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Determination of total iron as Fe(II) in multivitamins, haematinics and natural waters using a sequential injection (SIA) system.

J. F. van Staden[1] and E. B. Naidoo
Department of Chemistry, University of Pretoria, Pretoria, 0002 South Africa

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Abstract

The determination of total iron in pharmaceutical products and natural waters as Fe(II) using a sequential injection system was investigated. A cadmium reductor consisting of cadmium granules was used to reduce Fe (III) to Fe (II). The Fe (II) was then determined (by its reaction with 1,10 Phenanthroline) as a [$Fe(phen)_3^{2+}$] complex at 515 nm with a UV/Vis spectrophotometer. The linear range of the system is between 1 and 60 mg/l with a detection limit of 0.18 mg/l. The proposed system is suitable for the determination of total iron as Fe(II) in pharmaceutical products and natural waters at a rate of 24 samples/hour with a relative standard deviation of less than 2.5%. Statistical comparison between the proposed sequential injection (SIA) system, certified values and the standard methods (Inductively Coupled Plasma {ICP} and UV/Vis spectrophotometry) revealed that there is no significant difference at the 95% confidence level.

1. Introduction

Essential trace elements, of which iron is one, are vital and are required for various biological functions in the human body. Deficiencies of iron are known to occur in vulnerable population such as pregnant women, infants and children as well as malnutritioned individuals. In order to avoid such deficiencies, an adequate supply of iron that can be utilized for biological functions is needed. Individual components of the diet and the iron status of each individual will affect this bioavailability[1]. However, besides diet iron requirements may be supplied by administering multivitamins and

[1]To whom correspondence should be addressed;

Fax:27-12-362-5297; e-mail:Koos.vanStaden@chem.up.ac.za

haematinics orally which are available from drug stores, super markets, clinics, hospitals and from medical practitioners.

Heinrich *et al*[2] has shown that the amount of average daily requirement of iron is 1.3 mg/day in males and non menstruating females or 1.8 mg/day in menstruating females. Diminished food iron adsorption causes iron deficiency anaemia and gastric mucosa atrophy after the depletion of iron stores in such individuals. Hence, the determination of iron is imperative.

The determination of iron in its various oxidation states in a variety of matrices has been studied and described in detail by numerous researchers[3-8]. Methods used include kinetic spectrometry[4,5,7], polarography[9], Graphite Furnace Atomic Absorption Spectrometry (GFAAS)[9] and Flame Atomic Absorption Spectrometry (AAS)[10]. Most of these classical techniques have been modified for use in flow systems by making use of the flexibility and ease offered by flow injection analysis (FIA)[5,11-13].

Speciation of Fe(III) and Fe(II)[14-16] was also described. Faizullah and Townshend[17] determined Fe(II) after complexing with 1,10 Phenanthroline, then reducing the Fe(III) present with a reducing column. Lynch *et al*[18] describes the use of different complexing agents in the same manifold for determining Fe(II) and Fe(III) simultaneously. Masatoshi and Shigeki[19] developed a method for the sequential spectrophotometric determination of Fe(III) and Fe(II) by copper(II) catalyzed reaction with Tiron in a double-injection flow injection system. Oliveira *et al*[20] proposed an asynchronous merging zones method with simultaneous introduction of the sample and modifier reagent (ascorbic acid) for sequential determination of Fe(II) and Fe(III) in pharmaceutical products. Luque-Perez *et al*[21] indirectly determined ascorbic acid by reducing Fe(III) to Fe(II) with ascorbic acid, then complexing with 1,10 Phenanthroline and monitoring the Ferroin complex spectrophotometrically.

The aim of the work described in this paper was to find a simpler method for determining total iron as Fe(II) without splitting and merging zones and reagent streams. The method should also save on reagent and samples. To achieve this, a sequential injection analysis (SIA) system incorporating a cadmium reductor to reduce Fe(III) to Fe(II) was adopted. Van Staden and Kluever[22,23] modified existing FIA systems by incorporating solid-phase reactors into the manifold. This led to lowered reagent consumption.

SIA, launched in 1990[24,25] is a technique that has great potential for on-line measurements in many routine laboratories due to the simplicity and convenience with which sample manipulation can be automated. It saves on both reagent and sample consumption.

