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Sequential injection spectrophotometric determination of iron as Fe(II) in multi-vitamin preparations using 1,10-phenanthroline as complexing agent☆

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Abstract

A sequential injection analysis (SIA) system is proposed for the determination of iron (II). Fe(II) was determined by SIA based on the reaction between 1,10-phenanthroline and iron (II), yielding an orange–red colour complex with absorption maximum at 512 nm. The method involved aspiration of 187 μl sample/standard zone followed by a zone of a reagent solution containing 140 μl of 7.8×10^{-4} mol l^{-1} 1,10-phenanthroline into a carrier stream to be stacked inside a holding coil and flow reversed through a reaction coil to a detector. The optimum condition was evaluated and the calibration curve is linear over a range of 0.25 to 5.0 mg l^{-1} of Fe(II) with detection limit of 18 μg l^{-1}. A sample throughput of 40 h^{-1} was established. This technique is found to be simple, accurate, reproducible and sensitive. The proposed method was successfully applied for the determination of total iron as Fe(II) in pharmaceutical products (multi-vitamin tablets) and is especially useful for the determination of iron (II) in tablets with lower iron (II) contents. The results were found to be in good agreement with the results obtained by manual UV/Vis spectrophotometry and flame atomic absorption spectrometry (FAAS) and with claimed values by the manufacturers.

Keywords: Spectrophotometry; 1,10-phenanthroline; Sequential injection

1. Introduction

Iron is an essential nutrient in the human diet. In the body, it is complexed with hemoglobin, which carries oxygen from the lungs to the cells of the body, and plays an equally essential role in respiratory enzymes such as cytochromes, which allows us to use oxygen [1]. Iron deficiency is the most common cause of diseases mainly during infancy, pregnancy and adolescence [2]. When the dietary intake is deficient in iron, a condition called anaemia results. In order to avoid such deficiencies, an adequate supply of iron is needed. Some people take dietary supplements, which contain iron, such as multi-vitamins. Thus, it is necessary that an accurate, fast and a cheap method for the determination of iron in pharmaceuticals and food supplements should be developed.

Several methods for the analysis of iron in pharmaceuticals and environmental samples have been reported, including potentiometry [3], chemiluminescence [4], graphite furnace atomic absorption spectrometry [5], flame atomic absorption spectrometry [6], (these two methods are very expensive), fluorometric analysis [7,8], anodic stripping voltammetry [9], volumetric analysis [10] and spectrophotometry [11,12]. As compared with the other techniques, spectrophotometry is very simple, rapid and less expensive for determination of elements in a variety of samples. Most of these classical detectors are coupled with flow injection analysis (FIA) or sequential injection analysis (SIA) and are used for quantification of elements in a variety of real samples.

The application of SIA for the determination of iron (II) using α,α-bipyridyl [1] and 1,10-phenanthroline after reduction of iron (III) to iron(II) with a cadmium

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reductor [13] in pharmaceutical products has been described. The α,α-bipyridyl [1] procedure presented a linear dynamic range between 5–40 m l^{-1} with a detection limit of 0.97 mg l^{-1} and was suitable for the determination of iron (II) in anti-anemic pharmaceutical formulations with an iron content above 21 mg l^{-1} as described by the authors. A synchronous merging zones for sequential simultaneous determination of Fe(II) and Fe(III) in FIA have also been employed in the analysis of pharmaceutical products [14] and mathematical simulation of detector response [15].

The determination of iron (III) and total iron by SIA based on tiron [16–18] and thiocyanate methods [2,19] and by FIA based on tiron [20] and thiocyanate [21] has also been reported. All these methods involve an oxidation step in the process, i.e., oxidation of Fe(II) to Fe(III). In pharmaceutical products iron is in the Fe(II) oxidation state. Furthermore certain pharmaceutical products contain anti-oxidants in excessive amounts compared to the concentration of Fe(II) and this prevents the quantitative oxidation of Fe(II) to Fe(III). Sample preparation is therefore very difficult for the oxidation of Fe(II) before determination as Fe(III). It takes 8–12 h to prepare a sample by dry ashing [2], requires digestion and separation (2 days) of the organic part [13] and this is very time and reagent consuming. Because of this reason, a direct, simple and accurate SIA method for the determination of Fe(II) is needed in pharmaceutical products.

