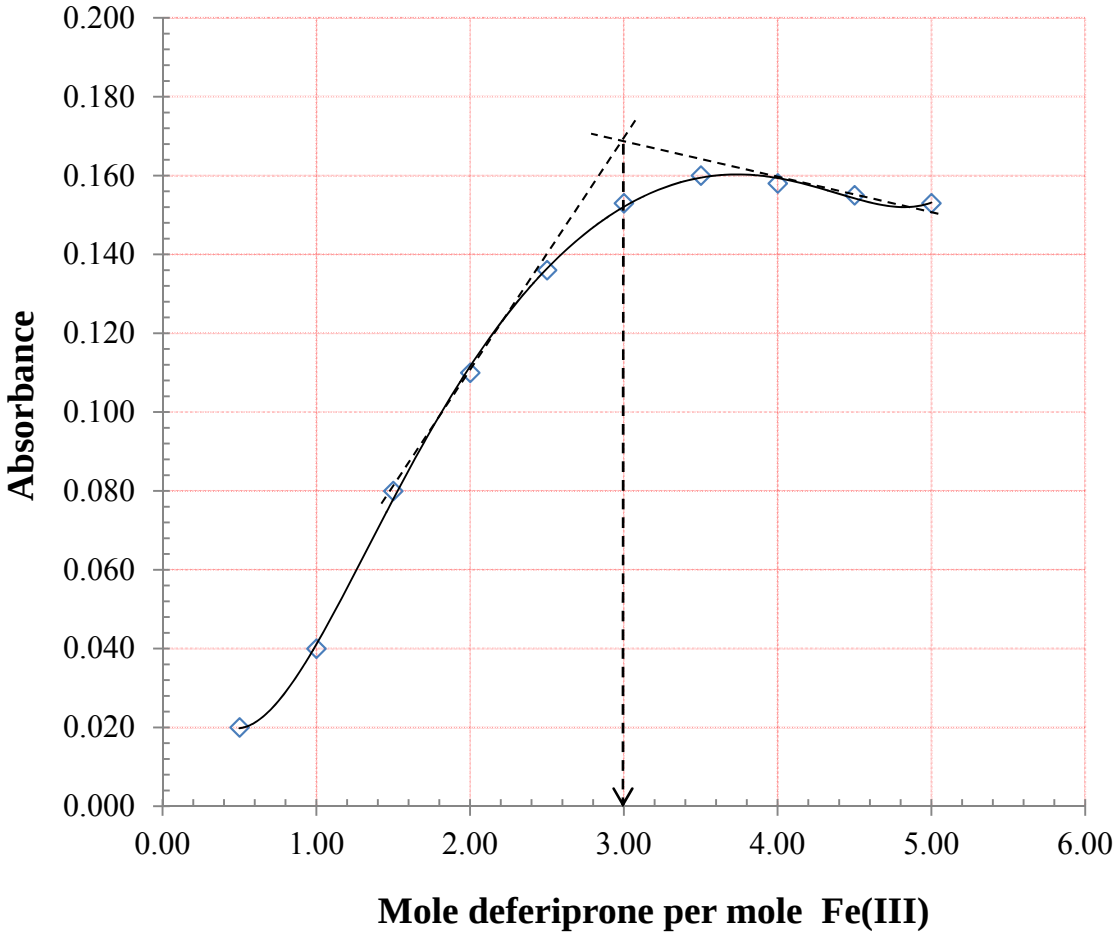

Fig. 3 Mole-ratio plots for Fe(III)-deferiprone complex.

