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# Kinetic Spectrophotometric Determination of Iron(II) Based on Its Inhibitory Effect on the Oxidation of Methylthymol Blue by Bromate

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A simple and sensitive kinetic-spectrophotometric method has been described for the determination of trace amounts of iron(II). The proposed method is based on the inhibitory effect of iron(II) on the oxidation of methylthymol blue by bromate in the sulfuric acid media and at 35ºC, which was monitored at 440 nm. The variables affecting the rate of the reaction were investigated and the optimum conditions were established. Iron(II) can be measured in the range of 1.0-200.0 ng/mL with a detection limit of 0.6 ng/mL. The relative standard deviations for ten replicate analysis of 50 and 160 ng/mL of Fe(II) were obtained 1.2% and 2.0 %, respectively. The method was found to be relatively selective in the presence of foreign ions. The developed method was successfully applied to the determination of iron(II) in pharmaceutical products and real water samples.

**Key Words: Iron, Methylthymol blue, Inhibitory effect, Spectrophotometry.**

## INTRODUCTION

Iron is the most important transition element involved in living systems, being vital to both plants and animals[1]. The adult human body contains about 4 g of iron, of which about 3 g are in the form of hemoglobin and this level is maintained by absorbing a mere 1 mg of iron per day. Proteins involving iron have two major functions: a) oxygen transport and storage; b) electron transfer[2]. Iron also plays a central role in the biospher, as an active center of proteins responsible for dioxygen and electron transfer by oxidase, reductase and dehydrase enzymes. Iron(II) probably the preferred nutrient for phytoplankton[3]. On the other hand, Fe(II) is important in the transport and storage of oxygen in higher animals by means of hemoglobine and myoglobine[4].

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Several methods have been used for the determination of iron, including spectrometry[5,6], voltammetry and polarogeraphy[7,8], ion selective electrode[9], neutron activation analysis[10], atomic absorption spectroscopy[11,12], inductively coupled plasma[13], flow and sequential injection analysis[14,15] and chromatography[16]. In spectrophotometric methods, reagent such as 1,10-phenanthroline, bathophenenthroline, 5-nitro-6-amino-1,10-phenanthroline and ferrozine were used for the selective determination of iron(II)[17-19]. Kinetic methods have progressed because of its extremely high sensitivity, selectivity and low detection limit. Kinetic-catalytic methods have been reported for determination of iron[20-24], but, there is no report from use of the inhibition effect of iron on the kinetic reactions in its determination.

In this work, a procedure proposed for determination of iron(II) using its inhibitory effect on the oxidation of methylthymol blue (MTB) by potassium bromate in sulfuric acid media at 35ºC. The reaction was monitored spectrophotometrically at wavelength of maximum absorbance of the methylthymol blue (440 nm) while measuring the change in the absorbance was carried with time. The proposed method shows a low detection limit and a wide linear range.

## EXPERIMENTAL

A 1000 µg/mL stock solution of iron(II) was prepared by dissolving 0.7010 g of $FeSO_4\cdot(NH_4)_2SO_4\cdot9H_2O$ (Fluka) in 100 mL of distilled water and 1 to 2 mL of conc. $H_2SO_4$ and standardized by potassium dichromate. Potassium bromate solution (0.4 M) was prepared by dissolving 6.6808 g of $KBrO_3$ (Merck) in water in a 100 mL volumetric flask. Stock solution of methylthymol blue (MTB) ($1.0 \times 10^{-3}$ M) was prepared by dissolving 0.0845 g methylthymol blue (Merck) in distilled water and diluting it to 100 mL. Sulfuric acid solution (1 M) was prepared by diluting the appropriate volume of concentrated acid (Merck) with water. Stock solutions (1000 mg/mL) of interfering ions were prepared by dissolving appropriate amounts of their suitable salts in water. Doubly distilled water was used throughout the study.