Van Staden *et al*[26] used SIA for determining Fe(III) in pharmaceutical samples using a dialyser. The Fe(III) was complexed with Tiron and determined spectrophotometrically. Araújo *et al*[27] described a procedure for the colorimetric determination of iron in infant fortified formulas based on an SIA system. The Fe(III) was complexed with thiocyanate as colour developing reagent. Rubi *et al*[28] proposed an SIA assembly for the atomic absorption determination of Fe(III) in natural waters. The iron was preconcentrated on a microcolumn packed with a chelating resin (Chelex 100) that was inserted into the SIA manifold.

In this work a cadmium reductor as a solid-phase reactor was incorporated into a SIA manifold to reduce on-line Fe(III) to Fe(II) and complexed the Fe(II) with 1,10 Phenanthroline to a red-orange complex. The reagent is a weak base that reacts to form the Phenanthroline ion, $phenH^+$ in acidic media. Complex formation can be described by the equation

$$Fe^{2+} + 3\ pheH^+ \circ Fe(phen)_3^{2+} + 3\ H^+ .$$

The formation constant for this equilibrium is 2.5×10^6 at 25EC. Fe(II) is quantitatively complexed in the pH range between 3 and 9. A pH of about 3.5 is ordinarily recommended to prevent precipitation of iron salts such as phosphates. An excess of reducing agent such as hydoxylamine or hydroquinone which is often necessary to maintain iron in the 2+ oxidation state is not necessary when using a solid-phase reactor as reductor. The complex formed is stable and was monitored at 515 nm with a UV/Vis spectrophotometer.