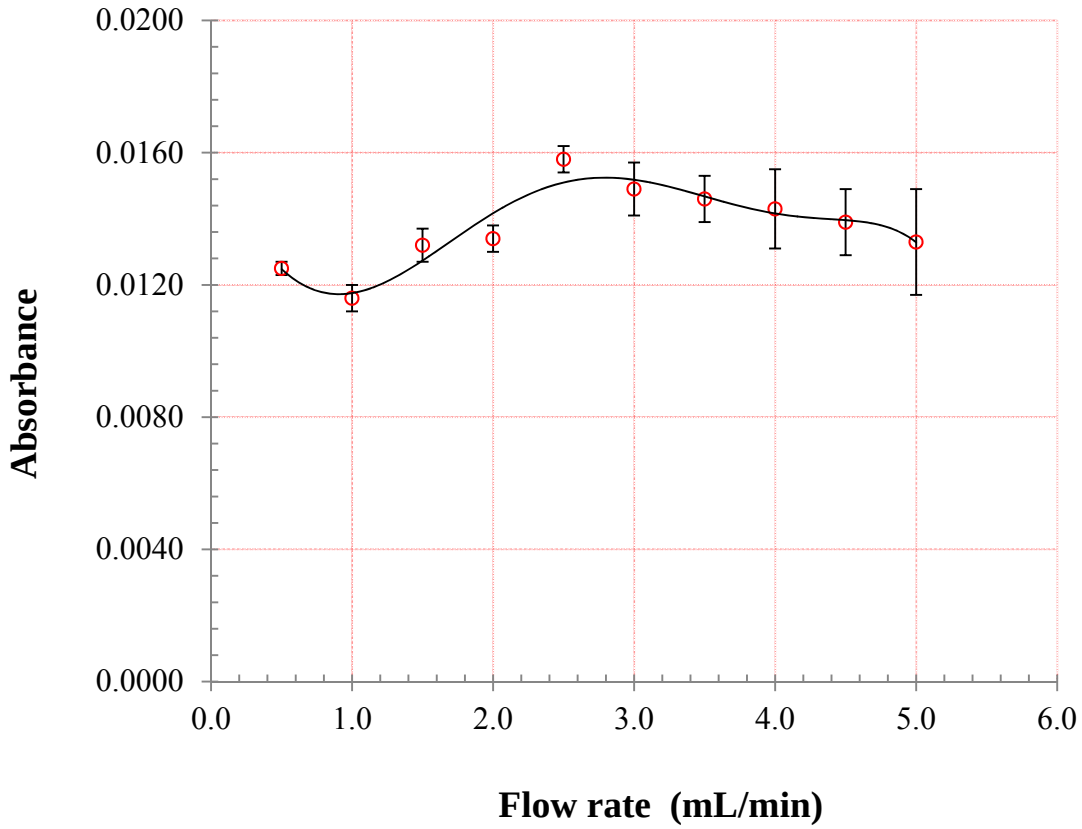

Fig. 4 Effect of total flow rate over the range from 0.5 to 5.0 mL min^{-1} on the absorbance of Fe(III)-deferiprone complex under the conditions of 1.0 µg mL^{-1} Fe(III), 0.10 mmol L^{-1} deferiprone and phosphate buffer pH 7.5.