The objective of the present work is therefore, to find a direct, simple and accurate method for the determination of Fe(II) in multivitamin prepartions without splitting and merging of sample zones and reagent streams and also to avoid any oxidation step. In this method, total iron was determined as Fe(II) in multi-vitamin preparations, with UV/Vis spectrophotometric detection using 1,10-phenanthroline as complexing agent. Since, the complex is stable in the pH range of 2–9 [13], it is not necessary to control the pH of the solution exactly.

2. Experimental

All reagents used were of analytical reagent grade and all solutions were prepared with double de-ionised water from a Modulab system (Continental Water System, Sant Antonio, TX, USA). These solutions were degassed before hand and stored under inert atmosphere.

Samples: Bettaway iron extra containing ferrous furmarate 40 mg (13 mg Fe/tablet) was supplied by Better Nutrition (Pty) Ltd., Sandton, S. Africa; Weigh-Less Daily Multi-Vitamin with anti-oxidants containing 7.5 mg iron, 7.5 mg zinc, 1 mg copper 35 mg magnesium/tablet was supplied by Weigh-Less S.A. (Pty) Ltd., Hout Bay, S. Africa; Vital Multitime containing 18 mg iron/ tablet was supplied by Vital Health Foods (Pty) Ltd., KuilsRiver, S. Africa; and Vita force 21-Plus containing 4.93 mg iron, 25 mg magnesium/tablet was supplied by Pharma Natura (Pty) Ltd., Sandton, S. Africa.

2.1. Reagents and solutions

The iron (II) stock solutions were prepared by dissolving 0.702 g of Mohr's salt ($Fe(NH_4)_2(SO_4)_2$ 6 H_2O) and 1 g of ascorbic acid (to prevent oxidation of Fe(II) to Fe(III)) in a 1 l volumetric flask. Working standard solutions ranging between 0.25 and 25 mg l^{-1} of Fe(II) were prepared by appropriate dilution of the stock solution with 0.01 mol l^{-1} of H_2SO_4. The acetate buffer pH 5.5 was prepared from 0.10 mol l^{-1} of acetic acid and 0.10 mol l^{-1} of sodium acetate. The colorimetric reagent of 0.25% (w/v) 1,10-phenanthroline solution was prepared by dissolving 0.625 g of 1,10-phenanthroline in 1 ml of concentrated HCl and 50 ml of water and then diluted quantitatively to the mark with the acetate buffer solution in a 250 ml volumetric flask. A concentration of 7.8×10^{-4} mol l^{-1} 1,10-phenanthroline was prepared by diluting 6 ml of 1.3×10^{-3} mol l^{-1}, 1,10-phenanthroline-into a 100 ml volumetric flask with acetate buffer solution. Double de-ionised water was used as carrier stream.

2.2. Apparatus

A single wavelength Unicam 5625 UV/VIS spectrometer (Cambridge, UK) equipped with a 10 mm Hellma-type flow through cell (Hellma GmbH and Co., Mulheim/Baden, Germany) was used in all SIA experiments. A ten position micro-actuated selection valve (Model E-10-230, Valco Instruments, Houston, TX, USA) and a Gilson Minipuls-3 peristaltic pump (Model M321, Gilson, Villiers-le-Bel, France) were also used in the SIA system. For the device control and data acquisition, a FlowTEK interface box and FlowTEK software package (obtained from MINTEK Randburg, South Africa) for computer aided flow analysis were used throughout. The SIA system is given in Fig. 1.