2. Experimental

2.1 Reagents and solutions

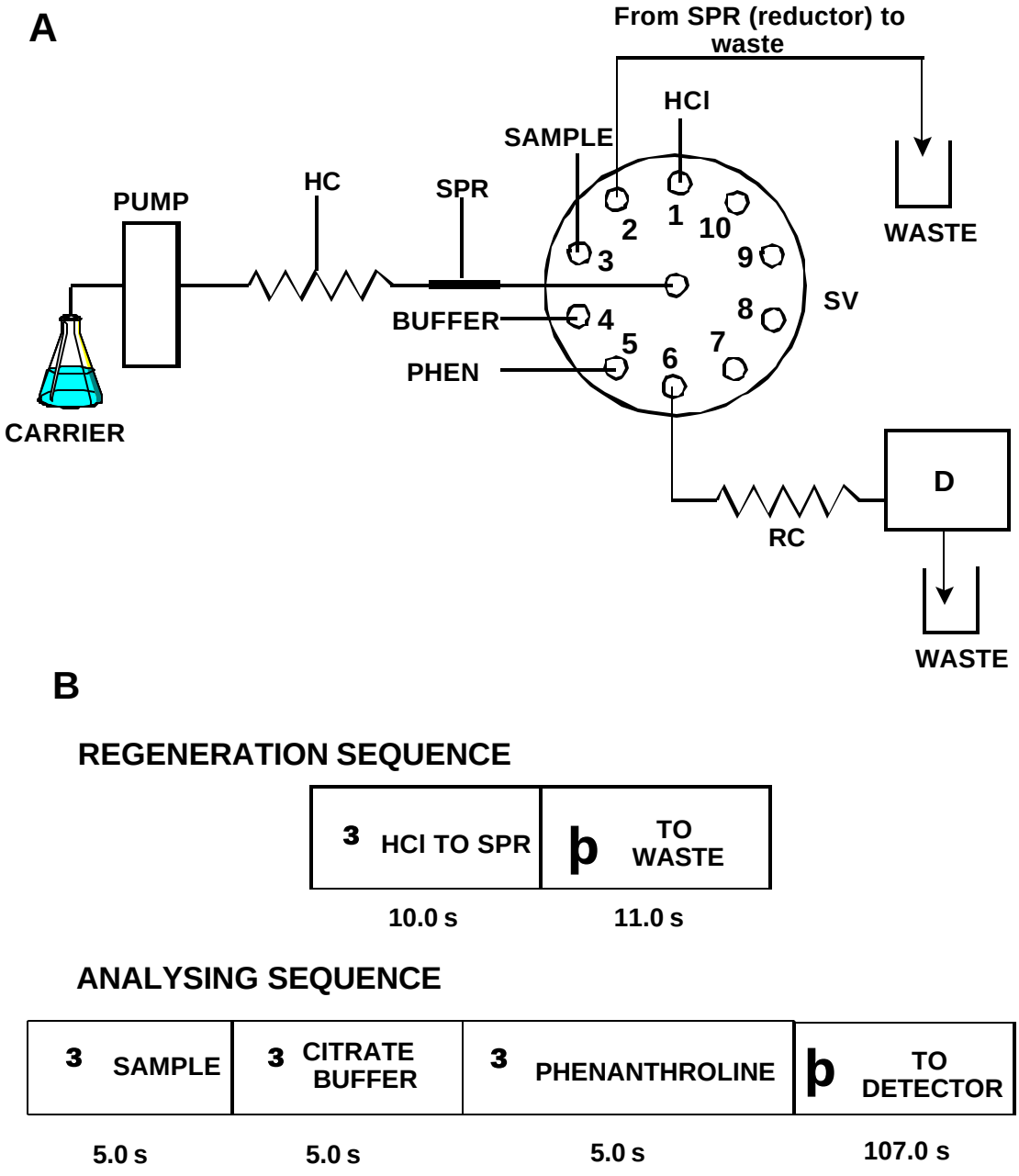
All solutions are prepared from analytical grade reagents unless specified otherwise. Deionised water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used to prepare all aqueous solutions and dilutions. A stock Fe(II) solution containing 1000 mg/l Fe(II) was prepared by dissolving $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$ (Kanto Chemical Co., extra pure) and diluting to 1 litre with water. Working standards in the range 1 to 100 mg/l were prepared by appropriate dilution of the stock solution with 0.01 mol/l $HClO_4$. The 0.01 mol/l $HClO_4$ was prepared by diluting 4.4 ml of $HClO_4$ (Merck, GPR, 70 %) to 5 l. A 0.25 % 1,10 Phenanthroline solution (Aldrich, 99+%) was prepared by dissolving 0.625 g of 1,10 Phenanthroline in 50 ml 0.01 mol/l $HClO_4$ and diluting to 250 ml with water. A 0.1 mol/l acetic acid solution was prepared by diluting 1.45 ml of acetic acid (Chemical suppliers, 99.9%) to 250 ml. A 10 % hydroxyl ammonium chloride

(Searle, GPR, 97%) was prepared by dissolving 10 g in water and making up to 100 ml. A 0.1 mol/l sodium acetate solution was prepared by dissolving 1.36 g of sodium acetate (Merck, extra pure) in water and making up to 100 ml. A buffer solution in the pH range 3 to 5 was prepared by mixing 65 ml of 0.1 mol/l acetic acid solution with 35 ml 0.1 mol/l sodium acetate solution and adding 1 ml of 10 % hydroxyl ammonium chloride to the resulting solution. A 1 mol/l HCl solution was prepared by diluting 100 ml of concentrated HCl (Merck, 32 %) and making up to 1 l. Chloroform (Merck, pro analysis) was used to extract the unwanted organic material from the samples.

2.2 Instrumentation

The sequential injection system depicted in Figure 1A was constructed from the following components: a Gilson minipuls peristaltic pump (Model M312, Gilson, Villiers-Le Bel, France); a 10-port electrically actuated selection valve (Model ECSDIOP, Valco Instruments, Houston, Texas) and a Unicam 8625 UV-Visible spectrophotometer equipped with a 10-mm Hellma-type (Hellma GmbH and Co., Mulheim/Baden, Germany) flow-through cell (volume 80 Fl) for absorbance measurements. The absorbance of the Fe-Phenanthroline complex, $[Fe(phen)_3^{2+}]$, at 515 nm was used to study the response and precision of the samples during dispersion in the SIA manifold. The working wave length of 515 nm was determined by scanning the solution complex from 200 to 1100 nm.