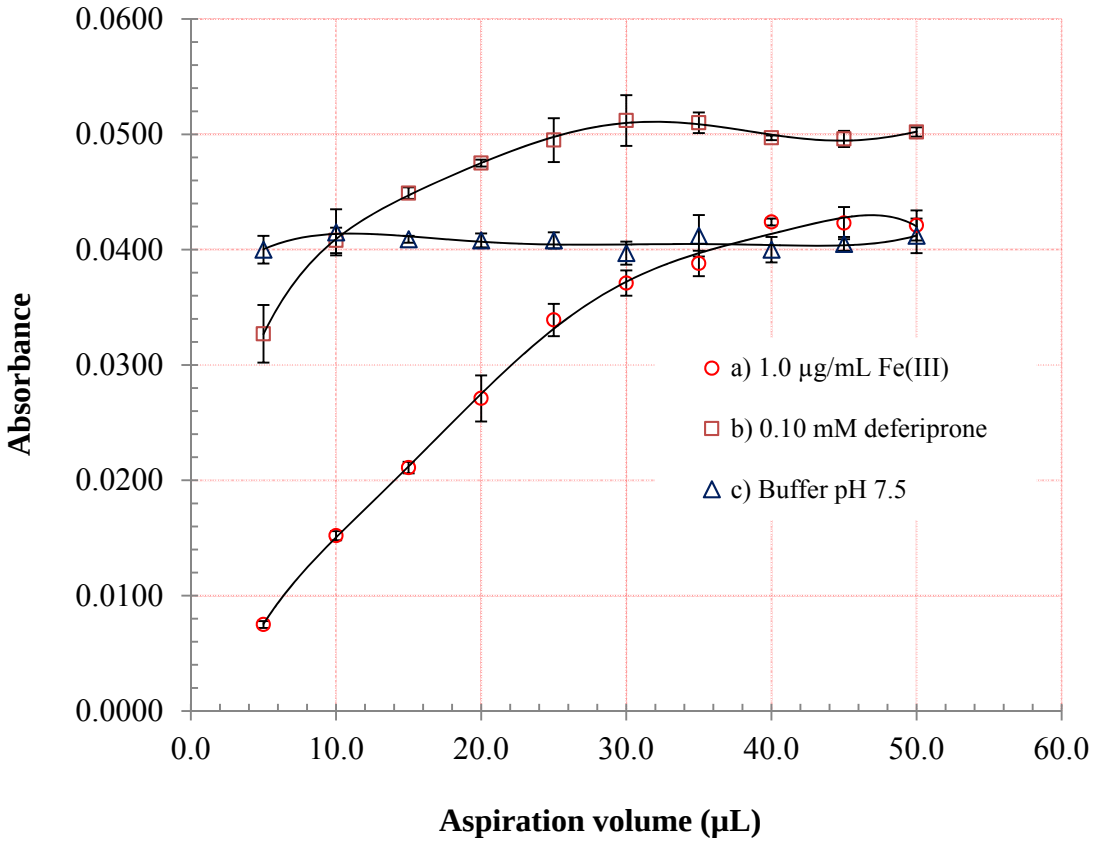

Fig. 5 Effect of the aspiration volume over the range from 5.0 to 50.0 µL on the absorbance of Fe(III)-deferiprone complex: a) 1.0 µg mL^{-1} Fe(III), b) 0.10 mmol L^{-1} deferiprone and c) phosphate buffer pH 7.5.