2.3. Sample preparation

Four tablets of each multi-vitamin were individually placed in a 100 ml conical volumetric flask. 25 ml of double distilled water and 1 ml of concentrated HCl (65% (v/v)) were added to the flask, heated on a hot plate for 30 min and filtered immediately into a 100 ml conical flask while it was hot. The flask and the residue were washed several times by hot double de-ionised water and cooled to room temperature. 1 ml of 1% m v^{-1} of ascorbic acid was added to each conical flask to prevent oxidation of iron (II) to iron (III). The individual dissolved sample solutions were quantitatively transferred to 100 ml volumetric flasks and diluted to the mark with de-ionised water. Further dilution was made (Weigh-Les Daily Multi-vitamin with anti-oxidants 20-fold, Vital force 21-plus 10-fold, Vital Multitime 40-fold and Bettaway iron extra 30-fold) before analysis.

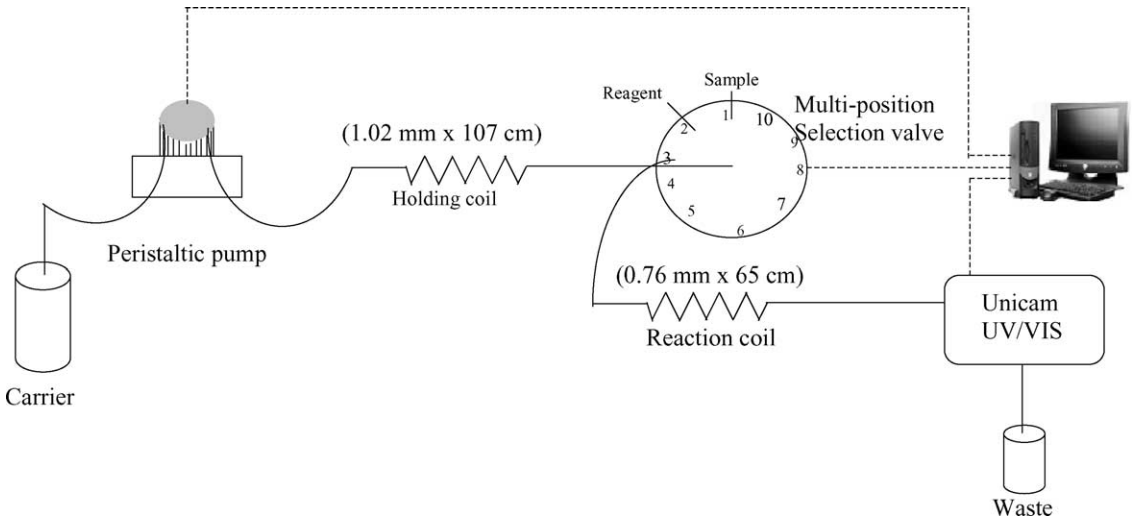

Fig. 1. Schematic diagram of the SIA system manifold used for the determination of iron in multivitamin preparations.

Table 1
Device sequence used for one cycle of the SIA system for the determination of iron (II) in multivitamin preparations

Time (s)	Pump	Valve	Description
0	Off	Sample	Pump off, valve select sample stream
3	Reverse		Draw up the sample solution
7	Off		Pump stop
8		Reagent	Select reagent solution (valve position 2)
9	Reverse		Draw up reagent solution
11	Off		Pump stop
12		Detector	Selects the detector stream. Valve position
13	Forward		Pump stack zones to the detector
89	Off		Pump stop
90	Home		Return back to position 1

2.4. Procedure

An illustration of the device sequence for the determination of iron in multivitamins is shown in Table 1. 187 μl of sample solution and 140 μl of reagent solution were aspirated sequentially through the selection valve into the holding coil. By using flow reversal the stack of well-defined zones were propelled by the peristaltic pump from the holding coil to the reaction coil. The zones penetrate each other as they pass through the reaction coil to the detector. The absorbance of the complex is measured at 512 nm using UV/Vis spectrophotometry.

