

Short communication

# New Induction Period Based Kinetic Spectrophotometric Method for the Determination of Iron (II) in Pharmaceutical Products

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## Abstract

A new simple, sensitive and selective kinetic spectrophotometric method was developed for the determination of iron (II) in pharmaceutical products. The method is based on the enhancing effect of iron (II) on the sodium periodate-potassium bromide-meta cresol purple reaction system in the presence of thiocyanate. The reaction starts after an induction period, which decreased by addition of iron (II). The reciprocal of the reaction induction period is proportional to the iron (II) concentration. The decolorization of meta cresol purple (MCP) at 525 nm was used to monitor the reaction spectrophotometrically. Under optimum conditions, iron (II) can be determined in the range of 0.020–4.0  $\mu\text{g mL}^{-1}$  with a  $3\sigma$  detection limit of 0.017  $\mu\text{g mL}^{-1}$ . The relative standard deviations for five replicate determinations of 0.030, 0.20, and 2.0  $\mu\text{g mL}^{-1}$  iron (II) were 2.9%, 2.7% and 2.3%, respectively. This method was successfully used in the determination of iron (II) in ferrous sulfate pharmaceutical products.

**Keywords:** Iron (II); m-Cresol purple; Induction period; Kinetic, Spectrophotometry

## 1. Introduction

Iron is present in the hydrosphere at two oxidation states of iron (II) and iron (III), which are thermodynamically stable under anoxic and oxic conditions, respectively.<sup>1</sup> From these two oxidation states, Fe(II) is a constituent of hemoglobin which is essential for the normal transportation of oxygen to the tissues, while Fe(III) will not bind oxygen.<sup>2</sup> The amount of iron in the foods ingested during a day is approximately 10–15 mg and studies indicate that normal subjects absorb ten percent of iron in the food.<sup>3</sup> The absence of iron in the organism causes anemia, the result of decreased red blood cell content. This deficiency is treated with iron salts via oral or intramuscular administration.<sup>4</sup> The excess of iron during treatment with iron salts may produce severe poisoning, causing symptoms of gastric irritation, vomit, pallor and circulatory collapse.<sup>5</sup> Owing to the presence of iron (II) in biological samples and pharmaceutical products, an accurate, fast and cheap method for the determination of iron in

pharmaceuticals and food supplements is of great interest. Although there are number of papers that deal with the total iron in the real samples, only a few have reported the concentration of iron (II). Some of these methods are based on the complex formation of iron (II) with specific chelating agents followed by spectrophotometric measurement. 1,10-Phenanthroline,<sup>6</sup> bathophenanthroline (4,7-diphenyl-1,10-phenanthroline),<sup>7</sup> ferrozine (3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine),<sup>8</sup> 2,4,6-tris(2'-pyridyl)-1,3,5-triazine (TPTZ)<sup>9</sup> and 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT)<sup>10</sup> are the common chelating agents for Fe(II). Preconcentration methods coupled with spectrophotometric detection,<sup>11–13</sup> hyphenated techniques (such as LC-ICP-AES,<sup>14</sup> LC-ICP-MS<sup>15</sup>) and fluorimetric methods<sup>16,17</sup> have been reported for high sensitive determination of iron, but these methods require expensive instruments and well-trained operators.

Kinetic methods of analysis have high sensitivity and sufficient accuracy. A number of kinetic methods for the determination of iron at trace levels based on its catalytic effect on the oxidation of various organic com-

pounds have been reported in several reviews<sup>18–20</sup> and experimental papers.<sup>21–31</sup> Although some of these methods are very sensitive, some suffer from interference or poor reproducibility and all these methods generally determine iron (III) or the total iron, rather than iron (II). Thus more sensitive and selective methods that can rapidly and conveniently determine the low concentrations of iron (II) in the presence of iron (III) are still needed.

Meta Cresol purple (MCP) has been used as an indicator for the catalytic determination of bromide<sup>32</sup> and simultaneous determination of nitrite and nitrate.<sup>33</sup> However, there has been no report on MCP as an indicator for the kinetic determination of iron (II). The objective of the present work is therefore, to report a direct, simple and accurate method for the determination of Fe(II) in pharmaceutical products based on the enhancing effect of iron (II) on the sodium periodate-potassium bromide-meta cresol purple reaction system in the presence of thiocyanate. The reciprocal of reaction induction period at 525 nm is proportional to the iron (II) concentration.

