

## Research Article

# A New Spectrophotometric Reagent for Fe(III): 2-(2,3-Dihydroxy-4-oxocyclobut-2-enylidene) Hydrozinecarbothiamide and Its Application in Real Samples

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A new reagent 2-(2,3-dihydroxy-4-oxocyclobut-2-enylidene) hydrozinecarbothiamide has been synthesized and used for developing a simple spectrophotometric method for the determination of Fe(III) which is based on a 2:1 complex formation between Fe(III) and new reagent in aqueous solution. The method is optimized in terms of the pH value, amount of reagent required, ionic strength, and stability of the complex, sensitivity, linearity, and tolerance limits of various foreign ions. The complex is a red-brown chelate, with  $\lambda_{\max} = 465$  nm at pH = 3 ( $\epsilon = 1.95 \times 10^3$  L·mol<sup>-1</sup>·cm<sup>-1</sup>). The ionic strength was kept constant at 0.02 by adding appropriate amounts of NaCl solution. The calibration curve is linear in the concentration range from 0.27–33.50  $\mu\text{g}\cdot\text{mL}^{-1}$ . The effects of foreign ions on the determination of Fe(III) were investigated in order to assess the selectivity of the method. The method was applied in determination of Fe(III) in tap water, cow milk, and human serum.

## 1. Introduction

Iron is the most important transition element involved in living system, being vital to both plants and animals. Its versatility is unique. It is at the active center of molecules responsible for oxygen transport and electron transport and is found in such diverse metalloenzyme as nitrogenase, various oxidases, hydrogenases, reductases, dehydrogenases, deoxygenases, and dehydrases. Iron is involved in enormous range of function and the whole range of life forms, from bacteria to man [1, 2]. Determination of oxidation state of iron in aquatic system is very important for environmental and biological studies because of the influence of the chemical forms on the bioavailability of iron [3]. Owing to simplicity, cheapness, and rapidity, UV-Visible spectrophotometric methods have been developed for the determination of metal ions. It involves using a number of chromogenic reagents for this purpose. Ferric ion is the most stable state of iron in the solutions; hence the determination of iron is generally

demonstrated on Fe(III) contents. In recent years, numerous chromogenic reagents, which are capable to form high stable complexes with Fe(III) ion, have been widely used for the determination of iron contents in numerous complicated matrices [4].

There are many spectrophotometric methods for the determination of iron [5]. Several techniques, such as spectrophotometry [6–9], atomic absorption spectrometry (AAS) [3, 7, 10–12], inductively coupled plasma-optical emission spectrometry (ICP-OES) [13, 14], ICP-mass spectrometry (MS) [15–17], cathodic or anodic stripping voltammetry [18, 19], chromatography [20, 21], cloud point extraction (CPE) [22–26], and spectroscopic sensors [27], have been reported for the determination of Fe.

In this study, 2-(2,3-dihydroxy-4-oxocyclobut-2-enylidene) hydrozinecarbothiamide is a new ligand used for spectrophotometric determination of Fe(III) ion. The procedure was applied to the determination of iron in tap water, cow milk, and human serum.

## 2. Experimental

Absorbance was measured and absorption spectra were recorded using a Cary 100 model Varian UV-VIS spectrophotometer (Australia) equipped with a quartz cell of 10 mm path length. pH measurement was made with 827 pH lab Metrohm pH meter (Switzerland). IR spectra were obtained on a Matson 1000, FT-IR spectrometer (USA). Peaks are reported in wave numbers ( $\text{cm}^{-1}$ ). All NMR spectra were recorded on a Bruker model DRX-500 AVANCE ( $^1\text{H}$ : 500 MHz) ( $^{13}\text{C}$ : 125 MHz) (Germany). Chemical shift is reported in parts per million (ppm) from tetramethylsilane (TMS) as an internal standard in DMSO- $d_6$  as a solvent.

All chemicals used were of analytical reagent grade (Merck). All solutions were prepared with deionized water. The stock standard Fe(III) solution was prepared by dissolving 0.6757 g of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in  $1 \times 10^{-3}$  M HCl and diluting to 250 mL. A  $1 \times 10^{-2}$  M stock complexing agent solution was prepared by dissolving 0.1871 g ligand in  $1 \times 10^{-3}$  M HCl and diluting to 100 mL. The working solution was obtained by diluting the stock Fe(III) and ligand solution in  $1 \times 10^{-3}$  M HCl. The ionic strength was kept constant at 0.02 by adding appropriate amounts of NaCl solution (0.5 M). All the glassware used was washed with aqueous HCl (1 : 1) and then thoroughly rinsed with tap, distilled, and finally deionised water.