3. Results and discussion

Iron (II) forms many coordination compounds in which the iron ions are surrounded by various ligands. Many of these coordination compounds are colored. The amount of color generated under a standard set of conditions can be used to measure the amount of iron present. In the current method, the compound used to form a colored complex with iron is 1,10-phenanthroline, which form a stable red–orange colored complex in the pH range of 2–9. The resulting complex is measured spectrophotometrically at 512 nm. Since iron (II) solution is easily oxidized to Fe(III) in the presence of acid and water, ascorbic acid is added to the standard solution and samples to make sure all the iron in solution is in the Fe(II) oxidation state. Ascorbic acid stabilizes the iron in the Fe(II) oxidation state and reduces Fe(III)–Fe(II) if present in the solution.

3.1. Optimisation of parameters

The condition for the determination of total iron as Fe(II) was optimised by studying the influence of the various parameters such as flow rate, sample and reagent volume, reaction coil length and diameter, holding coil length and diameter and reagent concentration. 20 mg l^{-1} of an iron (II) standard solution and 1.3×10^{-3} mol l^{-1} 1,10-phenanthroline concentrations were used to optimise these parameters. In all cases both the relative peak height and percentage of relative standard deviation were used as criteria for establishing the most appropriate parameter.

3.1.1. Flow rate

The flow rate is a very important parameter to be optimised because it regulates the amount of final product (complex) formed. The reaction between iron and 1,10-phenanthroline is rapid, resulting in an almost instant colour development. The flow rate was evaluated between 1.87 and 4.67 ml min^{-1} by changing the speed of the pump. The results (Fig. 2) revealed that there is a slight increase in response from 1.87 to about 3.7 ml min^{-1}, and this shows that the reaction is fast and develop colour immediately. Beyond 3.7 ml min^{-1} the response increases

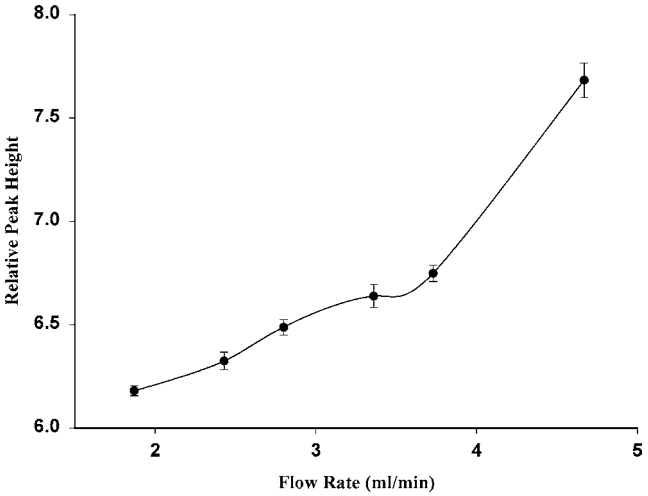

Fig. 2. Effect of flow rate on sensitivity and precision. Experimental conditions were: holding coil = 1.02 mm i.d. × 138 cm; reaction coil = 0.89 mm i.d. × 65 cm; aspiration volumes 140 μl each and 1.3 × 10^{-3} mol l^{-1} 1,10-phenanthroline and $n = 10$ (repetitive determinations).

