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Determination of Iron(II) in Pharmaceuticals Based on Its Catalytic Effect on the Bromate–Crystal violet Reaction by Spectrophotometric Detection

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Abstract

A simple, selective, and sensitive kinetic spectrophotometric method with no need for removing iron(III) interference is proposed for determination of iron(II) in pharmaceutical and water samples. This method is based upon the catalytic effect of iron(II) on the sodium bromate–crystal violet system in acidic media. Decolourization of crystal violet was used to monitor the reaction spectrophotometrically at 630 nm. The variables affecting the reaction rate were investigated. Under the optimum conditions, iron(II) can be determined in the range of 0.10 – 3.0 μg mL^{-1} with a 3σ detection limit of 0.025 μg mL^{-1}. The relative standard deviations for ten replicate determinations of 0.50, 1.0, and 1.5μg mL^{-1} were 1.94%, 0.43%, and 0.28%, respectively. The influence of various foreign species was studied and it was found that without addition of a masking agent, Fe(III) did not interfere with the Fe(II) determination up to 50-fold concentration of this ion. This method could be used successfully for determination of the iron(II) content of spiked water and pharmaceutical samples.

Keywords:

Iron(II); Crystal violet; Kinetic, Spectrophotometric

1. Introduction

Because of the different biological roles of iron in humans, animals, plants, and oceans, the need for analysis of iron in environmental and biomedical materials have been increased. Iron deficiency anemia is one of the world's most common nutritional deficiency diseases. Evidence has been presented that at low levels iron is an essential element in the diet, whereas at higher concentrations it is toxic [1].

A number of analytical methods for quantitative analysis of iron have been developed. These methods include spectrophotometry [2, 4] flourimetry [5], flow-injection analysis [6, 8], voltammetry [9, 10], chemiluminescence [7, 11], capillary electrophoresis [12], atomic emission and atomic absorption spectrometry [4, 13-15], and chromatography [16-17]. Although some of these methods are highly sensitive, they have disadvantages such as the necessity for expensive and sophisticated instrumentation and can only be used to determine iron(III) and/or total iron content.

Kinetic methods based on catalytic reactions have been applied for the determination of trace amounts of iron. Such methods have the general advantage of combining high sensitivity with relatively simple procedures and apparatus. Several kinetic methods based on the catalytic action of iron have been reported in several reviews [18-21] and original papers

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[6, 22, 23]. Most of these methods can be used to determine Fe(III). Therefore, methods for selective determination of iron (II) are necessary.

In this paper, the authors would like to develop a selective, accurate, and simple spectrophotometric method for the determination of iron(II) based on the catalytic oxidation of crystal violet with bromate in real matrixes such as pharmaceutical products and water samples.

2. Experimental

2.1. Reagents and chemicals

All solutions were prepared from analytical grade chemicals and double distilled water. A stock solution containing 1000 μg mL^{-1} of Fe(II) was prepared by dissolving 0.3516 g of $Fe(NH_4)_2(SO_4)_2\ 6H_2O$ (Merck) in a 50-mL volumetric flask. Stock sodium bromate solution of 0.030 mol L^{-1} was prepared by dissolving 0.4527 g $NaBrO_3$ (Merck) in a 100-mL volumetric flask. 0.0500 g crystal violet (Merck) was dissolved in water and diluted to mark in a 250-mL volumetric flask (4.9×10^{-4} mol L^{-1}). A 2.0 mol L^{-1} HCl solution was prepared by diluting the appropriate volume of concentrated hydrochloric acid (Merck) and standardized against sodium carbonate. A mixture of crystal violet and sodium bromate with known concentration was prepared by diluting the appropriate volumes of their stock solutions. Solutions of the interfering ions to be studied were prepared from their appropriate salts.

2.2. Apparatus

To record the UV-visible spectra, a Shimadzu UV-160 spectrophotometer with a 1.0 cm quartz cell pairs was used. The absorbance measurements were made at a fixed wavelength using a Milton Roy spectronic $20D^+$ with a 1.0 cm glass cell. A water bath thermostat (n-BIOTEK, INC, model NB-301) was used to control the reaction temperature. A stopwatch was used for recording the reaction time.