Data acquisition and device control was achieved using a PC30-B interface board (Eagle Electric, Cape Town) and an assembled distribution board (Mintek, Randburg). The flowTEK[29] software package (obtainable from Mintek) for computer-aided flow analysis was used throughout for device control and data acquisition. All data given (mean relative peak height values) are the average of 10 replicates and absorbance for the standard method are a mean of 5 replicate measurements.



B

REGENERATION SEQUENCE

3 HCl TO SPR	b TO WASTE
10.0 s	11.0 s

ANALYSING SEQUENCE

3 SAMPLE	3 CITRATE BUFFER	3 PHENANTHROLINE	b TO DETECTOR
5.0 s	5.0 s	5.0 s	107.0 s

Figure 1 Sequential injection manifold for the determination of total iron as iron(II).

(A) Hydrochloric acid solution is first aspirated through the sequential selection valve (SV), solid-phase reactor (SPR, 15 cm x 6 mm) into the holding coil (HC, 300 cm x 0.89 mm) to regenerate the SPR. Carrier solution propelled by the peristaltic pump then flushes the hydrochloric acid solution through the SPR to waste. Thereafter, sample, buffer and phen (1,10 phenanthroline) are sequentially aspirated through the SV and SPR into the HC. Finally the stack of zones with the final reaction product are transported via the SPR and the reaction coil (RC, 100 cm x 0.89 mm) towards the detector (D) for measurement and then to waste. (B) Device sequence for one SIA cycle.

2.3 Operation of the system

A schematic diagram for the SIA system is depicted in Figure 1A. The whole procedure, from sample injection to data processing and storage was computer controlled via the flowTEK program. The whole SIA procedure involved designing a method which allows a single cycle of the experiment to be run. Table 1 and Figure 1B shows the device sequence for one cycle.

TABLE 1. Device sequence for one cycle of the SIA system

Time (s)	Pump	Valve	Description
0	Off	Position 1	Pump off. Select HCl stream.
1	Reverse		Draw HCl solution through SPR for regeneration.
10	Off		Pump stop.
11	Off	Position 2	Select waste stream
12	Forward		Pump solution from SPR to waste
23	Off		Pump stop
24	Off	Position 3	Select sample stream
25	Reverse		Draw sample
29	Off		Pump stop
30	Off	Position 4	Select buffer stream
31	Reverse		Draw buffer solution
35	Off		Pump stop
36	Off	Position 5	Select orthophenanthroline stream
37	Reverse		Draw orthophenanthroline
41	Off		Pump stop
42	Off	Position 6	Select detector stream
43	Forward		Pump zones through reductor to detector
150	Off	Position 1	Valve return home

The zones were stacked in the holding coil and then transported by the carrier stream (0.01 mol/l $HClO_4$) through the reductor to reduce any Fe(III) that may be present to Fe(II). The Fe(II) then complexed with 1,10 Phenanthroline and was detected at 515 nm with a spectrophotometer. The data obtained is then converted to a response time

graph on the computer screen as a peak profile. The maximum relative peak height was then automatically processed and stored on a computer via the flowTEK program.

2.4 The cadmium reductor

The reductor columns were made of glass with varying lengths (12, 15, 17, 19 and 21 cm) but with the same internal diameter of 6 mm. The columns were then filled with cadmium granules (Merck, 0.3 - 1.5 mm). The particles were held by a glass frit at each end so that they did not block the SIA system. A vibrator was used for close packing of the columns. The cadmium granules used for packing the glass column were prepared by washing with acetone for 10 minutes, adding 2 mol/l HCl, deionised water and methanol and dried in a desiccator. An acidified cadmium reductor was chosen over a copperised cadmium reductor because copper has a tendency of interfering in the determination of iron. The cadmium reductor was regenerated by passing approximately 470 Fl of 1 mol/l HCl at the beginning of every cycle. This was to ensure consistency in the reduction efficiency and capacity of the reductor.

2.5 Sample preparation

The multivitamin and haematinic samples were digested in 50 ml 6 % v/v HCl on a hot plate. When a fifth of the solution was remaining a further 30 ml of the 6 % v/v HCl was added and the digestion continued until approximately 10 ml was remaining. Three 50 ml portions of chloroform (Merck, pro analysis) were added to the samples with vigorous shaking to separate the organic materials from the inorganic. The separation at each instance was allowed two hours. A final 50 ml portion was added and left overnight for final separation.