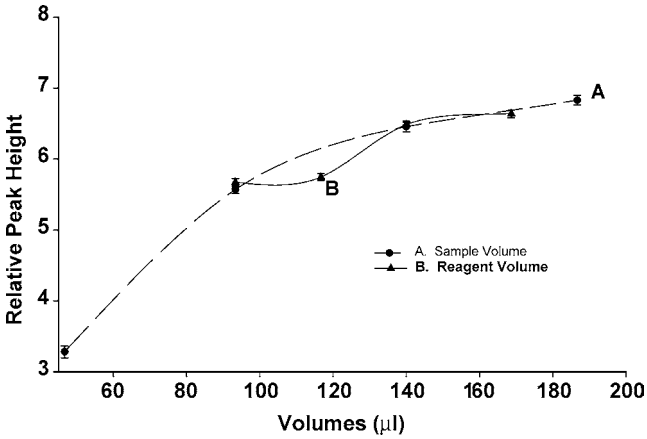

Fig. 3. Influence of sample (A) and reagent (B) volumes on precision and sensitivity. Experimental conditions were: 1.02 mm i.d. × 138 cm; reaction coil = 0.89 mm i.d. × 65 cm; flow rate = 2.8 ml min^{-1} and concentration of 1,10-phenanthroline 1.3 × 10^{-3} mol l^{-1}. (A) reagent volume 140 μl and (B) sample volume 187 μl. $n = 10$.

sharply, but the precision also deteriorates. Although the precision at 1.87 ml min^{-1} is slightly better than at 2.8 ml min^{-1}, the latter gave a better sample throughput and was chosen as the optimum working condition.

3.1.2. Sample volume and reagent volume optimisation

The aim of optimisation of these parameters is to minimize the consumption of reagent volumes while maintaining the best sensitivity and reproducibility of the procedure for the analyte to be determined. The procedure adopted for optimizing these parameters was to keep the volume of one of the reagents constant while varying the other one at different volumes. This was done by changing the period during which the specific sample/reagent volume was aspirated into the holding coil.

3.1.2.1. Sample volume. Optimum sample volume depends on two parameters: the flow rate and aspiration time. The influence of the sample volume was studied between 47 and 187 μl and the results are given in Fig. 3A. There is a sharp increase in response until about 100 μl, whereafter, the slope flatten off. Beyond 187 μl the length of the sample zone became too long and the system unstable and unreliable for routine analysis. The best working conditions were obtained with a sample volume of 187 μl and this was chosen as optimum for further work.

3.1.2.2. Reagent volume. The two parameters flow rate and aspiration time, are also responsible in obtaining the optimum reagent volume. The influence of reagent volume on response and precision was studied between 93 and 169 μl at constant sample volume and the results are given in Fig. 3B. A volume of 140 μl was chosen as an optimum reagent volume for subsequent measurements.

3.1.3. Coil length and diameter

3.1.3.1. Reaction coil. The reaction coil between the selection valve and the detector is usually kept as short as possible to avoid excessive dilution of the formed product zone

Table 2
Influence of reaction coil length and diameter (A) and holding coil length and diameter (B), on peak height (sensitivity) and precision

	Length (cm)				Diameter (mm)				
A									
Reaction coil	50	65	80	95	0.64	0.76	0.89	1.02	1.14
M.R.P. height[a]	7.07	6.9	6.79	6.77	6.67	6.92	6.82	6.59	6.17
% R.S.D.[b]	1.45	0.88	1.07	1.28	0.97	0.45	0.66	1.18	1.05
B									
Holding coil	107	138	168	200	0.76	0.89	1.02	1.14	
M.R.P. height[a]	6.29	6.29	5.99	5.99	6.04	5.99	6.00	5.90	
% R.S.D.[b]	1.08	1.91	2.2	1.86	1.16	1.86	0.66	1.05	

[a] M.R.P. height: mean relative peak height ($n = 10$ repetitive determinations).
[b] % R.S.D.: percentage relative standard deviation.