2.3. Procedure

The reagent solutions and water were kept at 20.0 ºC in the thermostatic water bath for 30 min. 1.0 mL of hydrochloric acid (2.0 mol L^{-1}) and an appropriate volume of sample or standard solution containing 1.0-30.0 μg iron(II) were added to a 10-mL volumetric flask and diluted with water to about 8 mL. Then 1.0 mL of crystal violet (2.5×10^{-4} mol L^{-1}) and sodium bromate (0.013 mol L^{-1}) mixed solution was added and the stopped clock was started. The solution was diluted to the mark with distilled water, then mixed and a portion of the reaction mixture was transferred into a spectrophotometer cell. The absorbance change at 630 nm was measured at 30 and 240 s from the initiation of the reaction (ΔA_s). A blank solution (without iron) was prepared and measured in a similar way (ΔA_b). The difference between absorbance changes for the catalyzed and uncatalyzed reactions ($\Delta A=\Delta A_s-\Delta A_b$) was used as analytical signal.

3. Results and discussion

Crystal violet is a dye that has been used as an indicator [24, 25] and reagent [26-28] in analytical chemistry. The reaction of crystal violet with sodium bromate in acidic medium at room temperature is slow, but the reaction rate sharply increases by addition of trace amounts of Fe(II). The reaction was monitored spectrophotometrically by measuring the decrease in absorbance at 630 nm *vs.* time (Fig 1). The influence of different parameters on the reaction rate was studied in the presence and absence of Fe(II) for choosing the optimum

conditions. The system was optimized by one-at-a-time procedure. Based on this method of optimization, each variable was altered in turn, while the others were kept constant.

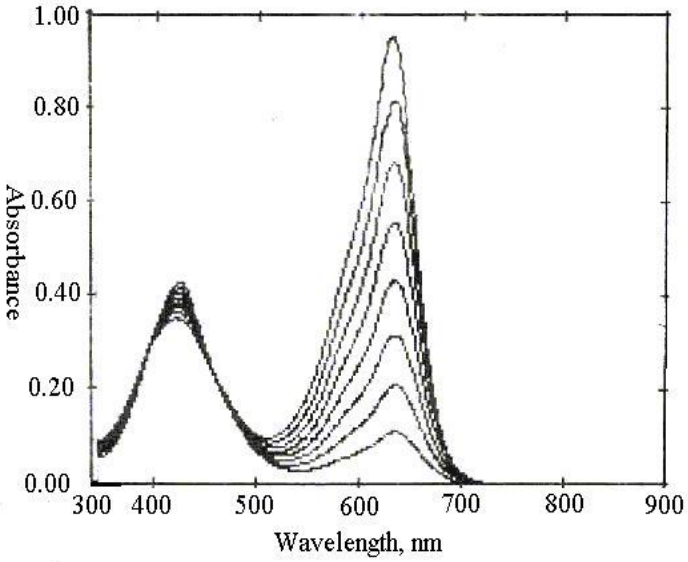

Fig. 1. Absorption spectra of reaction system.

Conditions: HCl (0.20 mol L^{-1}); $NaBrO_3$ (1.3×10^{-3} mol L^{-1}); crystal violet (2.5×10^{-5} M); Fe(II) (2.0 μg mL^{-1}), and temperature of 20 ºC with scan time intervals of 30 s.

Optimization of parameters was carried out at 630 nm by measuring the difference between absorbance changes for sample and blank at 30 and 240 s after initiation of reactions. Preliminary experiments were carried out for choosing the best type of acid. Solutions of the same acidity were tested using hydrochloric, nitric and sulfuric acid. Results obtained showed that hydrochloric acid had the best sensitivity, thus it was selected as the best reaction medium.

The effect of hydrochloric acid on the sensitivity of the proposed method was investigated while keeping the other conditions constant (Fig. 2). The results showed that with increase in hydrochloric acid concentration, ΔA_s and ΔA_b increased due to increase in the oxidation ability of bromate. Also the net analytical signal (ΔA) increased and reached a maximum value at 0.20 mol L^{-1} hydrochloric acid, and above that it decreased slightly. Thus the concentration of 0.20 M was selected for hydrochloric acid as the optimum concentration.

Dependence of sensitivity of the method on the sodium bromate concentration was investigated in the range of 2.0×10^{-4} to 1.5×10^{-3} mol L^{-1} bromate at 20 ºC. Fig. 3 shows that both ΔA_s and ΔA_b increase with increase in the sodium bromate concentration and that analytical signal (ΔA) reaches a maximum value at 1.2×10^{-3} mol L^{-1}, and at a higher concentration it is constant. The increase in both ΔA_s and ΔA_b is due to this fact that with increase in bromate concentration, the oxidation ability of bromate increases. According to the results, the sodium bromate concentration of 1.3×10^{-3} mol L^{-1} was chosen as the best concentration for further studies.