An UV-Vis spectrophotometer (Carry 100) with 1 cm cells was used for absorption spectra and absorbance measurements at a fixed wavelength. A thermostated bath (HAAKE-F3) was used to keep the reaction temperature at 35ºC. A stopwatch was used for recording the reaction time.

**Recommended procedure:** The temperature of the solutions was controlled at 35ºC in a thermostate bath. 2 mL of sulfuric acid (0.2 M), 1 mL of MTB ($1.0 \times 10^{-3}$ M) and suitable amounts of Fe(II) ion (1.0-200.0 ng/mL) transferred into a 10 mL volumetric flask. The water was added to make the solution volume up to *ca.* 7 mL. Then 2.0 mL $KBrO_3$ (0.4 M) was added into the solution and the solution was diluted to the make with water. Time was measured just after the addition of the last drop of the bromate solution. After shaking, the solution was transferred into the 1 cm cell of the spectrophotometer at 35ºC within 30 s. The decrease of the absorbance of MTB ($\lambda_{max}$ = 440 nm) *vs.* time was recorded for a duration of 300 s from initiation of the reaction ($\Delta A_s$). The measurements were repeated in the absence of Fe(II) ions to obtain the values for the blank solution ($\Delta A_b$). The Savitzky-Golay convolution method was used to calculate the reaction rate and the smoothing the reaction rate[25].

## RESULTS AND DISCUSSION

It was found that bromate ion oxidizes methylthymol blue in the sulfuric acid media at 35ºC and MTB solution becomes colourless quickly. Iron(II) decreased the rate of the reaction of methylthymol blue with bromate ion. The rate of the reaction decreased with increasing of the amount of Fe(II) (Fig. 1). As can be seen from Fig. 1, the colour-fading occurs after a induction period and the rate of the colour-fading reaction was characterized by the slope of the linear part of the curve. The slope of linear part of the curve (the variations of absorbance of MTB solution with time) decreased with increasing of concentration of Fe(II).

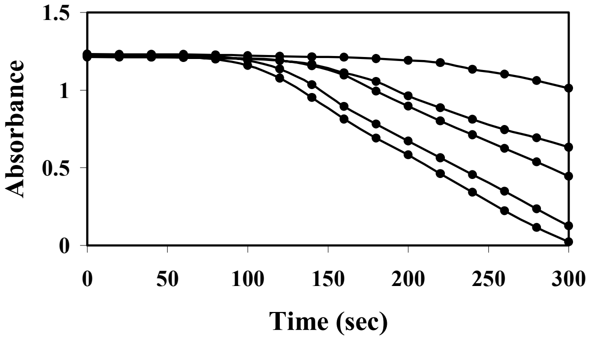

Fig. 1. Absorbance variations *vs.* time, conditions: MTB; 1 × $10^{-4}$ M, $BrO_3^-$; 0.08 M, $H_2SO_4$; 0.02 M and Fe(II); 1; 0, 2; 5, 3; 80, 4; 120, 5; 200 ng/mL

The bromate ion in acidic media produce $H_2BrO_3^+$ ion in a fast reaction. This ion is a strong oxidant that causes the MTB oxidized in a kinetic reaction and colour-fading of the MTB solution occurs in a period of time. In the presence of trace amounts of iron(II), the $H_2BrO_3^+$ ion reacts with $Fe^{2+}$ and therefore, the rate of reaction of $H_2BrO_3^+$ ion with MTB decreased. As a result, the iron(II) ion shows an inhibitory effect on the MTB-bromate ion reaction in sulfuric acid media[26].

**Optimization of the reaction variables:** To obtain the maximum sensitivity in the determination of iron(II), the effect of several variables investigated on the rate of uninhibited and inhibited reactions.