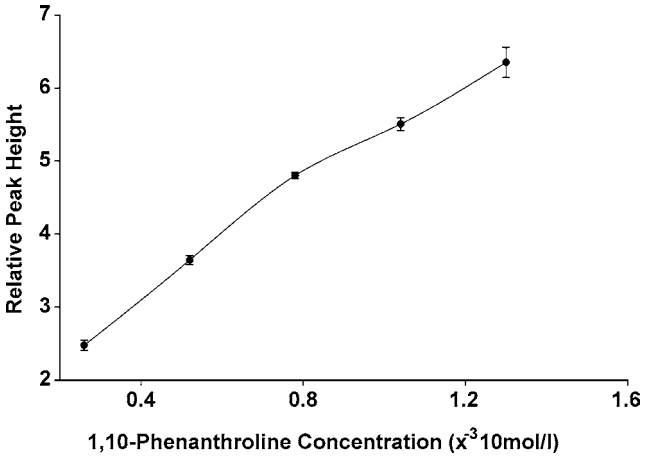

Fig. 4. Influence of reagent concentration on sensitivity and precision. Experimental conditions were: holding coil = 1.02 mm i.d. × 107 cm; reaction coil = 0.76 mm i.d. × 65 cm; flow rate: 2.8 ml min^{-1}; sample volume = 187 μl and reagent volume = 140 μl. n = 10.

[22]. The length of this coil was evaluated from 50–95 cm. The results obtained are given in Table 2A and indicate a slight decrease in sensitivity with an increase in line length confirming the rapidness of the reaction and that the decrease is mainly due to physical dispersion. An optimum line length of 65 cm for the reaction coil was chosen due to precision. The effect of reaction coil diameter was studied from 0.64–1.14 mm and the results are given in Table 2A. A reaction coil diameter of 0.76 mm gave the best results and this was used further on.

3.1.3.2. Holding coil. The main function of this device is to serve as a holding coil reservoir, which prevents the stack of zones from entering the conduit of the pumping tubing in the peristaltic pump where deformation could take place [22]. Thus the holding coil must be long enough to accommodate the stack of zones aspirated into it. The length of the holding coil was studied between 107 and 200 cm and the results are given in Table 2B. A holding coil with a line length of 107 cm gave the highest precision and was chosen as an optimum. The effect of holding coil diameter was also studied between 0.89 and 1.14 mm, the results are given in Table 2B and reveal that there is no significant difference on sensitivity, but difference in precision. A holding coil diameter of 1.02 mm was chosen as an optimum condition due to precision.

3.1.4. Concentration of reagent

The concentration of the reagent was studied between 2.6 × 10^{-4} and 1.3 × 10^{-3} mol l^{-1} of 1,10 -phenanthroline. It was observed that, the sensitivity steadily increases with an increase in the concentration of the reagent and the results are given in Fig. 4. The precision at a concentration of 7.8 × 10^{-4} mol l^{-1} was however far better than the others, was chosen as optimum concentration and used throughout.

Table 3
Optimum working conditions

Parameter		Value
Flow rate		2.8 ml min^{-1}
Holding coil	Diameter	1.02 mm
	Length	170 cm
	Configuration	Coiled
Reaction coil	Diameter	0.76 mm
	Length	65 cm
	Configuration	Coiled
Sample volume		187 μl
Reagent volume		140 μl
1,10-Phenanthroline concentration		7.8 × 10^{-4} mol l^{-1}
Ascorbic acid		0.01% m v^{-1} in sample solution
Sodium-acetate buffer		pH 5.5
Wavelength		512 nm

4. Method evaluation

The proposed sequential injection system was evaluated under the optimum conditions in Table 3 with regard to response, linearity, accuracy, precision, and interference and sampling rate.

4.1. Linearity, detection limit, accuracy and precision

The linearity of the method for the determination of total iron as Fe(II) was studied under optimum conditions described above. The relationship obtained between the relative peak height and iron (II) concentration is given by the equation.

Relative peak height = 0.467[Fe(II) mg l^{-1}] + 0.494, r^2 =0.999.

The method is linear between 0.25 and 5.0 mg l^{-1}.

The detection limit gives an indication of the lowest concentration of iron (II) that can be distinguished from the backgrounds signal with 99% certainty. The detection limit was calculated as follows:

$$\text{Detection limit} = \frac{(3S_b + I_b)(I_b - k)}{m}$$

where, S_b is the standard deviation of the background signal; I_b is the relative peak height of the background signal; k is the intercept of the calibration graph; m is the slope of the calibration graph. The calculated detection limit was 18 μg l^{-1} of iron (II).