## 2. Experimental

### 2.1. Reagents and Solutions

All reagents used were of analytical reagent grade and all solutions were prepared with double distilled water.

The iron (II) stock solution (1000 g mL<sup>-1</sup>) were prepared by dissolving 0.7080 g of Mohr's salt (Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6 H<sub>2</sub>O) with 0.01 M of H<sub>2</sub>SO<sub>4</sub> in a 100 mL volumetric flask. Working standard solutions ranging between 0.20 and 40 g mL<sup>-1</sup> of Fe(II) were prepared by appropriate dilution of the stock solution. A 1000 g mL<sup>-1</sup> stock standard solution of thiocyanate was prepared by dissolving 0.1706 g KSCN (Merck) in distilled water and diluting it to 100 mL. Working solutions were prepared by appropriately diluting the stock standard solution. A 100 mL 0.020 M potassium bromide solution was prepared by dissolving 0.2380 g of KBr (Merck) in distilled water and diluting to mark in 100 mL volumetric flask. A 2.35 × 10<sup>-4</sup> M m-Cresol purple (MCP) solution was prepared directly by dissolving 0.0090 g of MCP (Merck) in 20 mL of ethanol and diluting with distilled water in a 100 mL calibrated flask. A 100 mL sodium periodate solution (0.030 M) was prepared by dissolving 0.6420 g of NaIO<sub>4</sub> (Merck). Hydrochloric acid solution (0.50 M) was prepared by diluting a known volume of concentrated solution (Merck) and standardized against sodium carbonate.

### 2.2. Apparatus

A UV-160 double beam spectrophotometer (Shimadzu, Japan) equipped with 1.0 cm path length quartz cells, was used to get absorbance spectra and absorbance-time curves at fixed wavelength. The temperature was

controlled by a water bath (n-BIOTEK, INC, model NB-301). A stopwatch was used for measuring the reaction time.

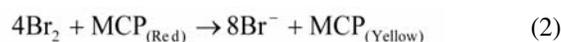
### 2.3. Procedure

All of the reagent solutions were brought to the 20 °C before mixing. An aliquot of sample solution containing 0.20–40.0 g iron (II) was transferred to a 10 mL volumetric flask, then 1.0 mL of 12.0 g mL<sup>-1</sup> thiocyanate, 1.0 mL of 0.50 M HCl, 1.0 mL of 2.35 × 10<sup>-4</sup> M MCP, and 1.0 mL of 0.020 M potassium bromide solutions were added. The solution was diluted to ca 8 mL with water. Then 1.0 mL of 0.030 M sodium periodate solution was added and solution was diluted to the mark. The variation in absorbance with time was recorded against water for the first 15–235 s from the initiation of the reaction at 525 nm. A blank solution (without iron (II)) was prepared and measured in the similar way. A calibration graph was constructed by plotting reciprocal of reaction induction period (1/t<sub>ip</sub>) against iron (II) concentration.

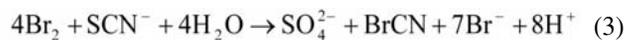
## 3. Results and Discussion

### 3.1. Reaction System

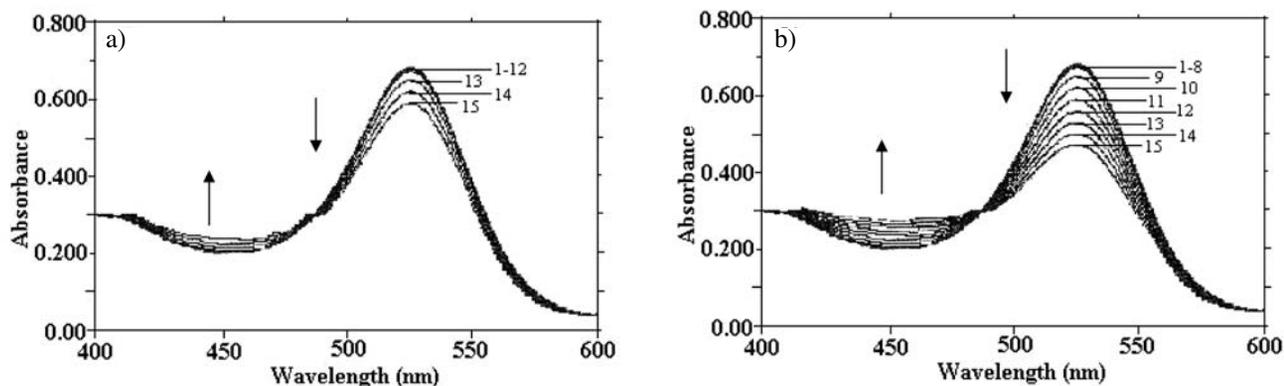
In the previous work we reported that the thiocyanate inhibits the bromide-catalyzed oxidation of MCP by periodate in acidic media.<sup>34</sup> The catalytic cycle of oxidation reaction may be shown as follows:



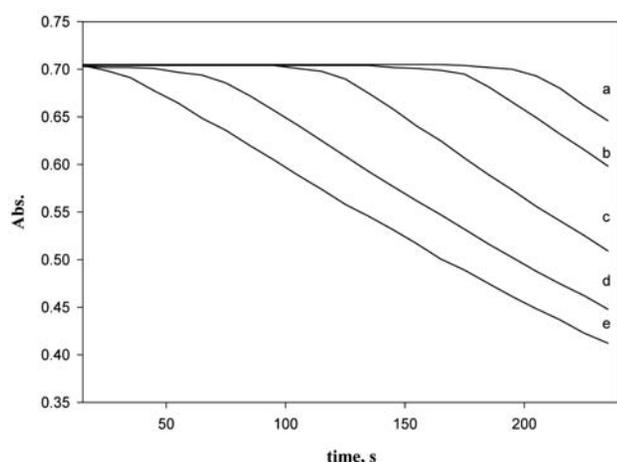
Thiocyanate has an inhibition effect on the catalytic reaction in acidic media due to possible perturbation in catalytic cycle via following reaction:<sup>35</sup>



It was found that in the presence of iron (II), reaction rate increases and as a result the reaction induction period decreases. This fact is due to possible complex formation of iron (II) with thiocyanate. Fig. 1a shows the time dependence of absorption spectra of the blank reaction (without iron (II)) system. From Fig. 1a can be seen that from 15 repeated scans with time intervals of 15 s, the spectra of 12 repeated scans overlay with each others which dedicates a long induction period for the reaction. The time dependence of absorption spectra for sample reaction system (in the presence of iron (II)) is shown in Fig 1b. These spectra show the decrease in the reaction induction period (only 8 repeated scans overlay). Fig. 2 shows that an increase in iron (II) concentration causes a decrease in induction period of the reaction. Therefore,



**Figure 1.** Absorption spectra of reaction system. Conditions:  $\text{SCN}^-$ ,  $1.2 \mu\text{g mL}^{-1}$ ;  $\text{HCl}$ ,  $0.050 \text{ M}$ ;  $\text{MCP}$ ,  $2.34 \times 10^{-5}$ ;  $\text{KBr}$ ,  $2.0 \times 10^{-3} \text{ M}$ ;  $\text{NaIO}_4$ ,  $3.0 \times 10^{-3} \text{ M}$  and temperature of  $20^\circ\text{C}$ . (a) In the absence of  $\text{Fe(II)}$  and (b) in the presence of  $0.20 \mu\text{g mL}^{-1}$   $\text{Fe(II)}$ . Spectra 1 to 15 show the repeat scans with scan time intervals of 15 s.



**Figure 2.** Absorbance change profile. Conditions: same as Fig. 1 with  $\text{Fe(II)}$  concentrations of (a) 0.00, (b) 0.030, (c) 0.20, (d) 1.0, (e)  $3.0 \mu\text{g mL}^{-1}$ .

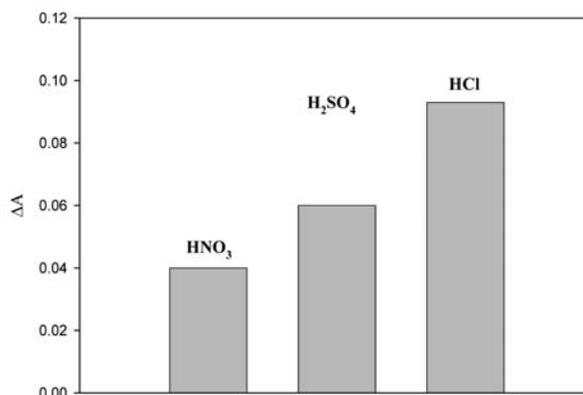
the inverse of induction period ( $1/t_{ip}$ ), evaluated from absorption-time curves of the reaction mixture at  $\lambda_{\text{max}} = 525 \text{ nm}$  was used as an analytical signal in the determination of iron (II).