**Effect of sulfuric acid concentration:** A reaction between MTB and bromate ion proceeds only in a strong acidic media. The effects of different inorganic acids were studied on the rate of reactions. The results showed that the sulfuric acid was the best choice for the study. The concentration of sulfuric acid varied in the range of 0.005-0.03 M and the reaction rate was followed for uninhibited and inhibited reactions in different interval times. The results showed that the maximum sensitivity is obtained in 0.02 M $H_2SO_4$ with using a fixed-time 30-330 s (Fig. 2). The fixed-time 30-330 s after initiation of the reaction and 0.02 M sulfuric acid was selected for consideration of other variables. It is obvious, in redox reactions with bromate ion as an oxidant in acidic media, the concentration of acid affect on the rate of reaction. In above concentrations of 0.02 M $H_2SO_4$, the rate of reaction is same as inhibited reaction and therefore the net of sensitivity of the system decreased with increasing of sulfuric acid concentration.

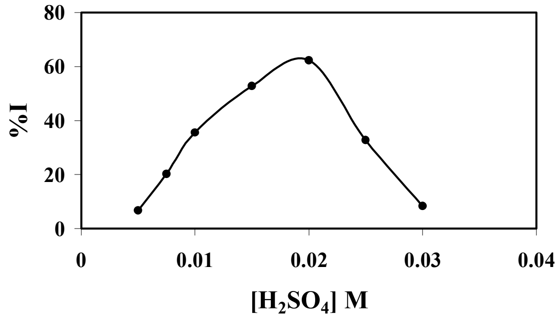

Fig. 2. Effect of sulfuric acid concentration on the inhibition (%) of iron(II) in the oxidation of MTB by bromate

**Effect of reagent's concentrations:** The effect of concentration of each reagent on the rates of uninihibited and inhibited reactions was studied. The sensitivity of the proposed system, increased with the increasing bromate concentration from 0.02 M up to 0.08 M and then decreased in higher concentrations (Fig. 3). Thus, 0.08 M $BrO_3^-$ was adopted for further studies. The presence of > 0.08 M bromate in blank and sample solution caused that the rate of sample reaction was same as the blank reaction and the net of signal decreased.

The influence of methylthymol blue concentration on the reaction rates was also studied. The results showed in Fig. 4. As seen from Fig. 4, an increase in the sensitivity of the reaction was seen with increasing in MTB concentration up to $1.0 \times 10^{-4}$ M. At higher concentration of $1.0 \times 10^{-4}$ M MTB, the rate of both reactions decreased. To establish precise conditions, this concentration ($1.0 \times 10^{-4}$ M) was chosen as the optimum concentration. The real limitation of Beer's law influence on the absorbance of solution at higher concentration of the reagent.

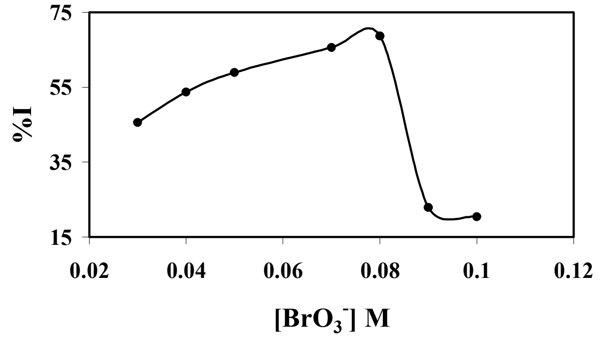

Fig. 3. Effect of $BrO_3^-$ concentration on the inhibition (%) of iron(II) in the oxidation of MTB by $BrO_3^-$

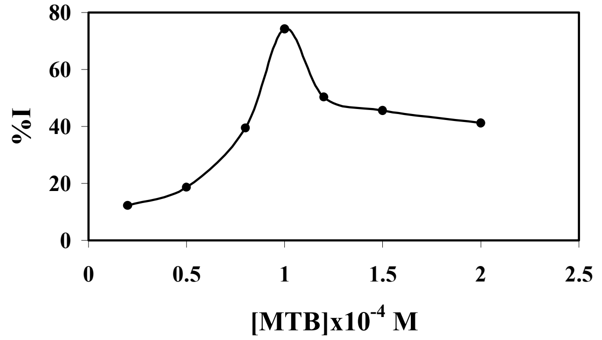

Fig. 4. Effect of Methylthymol Blue concentration on the inhibition (%) of iron(II) in the oxidation of MTB by $BrO_3^-$