Optimization of the crystal violet concentration in the range of 1.5×10^{-5}-2.7×10^{-5} mol L^{-1} was performed under the optimum concentration of hydrochloric acid and sodium bromate. According to the results (Fig. 4), ΔA_s and ΔA_b increase with increase in the crystal violet concentration, and sensitivity increases up to 2.2×10^{-5} mol L^{-1} and then it remains constant. Therefore, crystal violet concentration of 2.5×10^{-5} mol L^{-1} was selected for further studies.

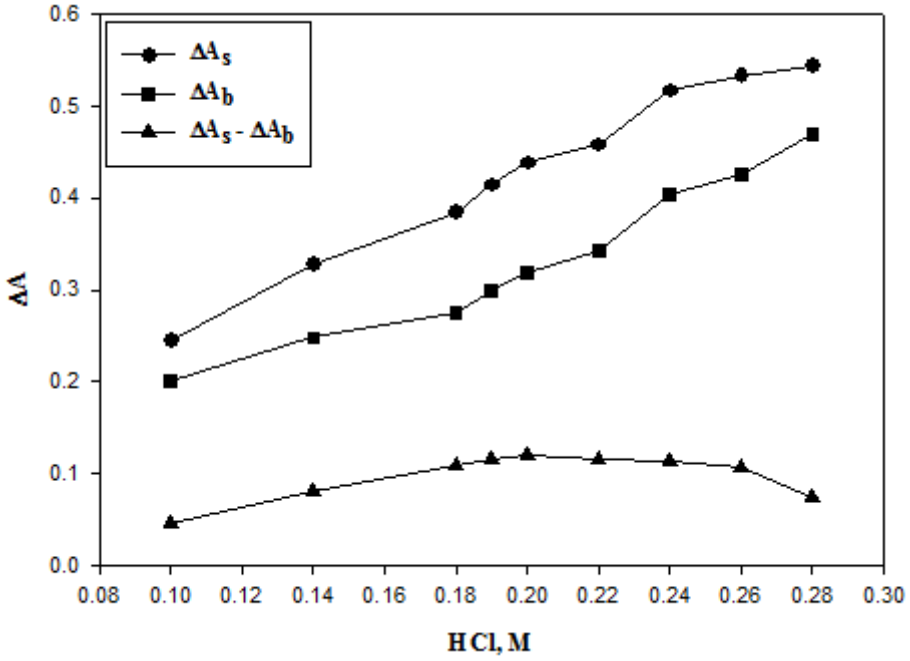

Fig.2. Effect of HCl concentration.

Conditions: $NaBrO_3$ (5.0×10^{-4} mol L^{-1}); crystal violet (2.5×10^{-5} mol L^{-1}); Fe(II) (2.0 µg mL^{-1}) at 20ºC.

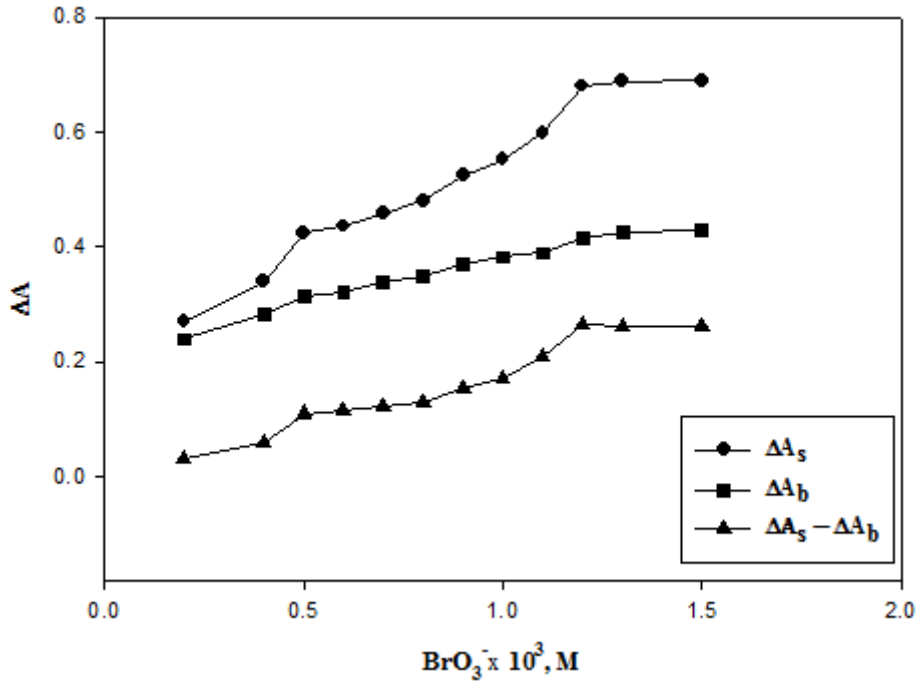

Fig. 3. Effect of sodium bromate concentration.