### 3. 2. Optimization of Variables

The conditions for the determination of iron (II) were optimized by studying the influences of the various parameters such as reagents concentrations, temperature and ionic strength in the presence of  $0.20 \mu\text{g mL}^{-1}$  of iron (II) standard solution. The difference between absorbance changes of sample and blank reaction ( $\Delta A = \Delta A_s - \Delta A_b$ ) during a fixed time of 15–235 s after the initiation of the reaction was measured. This measurement was repeated three times and the mean value of absorbance change was used as analytical signal in the one-at-a-time optimization procedure.

From the preliminary examination, it was found that kinetic reaction occurred in acidic medium. Some efforts

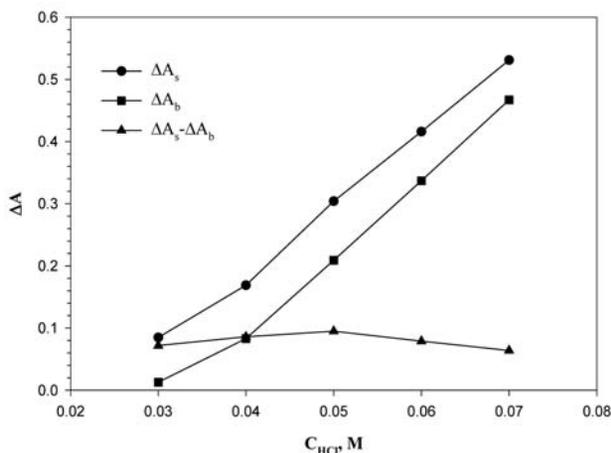
were made for selection of the best acid as reaction medium. Sulfuric, nitric, and hydrochloric acid were tested. The results in Fig. 3 show that hydrochloric acid has a better sensitivity and so it was chosen as the reaction medium.



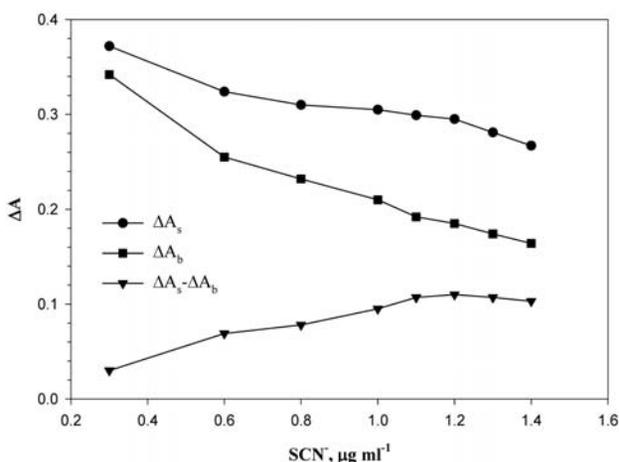
**Figure 3.** Effect of acid type. Conditions:  $\text{Fe(II)}$ ,  $0.20 \mu\text{g mL}^{-1}$ ;  $\text{SCN}^-$ ,  $1.0 \mu\text{g mL}^{-1}$ ;  $\text{MCP}$ ,  $2.6 \times 10^{-5}$ ;  $\text{KBr}$ ,  $2.0 \times 10^{-3} \text{ M}$ ;  $\text{NaIO}_4$ ,  $5.0 \times 10^{-3} \text{ M}$  and temperature of  $20^\circ\text{C}$ . All acids have the same concentration of  $0.050 \text{ M}$ .

The effect of hydrochloric acid concentration was examined over the ranges from 0.030 to 0.070 M. Fig. 4 shows that the absorbance changes of sample and blank mixtures increase rapidly with increasing in the hydrochloric acid concentration and analytical signal ( $\Delta A = \Delta A_s - \Delta A_b$ ) reaches to a maximum at 0.050 M and at the higher concentrations decreases. Therefore, the hydrochloric acid concentration of 0.050 M was used for the method.

The effect of thiocyanate concentration on the sensitivity was studied in the range of  $0.30$ – $1.4 \mu\text{g mL}^{-1}$  (Fig. 5). The results show that the sample and blank reaction rates decrease with increasing in thiocyanate concentration (due to inhibitory effect of thiocyanate) and the net reaction rate reaches to a maximum at  $1.2 \mu\text{g mL}^{-1}$  of thiocyanate, whereas greater amounts of thiocyanate decrease the



**Figure 4.** Effect of HCl concentration. Conditions: Fe(II), 0.20  $\mu\text{g mL}^{-1}$ ;  $\text{SCN}^-$ , 1.0  $\mu\text{g mL}^{-1}$ ; MCP,  $2.6 \times 10^{-5}$  M; KBr,  $2.0 \times 10^{-3}$  M,  $\text{NaIO}_4$ ,  $5.0 \times 10^{-3}$  M and temperature of 20 °C.