The aqueous layer was collected into a 100 ml standard flask and made to volume with a 0.01 mol/l $HClO_4$ acid solution. Further dilutions were made from the prepared samples to bring their concentrations within detectable range in the SIA system.

3. Results and discussions

3.1 Method optimisation

The method was optimised with regard to the following parameters: Fe(II) concentration, flow rate, sample and reagent volume, reactor length, carrier type and HCl concentration for reductor regeneration (reductor efficiency). Both the relative peak height and % RSD were used as criteria for establishing the most appropriate parameter value in each case.

3.1.1 Cadmium reductor parameters

The cadmium reductor forms the heart of the reducing manifold part of the proposed system. The performance of the SIA system depends on the efficiency of the reductor at the interface between the solid and the liquid phases of the cadmium reductor. In addition the reductor packing had to be thorough and the reductor length and efficiency had to be optimised.

TABLE 2. Effect of reactor length on response and precision

Length (cm)	12	15	17	19	21
Relative peak heights	5.262	5.281	5.31	5.872	6.521
%RSD	2	1.1	5.8	5.34	4.38

The response and precision of the system were studied by varying the reactor length between 12 and 21 cm with the internal diameter fixed at 6 mm. The five reactors (12, 15, 17, 19 and 21 cm) were compared for reductor efficiency. It was found from the results obtained (Table 2) that the first three cadmium reductors did not show a significant difference in response; there was, however for the longer lengths. The 15 cm reductor length was chosen as the optimum length because of its good precision as seen in Table 2.

3.2 Chemical parameters

The Fe(II) concentration was evaluated between 1 to 100 mg/l. The effect of concentration is presented in Figure 2. It is clear from Figure 2 that the response steadily increases with an increase in concentration. The 50 mg/l concentration gave the best precision and was chosen as the optimum concentration. The use of 1 mol/l HCl as both reductor regenerator and carrier could not work because bubbles were given off now and then. However, the use of 0.1 mol/l $HClO_4$ resulted in a better consistency in response and there were no bubbles.