Conditions: HCl (0.20 mol L^{-1}); crystal violet (2.5×10^{-5} mol L^{-1}); Fe(II) (2.0 µg mL^{-1}) at 20 ºC.

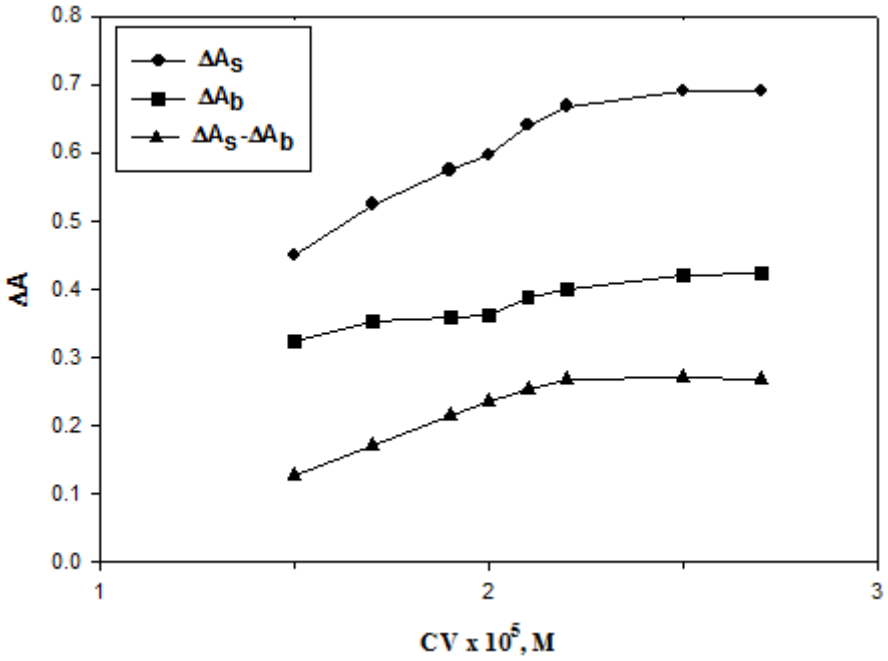

Fig. 4. Effect of crystal violet concentration

Conditions: HCl (0.20 mol L^{-1}); $NaBrO_3$ (1.3×10^{-3} mol L^{-1}), Fe(II) (2.0 µg mL^{-1}) at 20 ºC.

The effect of temperature on the rates of the catalyzed and uncatalyzed reactions was studied between 5 and 40 ºC at the optimum reagent concentrations. As the reaction temperature increases, the rates of both sample and blank reactions increase. However, the effect was more pronounced for the latter. The results showed that 20 ºC was the optimum temperature. Since this temperature provided a good reaction rate and could easily be maintained, thus reaction temperature of 20 ºC was used throughout the study.

The influence of measuring time on the analytical signal was studied. The results showed that the sensitivity increased up to 240 s, and above this it decreased. Thus the sample and blank signals were measured over the time interval of 30-240 s after the beginning of the reaction.

The effect of ionic strength was studied using potassium nitrate. The results showed that sensitivity of the method decreased slightly with increase in the ionic strength.

Based on the optimization results using the one-at-a-time optimization approach, the most suitable reaction conditions proved to be: λ=630 nm; temperature of 20 ºC; 0.20 M hydrochloric acid, 1.3×10^{-3} M sodium bromate and 2.5×10^{-5} M crystal violet.

3.1. Calibration graph and reproducibility

Under the optimum conditions cited above, a liner calibration graph was obtained in the range of 0.10-3.0 µg mL^{-1} Fe(II) using "fixed time method". The equation of the calibration graph is $\Delta A = -0.0044+0.1285\ C_{Fe(II)}$, where $C_{Fe(II)}$ is the concentration of iron(II) expressed in µg mL^{-1}. The correlation coefficient of the graph was 0.9992 (n=8). The relative standard deviation for ten replicate determinations of 0.50, 1.0 and 1.5 µg mL^{-1} was 1.94%, 0.43% and 0.28%, respectively. The experimental 3σ limit of detection is 0.025 µg mL^{-1}.