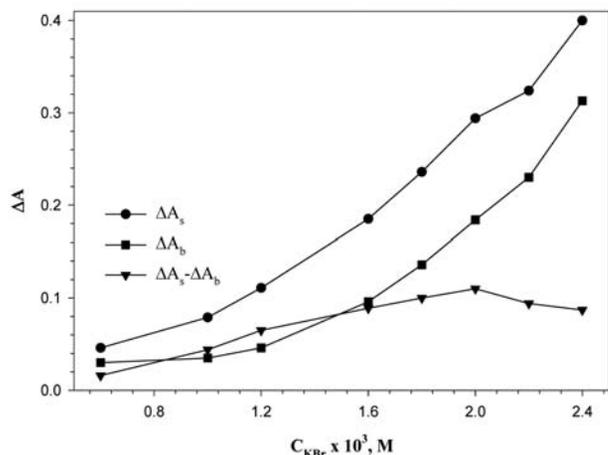


**Figure 5.** Effect of  $\text{SCN}^-$  concentration. Conditions: Fe(II), 0.20  $\mu\text{g mL}^{-1}$ ; HCl, 0.050 M; MCP,  $2.6 \times 10^{-6}$  M; KBr,  $2.0 \times 10^{-3}$  M,  $\text{NaIO}_4$ ,  $5.0 \times 10^{-3}$  M and temperature of 20 °C.

sensitivity slightly. Therefore, a thiocyanate concentration of 1.2  $\mu\text{g mL}^{-1}$  was selected for the study.

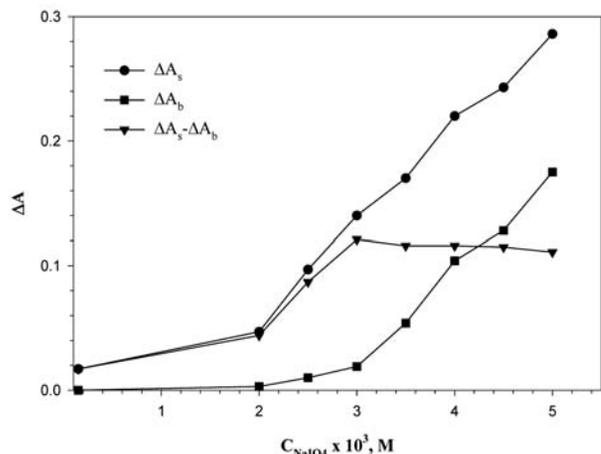
The dependence of sensitivity of the method on the potassium bromide concentration was studied in the range of  $6.0 \times 10^{-4}$  to  $2.4 \times 10^{-3}$  M bromide under the optimum concentration of hydrochloric acid and thiocyanate. Fig. 6 shows that both  $\Delta A_s$  and  $\Delta A_b$  increase with increasing KBr concentration and their difference reaches a maximum value at  $2.0 \times 10^{-3}$  M. The increases of  $\Delta A_s$  and  $\Delta A_b$  with increasing KBr concentration can be attributed to the catalytic effect of bromide ions. According to the results, the KBr concentration of  $2.0 \times 10^{-3}$  M was chosen as the best concentration for further studies.

The effect of sodium periodate concentration on the reaction rate was studied in the range  $1.5 \times 10^{-4}$  to  $5.0 \times 10^{-3}$ . As can be seen from the results (Fig. 7) the net reaction rate increases with sodium periodate concentration up



**Figure 6.** Effect of KBr concentration. Conditions: Fe(II), 0.20  $\mu\text{g mL}^{-1}$ ;  $\text{SCN}^-$ , 1.2  $\mu\text{g mL}^{-1}$ ; HCl, 0.050 M; MCP,  $2.6 \times 10^{-6}$  M;  $\text{NaIO}_4$ ,  $5.0 \times 10^{-3}$  M and temperature of 20 °C.