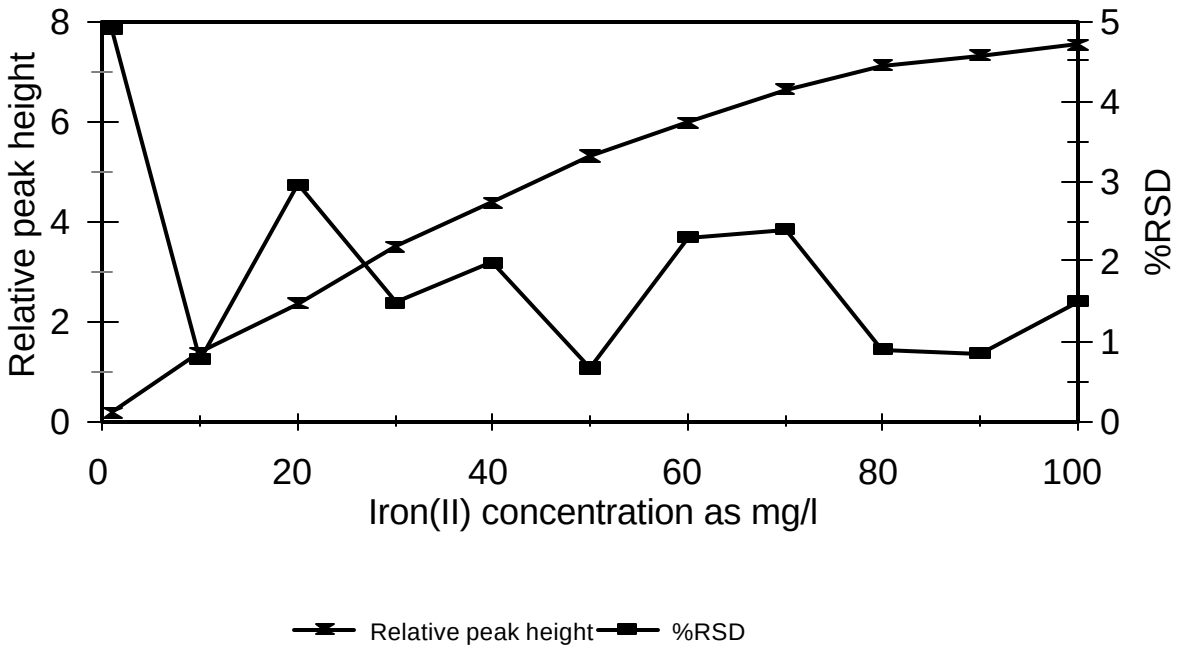


Figure 2 Effect of iron(II) concentration on response and precision

The $HClO_4$ concentration was then studied between 0.005 and 0.1 mol/l and the results given in Figure 3. The response increases up to a concentration of 0.05 mol/l. The best precision was however given by a concentration of 0.01 mol/l which was chosen as the optimum.

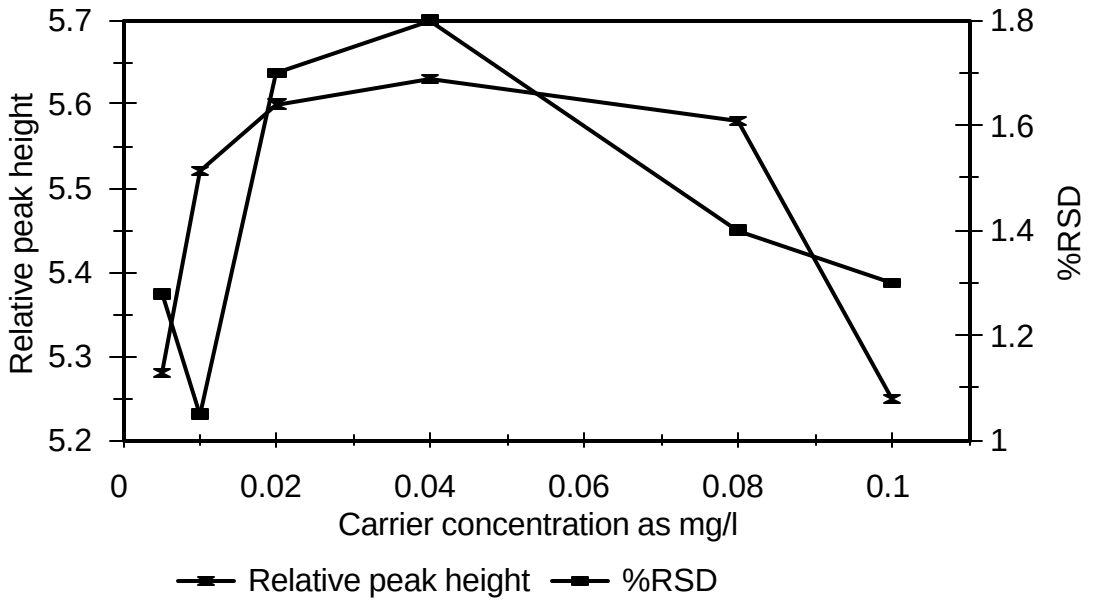


Figure 3 Effect of carrier concentration on response and precision

3.3 Physical parameters

The contact time between the iron and the cadmium reductor is of utmost importance. It was, however found that most of the iron in the pharmaceutical preparation is in the Fe(II) state, with very little in the Fe(III) state. Although the amount of iron present in the water samples analysed was lower, all the iron was in the Fe(III) state and had to be reduced. The 15 cm reactor was found to be optimum and effective with 470 Fl 1 mol/l HCl passed through the reactor for every SIA cycle. The flow rate was evaluated between 1.13 and 3.96 ml/min. The results and effect of this are illustrated in Table 3. The response increases with an increase in flow rate, due to less dispersion and better zone overlapping. The 2.83 ml/min flow rate however gave the best precision and was chosen as optimum. Sample volume was evaluated from 142 to 424 Fl and the results are given in Table 4. Although the sensitivity increases with an increase in sample volume, the best precision was obtained with a sample volume of 236 Fl which was chosen as optimum sample volume. The reagent volume was evaluated from 94 to 283 Fl (Table 5). 236 Fl was chosen as optimum reagent volume due to the best precision.