## 3. Preparation of Ligand

A solution of 3,4-Dihydroxy cyclo but -3 ene -1,2 dione (6.37 g, 48.97 mmol) was added in small portions to a solution of thiosemicarbazide (4.46 g, 48.97 mmol) in water (45 mL) and 12 M hydrochloric acid (4.18 mL) in ethanol (40 mL). The solution was stirred at room temperature for 2 hours. The resulting white precipitate was filtered, washed with cold water, and dried under vacuum at  $70^\circ\text{C}$  for 5 hours to give 9.05 g, 91% yield of 2-(2,3-dihydroxy-4-oxocyclobut-2-enylidene) hydrozinecarbothiamide as a white solid, White powder (Figure 1). FT-IR (KBr)  $\nu$  max ( $\text{cm}^{-1}$ ): 3210–3370 (N–H, NH<sub>2</sub>), 1780, (C=O), 1666 (C=N).  $^1\text{H}$ -NMR (DMSO  $d_6$ , 500 MHz);  $\delta$  (ppm): 14.89 (2H, OH), 8.75 (2H, NH<sub>2</sub>), 7.63 (1H, NH).  $^{13}\text{C}$ -NMR (DMSO  $d_6$ , 125.77 MHz);  $\delta$  (ppm): 190.89 (C=O), 179.50 (C=S), 162.43, 159.67, 156.21 (C=C, C=N).

**3.1. Spectrophotometric Determination of Fe(III) Ions.** For the determination of Fe(III), transfer aliquots of the standard or sample solution (pH = 3) containing 0.002–0.335 mg of iron to a 10 mL volumetric flask, add 4 mL of  $10^{-3}$  M reagent solution and 0.4 mL of 0.5 M of NaCl. Dilute to the mark with  $10^{-3}$  M HCl, Mix, and measure the absorbance at 465 nm.

## 4. Results and Discussion

**4.1. Absorption Spectra.** The absorption spectra of Fe(III), ligand and Fe(III)-ligand complex were recorded (Figure 2 curves a, b, and c, respectively). The absorption maximum

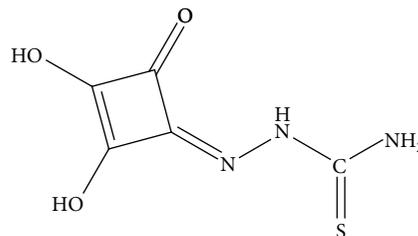


FIGURE 1: Proposed structure of the ligand (2-(2,3-dihydroxy-4-oxocyclobut-2-enylidene) hydrozinecarbothiamide). (z)-2-(2,3-dihydroxy-4-oxocyclobut-2-enylidene) hydrazinecarbothioamide.

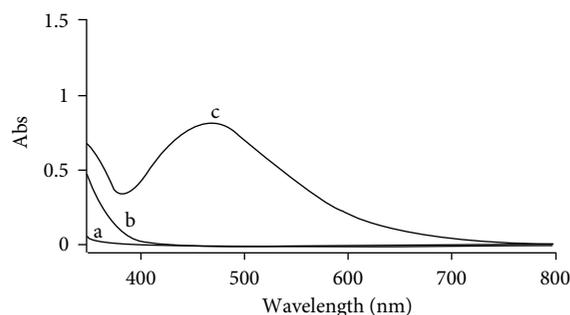


FIGURE 2: Visible absorption spectra of the Fe(III) (a), ligand (b), Fe(III)-ligand complex (c).

of the ligand is at 236 nm and that of complex is at 465 nm indicating the formation of a complex between the ligand and Fe(III). The molar absorptivity of the complex calculated from the absorbance data was found to be  $1.95 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$  at 465 nm.

**4.2. The Effect of pH.** The effect of pH on the determination of Fe(III) in aqueous medium was investigated spectrophotometrically. For this purpose, the absorbances were measured in the range of  $10^{-1}$ – $10^{-6}$  M HCl at 465 nm. The results obtained are shown in Figure 3. As it is clearly seen, the amount of Fe(III) can be determined quantitatively in the  $10^{-3}$  M HCl at pH ~ 3.

**4.3. Effect of Ionic Strength.** The effect of ionic strength was examined by establishing various concentrations of NaCl in the range of 0–0.1 M in the sample solutions. It was observed that ionic strength at 0.02 gave the most satisfactory results as evident by having obtained the maximum absorbance at 465 nm (Figure 4).