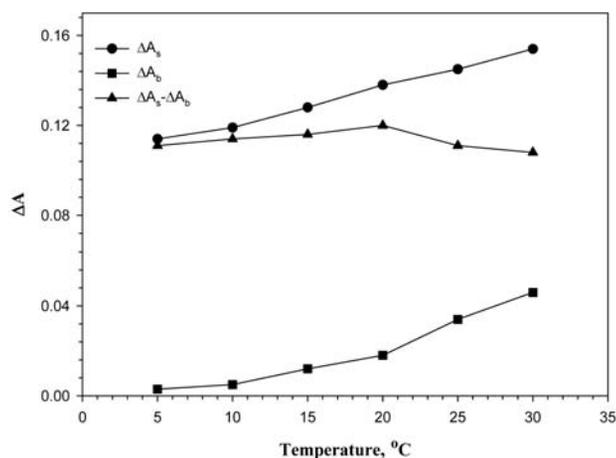
to  $3.0 \times 10^{-3}$  M, whereas the reaction rate decreases slightly with increasing sodium periodate concentration from  $3.0 \times 10^{-3}$  M to greater values. Thus the sodium periodate concentration of  $3.0 \times 10^{-3}$  was used for the study.



**Figure 7.** Effect of  $\text{NaIO}_4$  concentration. Conditions: Fe(II), 0.20  $\mu\text{g mL}^{-1}$ ;  $\text{SCN}^-$ , 1.2  $\mu\text{g mL}^{-1}$ ; HCl, 0.050 M; MCP,  $2.6 \times 10^{-6}$  M; KBr,  $2.0 \times 10^{-3}$  M and temperature of 20 °C.

The influence of MCP concentration on the sensitivity in the range of  $(1.6\text{--}2.9) \times 10^{-5}$  M was investigated. The results show that by varying MCP concentration over the studied range no considerable changes in the rates of blank and sample reactions were observed and thus the net reaction rate ( $\Delta A = \Delta A_s - \Delta A_b$ ) is nearly constant over the studied concentration range of MCP. This means that MCP not involved in the rate-determining step of reaction mechanism. Thus, with considering sensitivity and a reasonable absorbance at 525 nm, a  $2.35 \times 10^{-5}$  M of MCP was selected for further study.

The influence of reaction temperature on the sensitivity was studied in the range 5–30 °C with the optimum va-

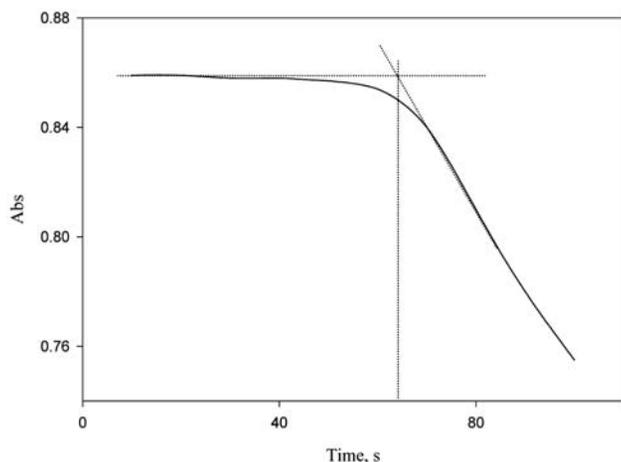


**Figure 8.** Effect of temperature. Conditions:  $\text{SCN}^-$ ,  $1.2 \mu\text{g mL}^{-1}$ ;  $\text{HCl}$ ,  $0.050 \text{ M}$ ;  $\text{MCP}$ ,  $2.34 \times 10^{-5}$ ;  $\text{KBr}$ ,  $2.0 \times 10^{-3} \text{ M}$  and  $\text{NaIO}_4$ ,  $3.0 \times 10^{-3} \text{ M}$ .

lues of reagents concentration. The results (Fig. 8) showed that by increasing temperature up to  $20^\circ\text{C}$ , the net reaction rate increases, whereas higher temperature values decrease the sensitivity. This is due to this fact that the rate of blank (without  $\text{Fe(II)}$ ) reaction increases with temperature to a greater extent than the sample (activated by  $\text{Fe(II)}$ ) reaction in higher temperature than  $20^\circ\text{C}$ . Thus, the difference between  $\Delta A_s$  and  $\Delta A_b$  diminishes at higher temperatures. Therefore, a temperature of  $20^\circ\text{C}$  was used in this study.