TABLE 3. Effect of flow rate on response and precision

Rate (ml/min)	1.13	1.71	2.26	2.83	3.29	3.96
Relative peak heights	0.557	1.929	2.544	3.612	3.951	4.033
%RSD	5.6	4.6	3.3	2.1	2.4	7.5

TABLE 4. Effect of sample volume on response and precision

Volume(µl)	142	236	330	424
Relative peak heights	2.847	3.951	4.404	4.376
%RSD	3.3	2.4	3.2	3.1

TABLE 5. Effect of reagent volume on response and precision

Volume(µl)	94	142	189	236	283
Relative peak heights	1.766	4.464	6.847	8.62	8.72
%RSD	2.9	3.2	1	0.8	4.2

3.4 Method evaluation

The linearity of the system was evaluated for the analyte concentration between 1 and 100 mg/l. The response was found to be linear in the range 1 to 60 mg/l (Figure 4). The relationship between the response and the concentration is given by the equation:

$$H = 0.1049x + 0.1722, (r= 99.99\%, n=10),$$

where H is the relative peak height and x the analyte concentrations in mg/l.

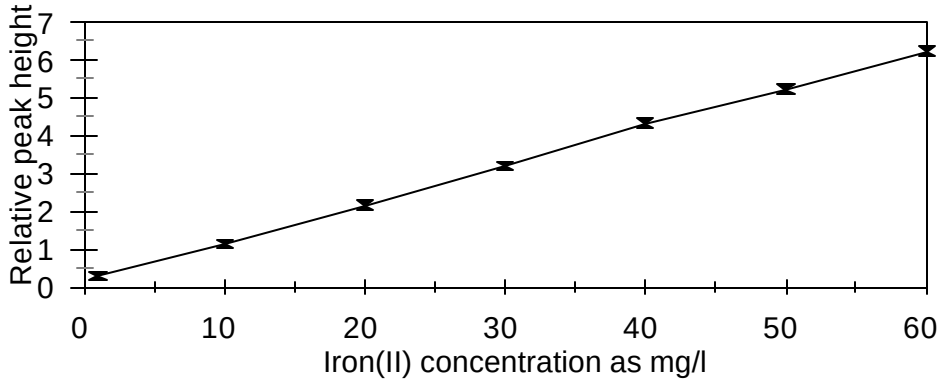


Figure 4 Calibration graph using optimum conditions

Real samples (multivitamins and haematinics) and water samples were analysed with the proposed system. The results obtained are a mean of 10 repetitive analyses of each sample. The accuracy was compared to certified values and the standard method (Tables 6 and 7).

TABLE 6. Iron in multivitamin and haematinics using SIA and spectrophotometric methods as well as certified values in mg/tablet (capsule) and % RSD's in brackets.

Sample	Certified values	Proposed SIA method	Standard spectrophotometric method
Filibon	15	16.04 (2.3 %)	15.3 (4.2 %)
Ferrimed	50	47.75 (1.6 %)	51.52 (4.3 %)
Pregamal	56	52.65 (1.8 %)	51.02 (4.5 %)
Ferrous C	28	27.19 (1.2 %)	27.16 (2.7 %)

TABLE 7. Iron in effluent streams using SIA and ICP methods

Sample	Concentration in mg/l		Relative standard deviation (%)	
	SIA	ICP-AES	SIA	ICP-AES
A	0.759	0.861	2.3	2.2
B	0.331	0.286	1.3	2.8
C	0.266	0.221	0.3	3.9

The precision of the method was determined by 10 repetitive analyses of the standard solutions as well as 10 repetitive analyses of the real samples. All these were carried out under optimum conditions. The relative standard deviation for the standard was 1.5 % and for the real samples was less than 2.5 %.

The detection limit was calculated using the formula $[(3F + k) - c]/m$ where F (0.00529) is the standard deviation of the baseline , k is the average response of the baseline (0.175) and c (0.1722) the intercept and m (0.1049) the slope of the calibration graph. The detection limit was found to be 0.178 mg/l.