Real samples (four different multi-vitamins of different compositions) were analyzed by the proposed SIA system.The accuracy of the method was evaluated by comparing the results with the values obtained by manual UV/Vis spectrophotometry, manual flame atomic absorption spectrophotometry and claimed values by the manufacturers. The results are given in Table 4 and reveal that the proposed

Table 4
Comparison of the results (as mg/tablet) obtained for Fe(II) from real samples. All values are averages from four determinations

Samples	Proposed SIA system	Manual FAAS method	Manual UV/Vis spectrophotometry	Claimed values
Weigh-Less daily multi-vitamin	7.34 ± 0.033	7.20 ± 0.20	7.30 ± 0.012	7.5
Bettaway Iron extra	12.74 ± 0.032	12.70 ± 0.30	12.63 ± 0.431	13
Vital Multitime (Multivitamin-mineral)	17.77 ± 0.070	18.24 ± 0.41	17.89 ± 0.357	18
Vital force (21-plus)	5.02 ± 0.047	5.37 ± 0.36	5.08 ± 0.328	4.93

SIA system is consistent with the manual methods and claimed values.

The precision of the method was evaluated with regard to its reproducibility of ten repetitive determinations of standard samples and the percentage R.S.D for the linear range from 0.25 to 5 mg l^{-1} was found to be between 0.7 and 1.6%.

4.2. Interferences

The influences of foreign ions, which are present in appreciable quantities in the tablets, were tested as possible interferences for the proposed SIA method. The study was conducted by analyzing a standard solution of iron (3 mg l^{-1} Fe^{2+}) to which increasing amounts of interfering species (Zn(II), Mg(II), and Cu(II)) were added (Table 5). It was found that Zn(II) starts interfering at the addition of 18 mg l^{-1}, Cu(II) at 3 mg l^{-1}while Mg(II) did not interfere. Therefore amounts of interfering species tolerated (an excess amount of 6:1 of Zn(II):Fe(II) and 0.5:1 of Cu(II):Fe(II) ratio, respectively, when present in the same medium) is higher than those found in the tablets (Zn(II):Fe(II) of 1:1 and Cu(II):Fe(II) of 0.13:1 that is the maximum ratio to iron (II) present in the tablet) and therefore did not interfere as is clear from the comparison results (Table 4). No interference was observed for Mg(II) ion at the maximum concentration ratio tested and the results obtained for iron (II) (Table 4) in different samples are in good agreement with the claimed values.

4.3. Statistical comparison

A comparison was made between the poroposed SIA system and a manual UV/Vis spectrophotometric method as well as between the proposed SIA system and a manual FAAS method and the results are given in Table 4. This comparison was made to establish whether the SIA system give reliable results and be accepted for the determination of iron (II) in multi-vitamin preparations. We conducted the null hypothesis testing and a *t*-test with multiple sample mean (paired by difference) was applied to examine whether the two methods differ significantly at 95% confidence level. The *t* calculated values for samples 1,2,3 and 4 are 1.87, 0.74, 0.63 and 0.44, respectively, for the manual UV/Vis spectrophotometric method and 1.4, 0.27, 2.26 and 1.93, respectively, for the manual FAAS method. The tabulated critical value of *t* at 95% confidence level and six degrees of freedom is 2.45 [23]. Since, the calculated *t*-values are much less than the tabulated critical value, the null hypothesis (H_o) cannot be rejected and which indicates that there is no significant difference between the proposed and manual methods for the determination of iron (II) in the multivitamin preparations.