### 3. 3. Calibration Graph, Detection Limit, Reproducibility and Accuracy

Under the optimum conditions described above the absorbance-time curves were recorded for a series of iron (II) standard solutions over an interval times of 15–235 s after the initiation of the reaction. The reaction induction period was measured by graphical method as illustrated in Fig. 9. The results show that there is a linear relationship



**Figure 9.** Graphical estimation of reaction induction period.

between inverse of reaction induction time and iron (II) concentration in the range of  $0.020\text{--}4.0 \mu\text{g mL}^{-1}$ . The regression equation was  $(1/t_{ip} = 0.0143C_{\text{Fe(II)}} + 0.0054)$  with a correlation coefficient of 0.9992 ( $n = 8$ ), where  $t_{ip}$  is induction time in second and  $C_{\text{Fe(II)}}$  is iron (II) concentration in  $\mu\text{g mL}^{-1}$ .

The limit of detection was given by  $\text{LOD} = \text{KS}_b/m$ , where  $K$  is a numerical factor chosen according to the confidence level desired,  $S_b$  is the standard deviation of the blank measurements and  $m$  is the slope of calibration curve. With ten replicate measurements of blank and  $K = 3$ , a  $0.017 \mu\text{g mL}^{-1}$  detection limit was calculated.

In order to examine the accuracy and precision of the method, standard solutions of  $0.030$ ,  $0.20$  and  $2.0 \mu\text{g mL}^{-1}$  of iron (II) were analyzed using recommended procedure. Ten replicate determination of each concentration gave relative standard deviation (RSD) of 2.9%, 2.7% and 2.3%, respectively. The results show that there is not any systematic error in the proposed method and thus indicates its reliability.

### 3. 4. Selectivity

The interference effect of common ions and compounds in the determination of  $0.20 \mu\text{g mL}^{-1}$  iron (II) was investigated. Synthetic mixture of solution containing  $0.20 \mu\text{g mL}^{-1}$  iron (II) and various excess amounts of diverse compounds or ions were analyzed. The tolerance limit was defined as the concentration of the added ions causing an error in the induction period (analytical signal) more than  $\pm 5\%$ . The results are summarized in Table 1. The results show that most cations and anions did not interfere even at high concentration. Interference of  $\text{I}^-$  and  $\text{Fe}^{3+}$  were observed because they could inhibit and enhance the indicator reaction, respectively. EDTA did not interfere up to  $800 \mu\text{g mL}^{-1}$ , but the presence of EDTA reduces the interference effect of iron (III) (up to  $20 \mu\text{g mL}^{-1}$ ). Therefore, in the presence of  $800 \mu\text{g mL}^{-1}$  of EDTA (as masking agent for  $\text{Fe(III)}$ ), it is possible to selective determine of iron (II) in the presence of relatively large amounts of iron (III) (up to  $20 \mu\text{g mL}^{-1}$ ).

**Table 1.** Interferences for the determination of iron(II) ( $0.20 \text{ g mL}^{-1}$ ).

Foreign species	Tolerated concentration ( $\mu\text{g mL}^{-1}$ )
$\text{Na}^+$ , $\text{K}^+$ , $\text{Li}^+$ , $\text{Sr}^{2+}$ , $\text{Ba}^{2+}$ , $\text{Ca}^{2+}$ , $\text{Al}^{3+}$ , $\text{Cu}^{2+}$ , $\text{Pb}^{2+}$ , $\text{Co}^{2+}$ , $\text{Mn}^{2+}$ , $\text{Ni}^{2+}$ , $\text{Cd}^{2+}$ , $\text{Zn}^{2+}$ , $\text{Cr}^{3+}$ , $\text{NO}_3^-$ , $\text{SO}_3^{2-}$ , $\text{SO}_4^{2-}$ , $\text{CO}_3^{2-}$ , $\text{C}_2\text{O}_4^{2-}$ , $\text{CH}_3\text{COO}^-$ , Glucose	200 <sup>a</sup>
$\text{Mg}^{2+}$ , $\text{PO}_4^{3-}$ and Fructose	160
$\text{F}^-$ , Sorbitol, Manitol	100
$\text{MoO}_4^{2-}$ , $\text{WO}_4^{2-}$	20
$\text{I}^-$ , $\text{Fe}^{3+}$	1