3.2. Effect of foreign ions

To study the selectivity of the proposed method, the effect of diverse ions on the determination of Fe(II) was investigated by adding a known quantity of the desired ion to a solution containing 20 µg/10mL of Fe(II) which was determined as described in the given procedure. The tolerance limit was defined as the concentration at which the species caused an error of less than ±5%. The results obtained are summarized in Table 1. Based on the results, most cations and anions did not interfere with the Fe(II) determination even in their presence in the amounts 1000-fold greater than Fe(II). In the absence of a masking agent, Fe(III) did not interfere with the Fe(II) determination up to 50-fold concentration of this ion. Interferences caused by Cr^{3+}, Hg^{2+}, and Fe^{3+} could be masked with EDTA (100 µg mL^{-1}) up to 100-, 30-, and 80-fold, respectively. An advantage of this method is low interference effect of Fe(III) on the determination of Fe(II). The results clearly confirm selectivity of the method.

Table 1: Tolerance limit of interfering ions in the determination of 2.0 µg mL^{-1} of Fe(II)

Ion	interferent– to– analyte ratio (w/w)
Na^{+}, K^{+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Mn^{2+}, Al^{3+}, Li^{+}, Co^{2+}, Pt^{2+}, Au^{3+}, SO_4^{2-}, Pd^{2+}, Cu^{2+}	1000[a]
Sr^{2+}	400
CN^{-}	300
EDTA	100
Fe^{3+}	50
I^{-}, Cr^{3+}, Hg^{2+}	2

[a]. Maximum ratio tested

3.3. Application

To confirm the usefulness of the proposed method, Fe(II) was determined in spiked water and pharmaceutical samples. The tap water sample was obtained from a local pipeline. The results were tabulated in Table 2. The analytical results obtained by the proposed method are in good agreement with the amounts of Fe(II) added.

The tablets and oral drops of ferrous sulfate were used for determination of iron(II) in pharmaceutical samples. Three tablets were accurately weighted and milled in a mortar. Exactly one–third of the powdered sample was weighted and transferred into a beaker and then 10.0 ml H_2SO_4 (4.0 mol L^{-1}) was added and the beaker was subsequently heated in a water bath (70 ^{o}C) to completely dissolve the solute in the solvent. The solution obtained was filtered into a 1000-mL volumetric flask through a filter paper (Whatman No. 1) and diluted to mark with distilled water. 10.0 mL of the resulting solution was diluted to mark in a 100-mL volumetric flask with water and 1.0 mL of this solution was used for the determination of Fe(II) by the standard addition method. For the oral iron drop pharmaceutical sample, 5.0 mL of the sample was diluted to mark with water in a 500-mL volumetric flask. 10.0 mL of the solution was diluted ten-fold with water and 1.0 mL of this solution was used to determine Fe(II) by the standard addition method. The results were summarized in Table 3. As it can be seen in this table, the recovery values for the spiked samples are excellent, and therefore the proposed method could be successfully applied for determination of iron(II) in drug samples containing iron.

Table 2: Recovery values for iron(II) determination in water samples.

Sample No	Sample	Spiked ($\mu g\ mL^{-1}$)	Found[a] ($\mu g\ mL^{-1}$)	Recovery (%)
1	Tap water	1.00	1.01 ± 0.023	101
2	Tap water	1.50	1.53 ± 0.057	102

[a] mean ± S.D. (n= 5)

4. Conclusion

A new reaction system was suggested for the kinetic spectrophotometric determination of iron(II) in pharmaceutical samples. This method offers several advantages, as follow: high selectivity and sensitivity, ease of operation and rapidity, and cheaper reagents. The reliability and simplicity of this method permit the analysis of pharmaceutical samples with satisfactory results.

Table 3: Analysis of pharmaceutical products by the proposed method.

Sample	Added ($\mu g\ mL^{-1}$)	Found[a] ($\mu g\ mL^{-1}$)	Recovery (%)	Taken (mg/tablet)	Found[b]
Ferrous sulfate tablet	---	0.508 ± 0.025	---	50.0	50.8
	0.100	0.604 ± 0.028	96.0	50.0	50.4
	0.300	0.782 ± 0.019	91.3	50.0	48.2
Oral drop	---	0.792 ± 0.01	---	40.0	39.6
	0.100	0.906± 0.016	114	40.0	40.3
	0.300	1.090±0.020	98.4	40.0	39.4

[a] mean ± S.D. (n = 5).
[b] mg Fe(II) per tablet or mg Fe(II) in 5.0 mL of oral drop.

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