**4.4. Effect of Reagent Concentration.** The effect of ligand concentration on the complex formation was examined in the range of  $0.5$ – $6 \times 10^{-4}$  M using the solutions in which the concentrations of Fe(III) were fixed on  $1 \times 10^{-4}$  M. As shown in Figure 5, the absorbance measurements established that the requested ligand concentration to complete the complex formation should be  $4 \times 10^{-4}$  M.

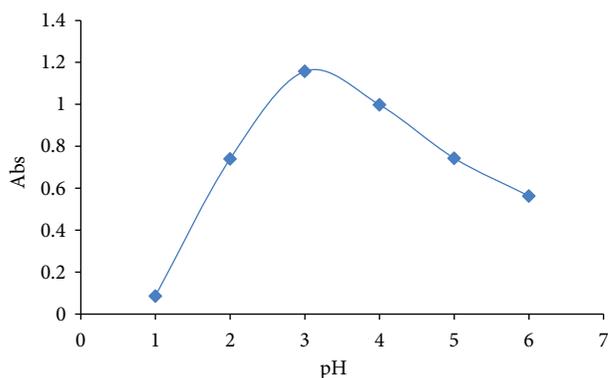


FIGURE 3: Effect of pH on the formation of Fe(III)-ligand complex.

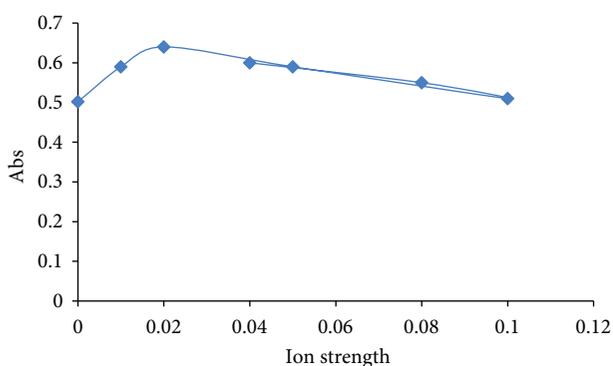


FIGURE 4: Effect of ion strength on the formation of Fe(III)-ligand complex.

**4.5. Nature and Stability of the Complex.** The stoichiometric ratio of Fe(III) and ligand in the complex was determined using the Job's method of continuous variation. Solutions of Fe(III) and ligand of the same concentration ( $10^{-4}$  M) were prepared and then mixed in the volume ratio from 1:9 to 9:1. The Job curve of this system at HCl concentration of  $10^{-3}$  M and  $I = 0.02$  is shown in Figure 6. The maximum at M:L = 0.33 indicates that the formation of the complex is in the metal: ligand ratio of 2:1. The color of Fe(III) complex was instantaneous, and the intensity remained constant for at least 48 h.

The composition of the complex was also determined by applying the mole ratio method. A series of solutions were prepared with a constant concentration of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  ( $10^{-4}$  M) and variable 2-(2,3-dihydroxy-4-oxocyclobut-2-enylidene) hydrozinecarbothiamide concentrations ( $0.1 \times 10^{-4}$ – $5 \times 10^{-4}$  M). It can be seen (Figure 7) that the metal: ligand ratio in the complex is 2:1, which agrees with result obtained by the Job's method.

**4.6. Beer's Law and Sensitivity.** Table 1 compares the analytical characteristics of the proposed with those of previously published spectrophotometric methods for the determination of Fe(III). A calibration graph for the determination

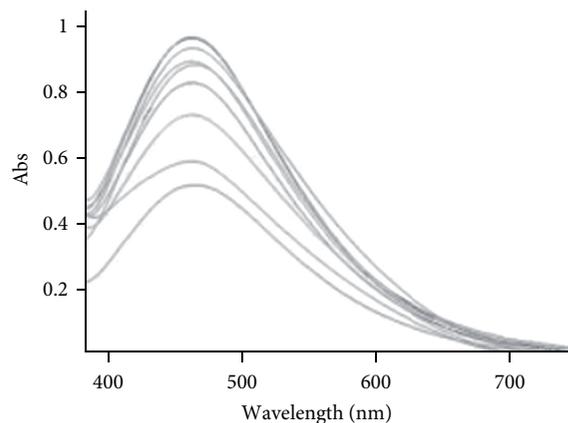
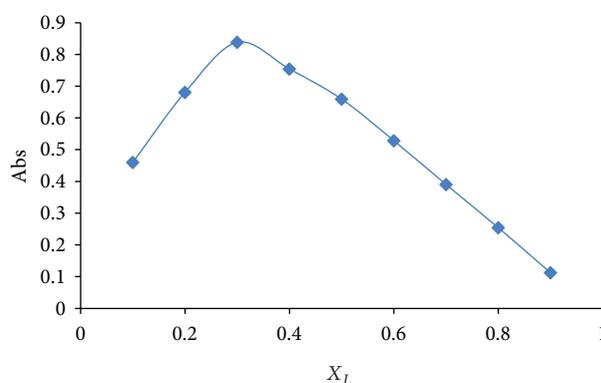


FIGURE 5: The effect of ligand concentration on the complex formation.