<sup>a</sup> Maximum concentration tested

### 3. 5. Analysis of Pharmaceutical Products

In order to test the accuracy of the developed method, the determination of Fe(II) in drug samples (tablets and oral drops) was performed. Pharmaceutical tablets were purchased from Rooz-Iran Co. with ferrous sulfate as active pharmaceutical ingredient (API). The sample solutions were prepared by dissolving each tablet in 10 mL of 4.0 M sulfuric acid, and heating in a water bath (70 °C) to completely dissolving. The solution was then filtrate with a filter paper (Whatman No 1), and the filtrate was diluted with water in a 1000-mL volumetric flask. 10.0 mL of the solution was diluted to 50.0 mL with water and 1.0 mL of the sample solution was used for the determination of Fe(II) by the standard addition method. For the oral iron drop pharmaceutical sample (from Exir Co., Iran), 1.25 mL of the sample was diluted with water in 1000-mL volumetric flask and then iron content in 1.0 mL of the sample solution was measured according to recommended procedure by standard addition method. Spikes of various concentrations of iron (II) and iron (III) were carried out in order to evaluate the validity of the proposed method. The results are summarized in Table 2. As it can be seen in Table 2, the recoveries of Fe(II) of the spiked samples were excellent. Comparison of the results obtained with certified values and those obtained by the already reported flow injection kinetic procedure<sup>36</sup> was carried out using t-test. The calculated t-values showed that at the 95% confidence level there was no systematic error in the proposed method. This confirms that the proposed method could be successfully applied for iron (II) determination in various drug samples containing iron.

### 4. Conclusion

The results presented clearly demonstrated that activation effect of iron (II) on the catalytic oxidation of MCP by periodate in the presence of thiocyanate can be used for the trace determination of iron (II). The results obtained

confirm that the spectrophotometric-kinetic method described in this paper, under adequate working conditions, could be successfully applied for selective iron (II) determination without requirement of sample cleanup and sophisticated instruments. The method was found to be accurate, reproducible, sensitive, and selective (free from iron (III) interference). Also the method is simple and can be performed with available and cheap chemicals. Therefore, the method could be proposed for pharmaceutical analyses.

### 5. Acknowledgment

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### 6. References

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Table 2: Analysis of pharmaceutical samples

Sample <sup>a</sup>	Added ( $\mu\text{g mL}^{-1}$ )	Found <sup>b</sup> ( $\mu\text{g mL}^{-1}$ )	Recovery (%)	Content in sample <sup>b,c</sup>	Certificate values <sup>c</sup>	FIA method <sup>c</sup> ref. 36
Tablet	–	0.99 (0.05)	–	49.5 (0.6)	50.0	48.5 (0.7)
	0.50	1.47 (0.07)	96.4			
	1.00	2.03 (0.07)	104			
	1.50	2.45 (0.08)	97.3			
Oral drop	–	1.02 (0.05)	–	8.2 (0.5)	8.0	8.3 (0.4)
	0.50	1.51 (0.08)	97.6			
	1.00	1.98 (0.07)	95.4			
	1.50	2.47 (0.08)	96.5			

<sup>a</sup> Samples with API of ferrous sulfate.

<sup>b</sup> Numbers in parentheses are standard deviations and were calculated from five replicate determinations.

<sup>c</sup> The values are in mg of iron (II) per tablet and concentration of iron (II) in  $\text{mg mL}^{-1}$  for oral drop samples.

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## Povzetek

Razvili smo novo, enostavno, občutljivo in selektivno spektrofotometrično metodo za določevanje Fe(II) v farmacevtskih proizvodih. Metoda temelji na vplivu Fe(II) v reakcijskem sistemu natrijev perjodat-kalijev bromid-meta-krezolsulfonftalein, v prisotnosti tiocianata. Za reakcijo je značilna indukcijska doba, ki je krajša v prisotnosti Fe(II). Recipročna vrednost indukcijske dobe je sorazmerna koncentraciji Fe(II), razbarvanje meta-krezolsulfonftaleina pa merimo spektrofotometrično pri 525 nm.

V optimalnih pogojih lahko z novo metodo določujemo Fe(II) v koncentracijskem območju 0,020–4,0  $\mu\text{g mL}^{-1}$  s spodnjo mejo detekcije 0,017  $\mu\text{g mL}^{-1}$ . Pri petkratni ponovitvi določitve koncentracij Fe(II) 0,03, 0,2 in 2,0  $\mu\text{g mL}^{-1}$  smo ugotovili relativne standardne odklone meritev 2,9%, 2,7% in 2,3%. Metodo smo uspešno uporabili za določevanje Fe(II) v farmacevtskih izdelkih, ki vsebujejo železov (II) sulfat.