The recovery of the proposed system was determined by comparing the expected results with those obtained with the proposed system and ranged between 90.5 and 109.4 %.

The sample interaction carryover effect between consecutive samples was determined by analysing sample with low analyte concentration followed with a high

analyte concentration which was again followed by the sample with a low analyte concentration. The sample interaction was then calculated using the following formula:

$$\text{Sample interaction} = (A_3 - A_1)/A_2 \times 100\%,$$

where A_1 is the peak height (1.35) of a sample containing 10 mg/l Fe (II), A_2 is a peak height (5.52) containing 50 mg/l Fe (II) and A_3 is a peak height (1.42) containing 10 mg/l Fe (II). The sample interaction was ± 1.3 % which may be considered negligible.

The only possible interferences that may disturb this analysis are Ag, Bi, Ni, Cu and Co. Fortunately of these only Cu was found in very low levels which may not affect the results. Table 8 gives the elements present in samples analysed and their amounts. In the work done by Van Staden and Kluever[21] this levels of cations did not interfere with the analysis of Fe(II) as was shown by the recoveries obtained.

TABLE 8. Elements present in samples analysed as mg/tablet or capsule

Element	**Amount/tablet (capsule)**	**Element**	**Amount/tablet (capsule)**
Ca	< 5 mg	Na	< 1 mg
K	< 0.83 mg	Mg	< 0.15 mg
Mn	< 0.05 mg	Zn	< 0.085 mg
Mo	< 0.025	Cu	< 0.15 mg

3.5 Statistical comparisons

The comparison was done between the SIA system and the certified values (Table 6) as well as SIA and the standard spectrophotometric method for the pharmaceutical products. A further comparison was done between SIA and the standard ICP method (Table 7) for the water samples. The comparison was done to establish whether the SIA system can be accepted as giving reliable results in the iron determination or not. The null hypothesis was used[30, 31]. For the null hypothesis the two methods should agree ideally when the population, H_0, mean difference, μ_D, is zero; H_0: $\mu_D = 0$. The alternative hypothesis, $\mu_D \neq 0$, implies that the two methods failed the test. The t-test with multiple samples (paired by difference) was applied to examine whether the two methods differed significantly at 95 % confidence level.

In the determination of iron in pharmaceutical products, the mean tabulated results for Table 6 between SIA and certified results was $\theta_{D1} = 1.34$ and the standard deviation was $s_{D1} = 1.90$. For SIA and the standard method, the mean tabulated results

was θ_{D2} = 0.40 and the standard deviation s_{D2} = 2.32. In the determination of iron in water samples, the mean tabulated results for Table 7 was θ_{D3} = 0.004 and the standard deviation was s_{D3} = 0.085.

In the determination of iron in pharmaceutical products there are five determinations (n = 5), therefore < = 4 and at 95 % confidence level $t_{0.05,4}$ = 2.78. The critical values are therefore ± 2.78. Finally the $t_{calculated}$ are 1.42 and 0.35 respectively. The results indicates that there is no significant difference between the methods at 95 % confidence level.

In the determination of iron in water samples there are three determinations (n = 3), therefore < = 2. At 95 % confidence level $t_{0.05,2}$ = 4.30. The critical t-values are therefore ± 4.30. The $t_{calculated}$ value is 0.082. There is no significant difference between the methods at 95 % confidence level.

It can be concluded that, in the determination of iron in pharmaceutical products, the SIA and the standard method (spectrophotometry) at 95 % confidence level give the same results. It can also be concluded that SIA and the ICP method in the determination of iron in water samples gives the same results at 95% confidence level. The null hypothesis can therefore be accepted that the results are the same at 95 % confidence level.

4. Conclusions

Total iron determination by SIA with a cadmium reductor incorporated into the SIA manifold is an improvement on the homogeneous methods applied in FIA and SIA systems. In contrast to the FIA system, the cadmium reductor in SIA was regenerated on-line without having to disconnect the system and replace with a new reductor. Thus, once designed it does not have to be physically reconfigured. The SIA system is easier to use and was found suitable for the determination of total iron as Fe (II) in pharmaceutical products and water samples within a wide range as shown by the detection limit value.

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