**Effect of temperature:** A study of the influence of the temperature on the rate of the oxidation of MTB by $BrO_3^-$ in sulfuric acid media was made in the temperature of 15-45ºC. The results showed in Fig. 5. As seen, at > 35ºC, the inhibition effect of iron(II) is decreased, causing a increasing in the rate, thus 35ºC was used throughout the study.

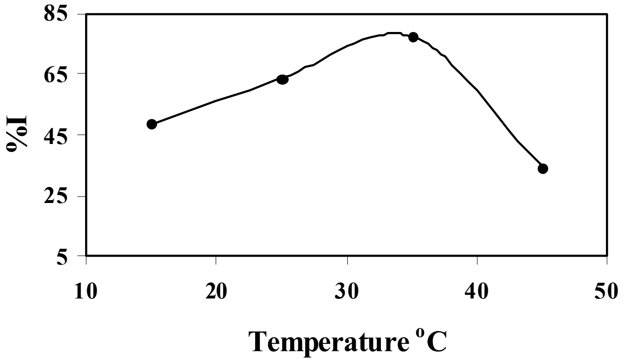

Fig. 5. Effect of temperature on the inhibition (%) of iron(II) in the oxidation of MTB by $BrO_3^-$

**Effect of ionic strength:** The effect of the ionic strength on the rate of reaction was studied by the addition of different concentration of $NaNO_3$. The results showed that the reaction rate decreased with increasing of the ionic strength of the solution.

**Calibration graphs and analytical figures of merit:** The method used to construct calibration plots in the kinetic determination of inhibitors, as described by Perez-Bendito, was commonly run by plotting the percentage inhibition as a function of the inhibitor concentration[27]. The percentage inhibition, (I %) was calculated from:

$I\ \% = 100\ (\Delta\nu)_{sample}/(\Delta\nu)_{blank}$,

where $(\Delta\nu)_{sample}$ and $(\Delta\nu)_{blank}$ denote the measured variable (initial rate, absorbance, *etc.*) for the inhibited reaction and uninihibited reaction, respectively.

Under the optimum conditions of all variables affected which reaction rate: $H_2SO_4$ 0.02 M, $BrO_3^-$ 0.08 M, MTB $1.0 \times 10^{-4}$ M and temperature 35ºC, a calibration plot is obtained in the concentration range 1.0-200.0 ng/mL iron(II). In the concentration range 1.0-200.0 ng/mL of $Fe^{2+}$, a regression equation, $I\ \% = 0.977 + 0.465C$, with a correlation coefficient of 0.9978 was obtained, where C is the ng/mL of iron(II).

The theoretical limit of the detection $LOD = KS_b/m$[28] was 0.6 ng/mL $Fe^{2+}$, where K = 3, $S_b$ is the standard deviation of the blank signals and m is the slope of the calibration plot[28]. The experimental limit of detection was 1.0 ng/mL iron(II).

A study of precision was made with ten replicate analysis of 50 and 160 ng/mL of Fe(II). The relative standard deviations were obtained 1.2 and 2.0 %, respectively.

**Interference study:** The influence of foreign ions was investigated on the determination of 50 ng/mL iron(II) under optimum conditions. The tolerance limit was defined as the maximum concentration of the foreign ion causing a relative error of less than 5 %. The results are summarized in Table-1. Many ions did not interefere, even when present in 100 to 1000 fold excess over Fe(II).