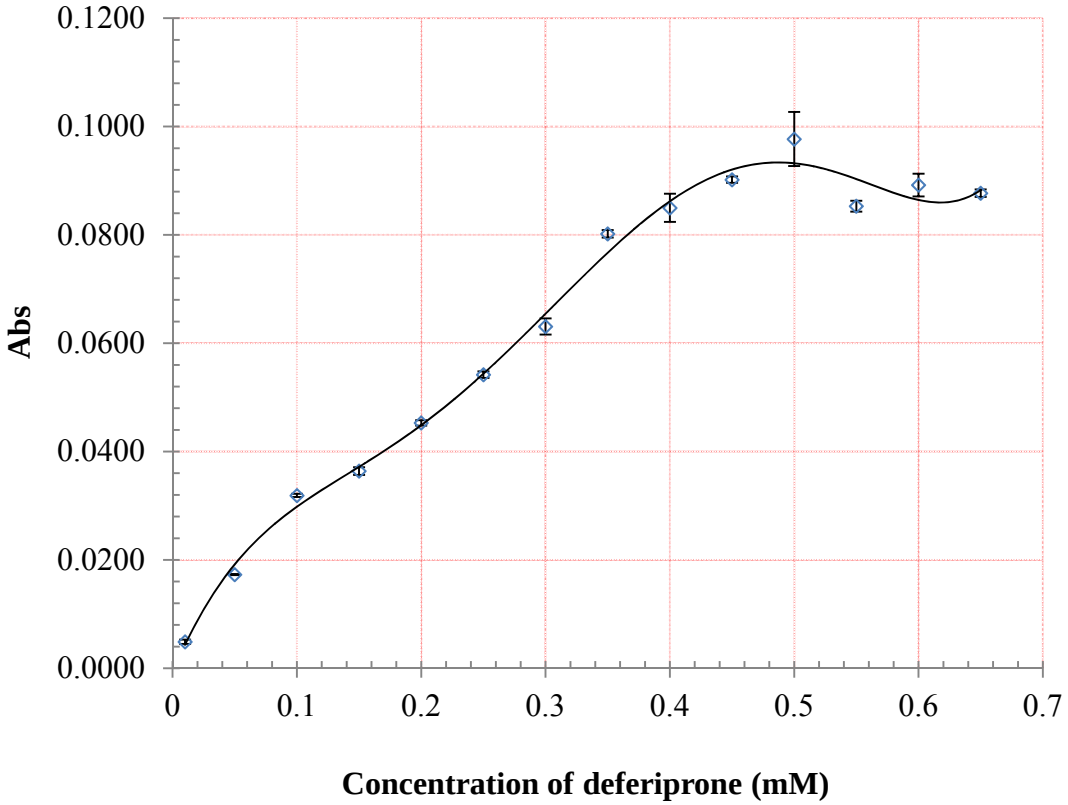

Fig. 6 Effect of concentration of deferiprone over the range from 0.01 to 0.65 mmol L^{-1} on the absorbance of Fe(III)-deferiprone complex.

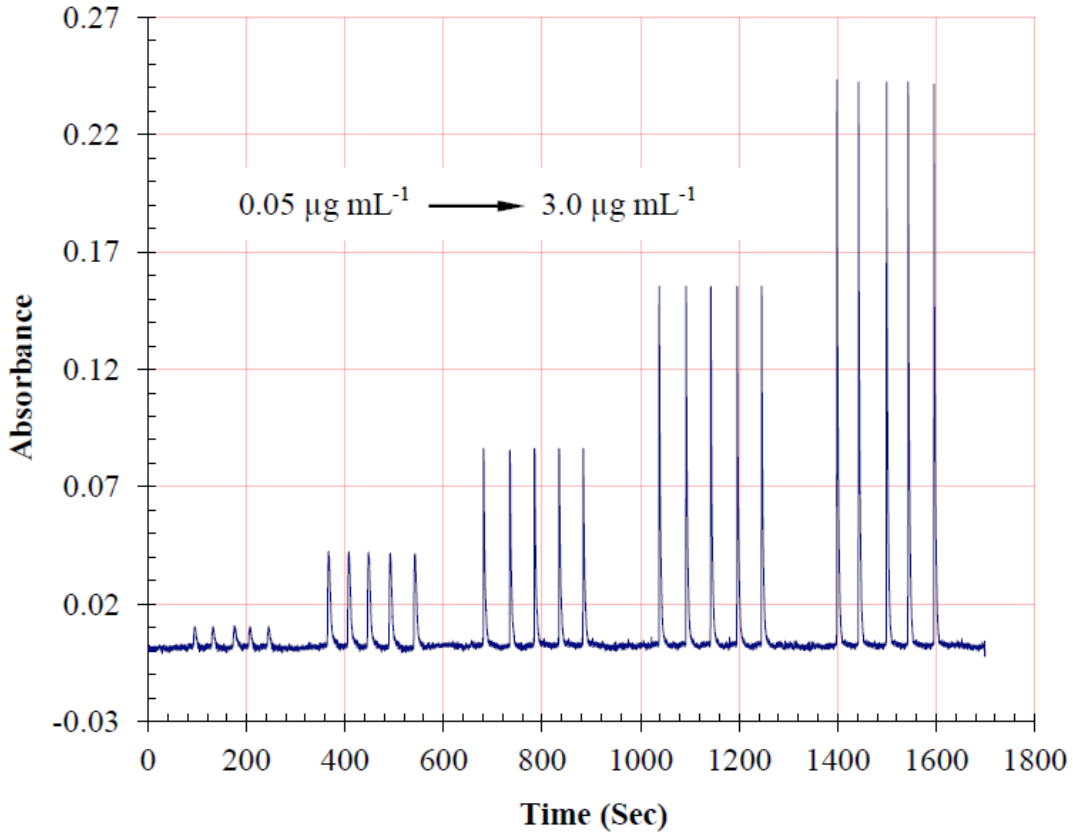

Fig. 7 SIA gram of Fe(III) at the concentration range of 0.05, 0.50, 1.0, 2.0 and 3.0 µg mL^{-1}

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Table 1 Optimum conditions of the proposed method.