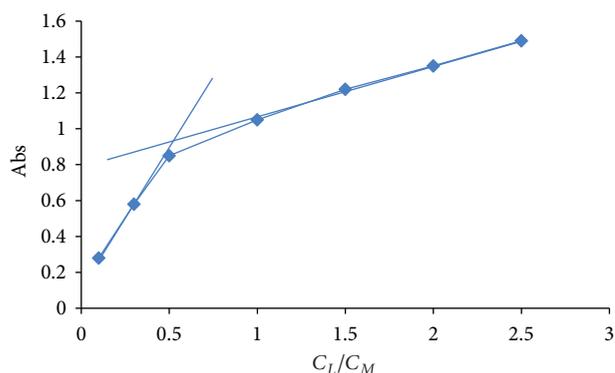
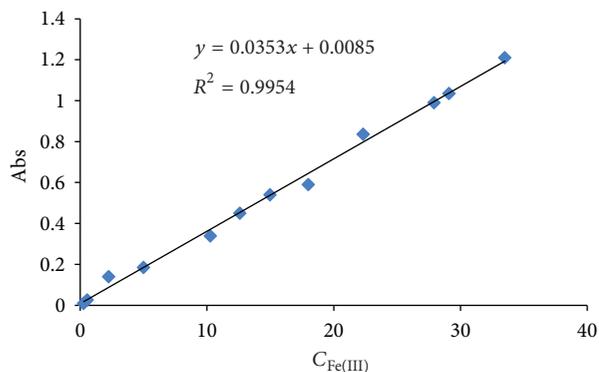
FIGURE 6: Determination of the stoichiometry of Fe(III)-ligand complex by the Job method of continuous variations:  $C_{\text{Fe(III)}} = C_L = 10^{-4}$  M; pH = 3;  $I = 0.02$ .

of Fe(III) was prepared under optimum experimental conditions (Ionic strength = 0.02 and  $4 \times 10^{-4}$  M ligand in  $10^{-3}$  M HCl) (Figure 8). Beer's law is obeyed within a wide range of  $0.27$ – $33.50 \mu\text{g}\cdot\text{mL}^{-1}$  of Fe(III) at 465 nm (Table 2). The calibration graph can be represented by a linear regression equation:  $y = 0.035x + 0.008$  ( $r^2 = 0.995$ ). Here,  $y$  is the absorbance and  $x$  the concentration of Fe(III) in  $\mu\text{g}\cdot\text{mL}^{-1}$ . The molar absorptivity is  $1.95 \times 10^3 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$  and the Sandell's sensitivity calculated on the basis of total Fe(III) present is  $0.114 \mu\text{g}\cdot\text{cm}^{-2}$ .

**4.7. Precision.** The precision of the method was checked by taking 10 replicate measurements on solutions each containing  $0.3$ – $30 \mu\text{g}\cdot\text{mL}^{-1}$  of Fe(III). The relative standard deviation (RSD) of the results of the determination of Fe(III) was in the range of 0.8–1.2%. Detection limit estimated from the standard deviation (SD) of the blank and calibration sensitivity (slope of calibration line), (LOD = 3 SD/sensitivity [32]) amounted to  $0.08 \mu\text{g}\cdot\text{mL}^{-1}$  of Fe(III) (Table 1). Limit of quantification (LOQ) is  $0.26 \mu\text{g}\cdot\text{mL}^{-1}$  of Fe(III), and the LOD/LOQ ratio is 0.3.

TABLE 1: Analytical characteristics of the proposed method and comparison with some other spectrophotometric methods.