Table 5
Influence of interferences

Foreign ions added to 3 mg l^{-1} of Fe(II)	Quantity (mg l^{-1}) of metal ions added	Fe(II) recovery (%)
Zn(II)	9	100
	18	95.5
	27	91.8
	36	90.5
Cu(II)	1.5	100
	3.0	88.4
	4.5	85.6
Mg(II)	15	100.5
	30	102.3
	60	101.3
	90	99.7

5. Conclusion

The proposed SIA method is direct, simple and reliable for the determination of iron (II) in pharmaceutical products. The advantage of the proposed method compared to the other methods is that it avoids the oxidation step, splitting and merging of sample zones and reagent streams. The results obtained by this method also revealed that this method offes a better analytical performance in terms of sensitivity and sample throughput (40 samples h^{-1}) with a linear range from 0.25 to 5 mg l^{-1} and with detection limit of 18 μg l^{-1}, maintaining all its intrinsic advantages of flexibility, reagent saving, reliability and rapid parameter optimization. The proposed SIA system is more sensitive (lower linear range) with a lower detection limit than the α,α-bipyridyl procedure reported before [1] making it more suitable for the determination of iron (II) in multivitamin tablets with low iron content. The results obtained by the proposed SIA system agree very well with the manual spectrophotometric and FAAS methods. This is confirmed statistically, which shows no significant difference at the 95% confidence level.

References

[1] P.C.C. Oliveira, J.C. Masini, Anal. Lett. 34 (2001) 389.
[2] A.N. Araujo, J. Gracia, J.L.F.C. Lima, M. Poch, M. Lucia, M.F.S. Saraiva, Fresenius J. Anal. Chem. 357 (1997) 1153.

[3] W.H. Mahmoud, Anal. Chim. Acta 436 (2001) 199.
[4] P.L. Croot, P. Laan, Anal. Chim. Acta 466 (2002) 261.
[5] P.C. Aleixo, J.A. Nobrega, Food Chem. 83 (2003) 457.
[6] S. Xing Li, N. Sheng Deng, J. Pharm. Biol. Anal. 32 (2003) 51.
[7] T. Palanche, F. Marmolle, M.A. Abdallah, A. Shanzer, A-M. Albrecht-Gary, J. Biol. Inorg. Chem. (JBIC) 4 (1999) 188.
[8] G. Zhu, Z. Zhu, L. Qiu, Anal. Sci. 18 (2002) 1059.
[9] S. Sundd, S.K. Prasad, A. Kumar, B.B. Prasad, Talanta 41 (1994) 1943.
[10] D. Tzur, V. Dosortzev, E. Kirowa-Eisne, Anal. Chim. Acta 392 (1999) 307.
[11] A. Huberman, C. Perez, Anal. Biochem. 307 (2002) 375.
[12] S. Zareba, H. Hopkala, J. Pharm. Biol. Anal. 14 (1996) 1351.
[13] J.F. van Staden, E.B. Naidoo, S. Afr. J. Chem. 53 (2000) 191.
[14] A.F. Oliveira, J.A. Nobrega, O.F. Filho, Talanta 49 (1999) 505.
[15] M. Novic, M. Novic, J. Zupan, N. Zafran, B. Pihlar, Anal. Chim. Acta 348 (1997) 101.
[16] M. Endo, S. Abe, Fresenius J. Anal. Chem. 358 (1997) 546.
[17] M. Kass, A. Ivaska, Talanta 58 (2002) 1131.
[18] L.V. Mulaudzi, J.F. van Staden, R.I. Stefan, Anal. Chim. Acta 467 (2002) 35.
[19] R.C.C. Costa, A.N. Araujo, Anal. Chim. Acta 438 (2001) 227.
[20] J.F. van Staden, L.G. Kluever, Fresenius J. Anal. Chem. 362 (1998) 319.
[21] J.A. Sweileh, Microchim. J. 65 (2000) 87.
[22] R.E. Taljaard, J.F. van Staden, Laboratory Robotics and Automation 10 (1998) 325.
[23] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, third ed., Ellis Horwood, Chichester, 1984.