Parameter	**Studied range**	**Optimum value**
Maximum absorption wavelength (nm)	300-600	460
pH	3.0-9.0	7.5
Aspiration sequence; Sample (S), Reagent (R), Buffer (B)	-	R-S-B
Flow rate (mL min^{-1})	0.5-5.0	2.5
Aspiration volume of the solution (μL);		
Sample and/or standard Fe(III)	5.0-50.0	40.0
Deferiprone solution	5.0-50.0	35.0
Buffer solution	5.0-50.0	15.0
Concentration of deferiprone (mmol L^{-1})	0.01-0.65	0.45

Table 2 Comparative determination of Fe(III) in various samples by using the proposed method and the FAAS method.

Samples	Concentration of iron found ±SD (n=5)		
	SIA	FAAS	Label
Ferrous fumarate tablets		mg per 1 tablet	
S1	63.9 ±2.1	63.7 ±1.5	65.7
S2	62.8 ±3.1	64.0 ±2.2	65.7
S3	63.8 ±1.8	63.1 ±1.7	65.7
S4	62.8 ±2.4	61.5 ±3.0	65.7
S5	59.6 ±3.0	59.0 ±3.1	60.8
S6	62.1 ±2.5	60.4 ±2.7	60.8
S7	59.0 ±2.1	58.2 ±2.3	60.8
S8	45.8 ±3.1	46.8 ±3.0	49.6
S9	47.0 ±2.5	48.0 ±2.6	49.6
S10	51.5 ±3.4	50.3 ±3.0	49.6
Water sample		$\mu g\ mL^{-1}$	
Rain water	0.06 ± 0.01	0.07 ± 0.01	-
Tap water	2.58 ± 0.05	2.71 ± 0.08	-
Drinking water	ND	ND	-
Mahasarakham university canal	6.92 ± 0.15	7.34 ± 0.32	-
Chee river (Mahasarakham province)	1.46 ± 0.07	1.77 ± 0.05	-
Food sample		$\mu g\ mg^{-1}$	
Chicken liver	9.82 ± 0.20	10.33 ± 0.17	-
Cow liver	16.31 ± 0.30	16.50 ± 0.24	-
Pork liver	12.70 ± 0.26	13.10 ± 0.30	-
Tofu	5.54 ± 0.08	4.71 ± 0.07	-
soybean	7.03 ± 0.15	7.82 ± 0.12	-

Table 3 Comparison of the analytical characteristics of the proposed method for determining iron with other published using a flow analysis method.

Analytical Method	Sampling rate (h^{-1})	Sample volume	Linear range	Waste (mL h^{-1})	LOD
This method	60	40 µL	0.05-3.0 µg mL^{-1}	~200	0.032 µg mL^{-1}
µFA nitroso-R [15]	40	5.0 µL	0.2-20 µg mL^{-1}	< 2.0	0.021 µg mL^{-1}
FIA nitroso-R [16]	110	70 µL	0.05-4.0 µg mL^{-1}	~300	0.011 µg mL^{-1}
FIA ferrozine [17]	90	600 µL	0.5-6.0 µg mL^{-1}	~400	0.012 µg mL^{-1}
Reverse FIA ferrozine [17]	50	-	0.1-5.0 µg mL^{-1}	~400	0.010 µg mL^{-1}
SIA 1,10-phenanthroline [19]	40	185 µL	0.25-5.0 µg mL^{-1}	~200	0.018 µg mL^{-1}
SIA-FAAS [20]	8 10	27 mL 9 mL	0.02-0.40 µg mL^{-1} 0.05-1.2 µg mL^{-1}	> 500	6.0 ng mL^{-1} 12.0 ng mL^{-1}
SIA-FAAS [19]	~18	1.83 mL	0.10-6.0 µg mL^{-1}	> 500	0.03 µg mL^{-1}