Reagent	Condition pH	LDR ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	LOD ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	$\lambda_{\text{max}}$ (nm)	Reference
2-(2,3-Dihydroxy-4-oxocyclobut-2-enylidene) hydrozinecarbothiamide	3, HCl	0.27–33.50	0.08	465	This work
Morin	—	0.4–15	0.36	412	[28]
2,6-Dihydroxy benzoic acid	3, Formic acid/sodium formate	3.24–97.2	0.4	560	[4]
2-Ethanolimino-2-pentylidino-4-one	3.5, HNO <sub>3</sub>	2–17	Not reported	440	[29]
thiocyanate and cetyltrimethylammonium bromide	3-4 HNO <sub>3</sub>	0–6	Not reported	474	[30]
2-Carboethoxy-1,3-indandione	1.5–3.5, HCl	0.27–27.92	0.04	500	[31]

FIGURE 7: Determination of the stoichiometry of Fe(III)-ligand complex by the mole ratio method:  $C_{\text{Fe(III)}} = 10^{-4}$  M,  $C_L = (0.1 \times 10^{-4} - 5 \times 10^{-4})$  M; pH = 3;  $I = 0.02$ .FIGURE 8: Calibration curve for Fe(III),  $C_L = 4 \times 10^{-4}$  M; pH = 3;  $I = 0.02$ .

4.8. *Effect of Diverse Ions.* The interference effect of cations and anions on the determination of iron ( $C_{\text{Fe}} = 10^{-4}$  M) and the tolerance limits of the interfering ions are given in Table 3.

## 5. Applications

The proposed method was applied for the determination of Fe(III) in real samples such as tap water, cow milk, and human serum.

TABLE 2: Conditions for the spectrophotometric determination of Fe(III).

Wavelength of maximum absorbance (nm)	465
Limit of detection (LOD) ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	0.08
Limit of quantification (LOQ) ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	0.26
LOD/LOQ	0.3
Linear range ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	0.27–33.50
Molar absorptivity ( $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ )	$1.95 \times 10^3$
Optimized pH	~3
Calibration graph slope	0.035
Intercept	0.008
Correlation coefficient ( $r^2$ )	0.995
Relative standard deviation (%) ( $n = 10$ ) <sup>a</sup>	0.8–1.2%

<sup>a</sup>RSD was determined for Fe(III) in the range of 0.3–30  $\mu\text{g}\cdot\text{mL}^{-1}$ .

TABLE 3: Effect of diverse ions on the determination of Fe(III).

Ion added	Tolerance level $C_{\text{ion}}/C_{\text{Fe(III)}}$
Hg <sup>+2</sup> , Ag <sup>+1</sup>	100
Mg <sup>+2</sup> , Co <sup>+2</sup>	50
Ni <sup>+2</sup> , Zn <sup>+2</sup> , Cu <sup>+2</sup> , Fe <sup>+2</sup> , CO <sub>3</sub> <sup>-2</sup> , SO <sub>4</sub> <sup>-2</sup>	10

Tap water samples were obtained from Rafsanjan City. To determine Fe(III), 10 mL of water sample was spiked with solution of  $10^{-3}$  M Fe(III) and was analyzed by the analytical procedure.

To 10 mL of cow milk, few drops of concentrated nitric acid were added, and the sample was centrifuged for few minutes. Then the supernatant solution was taken, its pH was adjusted to ~3, and the resulting solution was spiked. The solution was then analyzed according to the given procedure.

To 1 mL of human serum added 5 mL of 30 wt% trichloroacetic acid to precipitate protein. The mixture was centrifuged to remove protein. Supernatant liquid was transferred to a fresh test tube and then pH was adjusted to ~3 and the resulting solution was spiked. The solution was then analyzed according to the given procedure. The obtained results are given in Table 4.

TABLE 4: Determination of Fe(III) in tap water, cow milk, and human serum.

Sample	Fe(III) ( $\mu\text{g}\cdot\text{mL}^{-1}$ )		Recovery (%)
	Added	Found	
Tap water	10.45	9.81 $\pm$ 0.7	93.87
Cow milk (1.5% fat)	8.1	7.56 $\pm$ 0.4	93.33
Cow milk (2.5% fat)	8.10	7.29 $\pm$ 0.6	97.40
Human serum	13.50	12.13 $\pm$ 0.7	89.85

## 6. Conclusions

The proposed method is simple, rapid, and selective. The performance of the method described here allows the determination of iron(III) in tap water, cow milk, and human serum. The stoichiometry of complex was determined (2 : 1 for Fe(III): ligand). This method provides an appropriate selectivity for easy determination of 0.27–33.50  $\mu\text{g}\cdot\text{mL}^{-1}$  Fe(III) ions. The ligand used in this study has advantage over other ligands currently utilized for photometric Fe(III) determination and there was no interference from Fe(II) at the detection of Fe(III) at concentration ratio = 10. As a result, this ligand can be used in speciation analysis.

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