TABLE-1
EFFECT OF VARIOUS IONS ON THE DETERMINATION OF 50.0 ng/mL OF IRON(II) AT THE OPTIMUM CONDITIONS

| Foreign ion | Tolerance limit $W_{ion}/W_{Fe(II)}$ |
|---|---|
| $Li^+$, $Na^+$, $K^+$, $NH_4^+$, $Ca^{2+}$, $Mg^{2+}$, $Sr^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Al^{3+}$, $SO_3^{2-}$, $SO_4^{2-}$, $NO_3^-$, $CH_3COO^-$, $PO_4^{3-}$ | 1000 |
| $W^{6+}$, $Mo^{6+}$, $Ce^{3+}$, $Mn^{2+}$, $Co^{2+}$, $Ni^{2+}$ | 500 |
| $Ba^{2+}$, $Pb^{2+}$, $Cu^{2+}$, $Cr^{3+}$, $NO_2^-$, $C_2O_4^{2-}$, $Cl^-$, $Br^-$, $I^-$ | 100 |
| $V^{4+}$, $V^{5+}$, $Fe^{3+}$, $Ce^{4+}$, $Cr^{6+}$, $Hg^{2+}$, $Ag^+$ | 5 |

**Application:** In order to confirm the usefulness of the proposed method, it was applied to the determination of iron(II) in pharmaceutical products such as hematinic capsule, ferrous sulfate tablet and oral drop and under ground water samples (Table-2). The standard addition method is used for analysis of real samples. The spectrophotometric method with 1,10-phenanthroline was used as a standard method.

TABLE-2
DETERMINATION OF IRON(II) IN REAL SAMPLES

| Sample | Fe(II) added (ng/mL) | Fe(II) found (ng/mL) | Recovery (%) | RSD (%) (n = 5) | Standard method[c] Fe(II) (ng/mL) |
|---|---|---|---|---|---|
| [a]Hematinic capsule | – | $2.12 \times 10^3$ | – | 1.5 | $2.15 \times 10^3$ |
| Hematinic capsule | $1.00 \times 10^3$ | $3.08 \times 10^3$ | 96 | 1.4 | $3.10 \times 10^3$ |
| [b]Ferrosulfate tablet | – | $0.94 \times 10^3$ | – | 1.4 | $0.98 \times 10^3$ |
| Ferrosulfate tablet | $0.50 \times 10^3$ | $1.46 \times 10^3$ | 104 | 1.5 | $1.49 \times 10^3$ |
| [b]Oral drop | – | $1.10 \times 10^3$ | – | 1.3 | $1.12 \times 10^3$ |
| Oral drop | $0.80 \times 10^3$ | $1.88 \times 10^3$ | 97 | 1.4 | $1.90 \times 10^3$ |
| Under ground water | – | 68.5 | – | 1.2 | 70.0 |
| Under ground water | 50 | 119.2 | 101 | 1.4 | 120.4 |

a: Zahravy Co., b: Darropaksh Co., c: 1,10-phenanthroline spectrophotometry.

Pharmaceutical tablet and capsule solutions were prepared by dissolving each tablet in 10 mL of 4.0 M sulfuric acid and heating it in a water bath (70ºC) to complete dissolution. The solution was then filtered with a filter paper (Whatman No. 1) and the filtrate was diluted with water into a 1000 mL volumetric flask. Then, with 50000-times dilution with water, the iron contents were measured. For the oral iron drop pharmaceutical samples, 1.0 mL of the sample was diluted with water in a 250 mL volumetric flask and diluted solution was diluted 100000-times again with water. Then, the iron content was measured[29]. In order to evaluate the validity of the proposed method for determination of iron(II) ions, a recovery study was carried out on samples to which definite amounts of Fe(II) standards were added (Table-2).

## Conclusion

The oxidation of methylthymol blue (MTB) reagent by $BrO_3^-$ in the sulfuric acid media inhibited by iron(II). The inhibition of the reaction (the rate of variations of absorbance MTB solution) depends on the concentration of Fe(II) and increased with increasing of concentration of iron(II). In optimized conditions of reaction, the proposed system shows a simple, sensitive and selective method for determination trace amounts of iron(II). This procedure also has a wide dynamic range and a low detection limit.

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