



Spectrophotometric determination of Fe (III) in real samples by complexation

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Abstract: A New simple and selective spectrophotometric method has been described for determination of Fe (III) in synthetic mixture. The method is based on complex formation of Fe (III) with N'-(2-hydroxybenzylidene)-3-(4-o-tolylpiperazin-1-yl) propanehydrazide (HTP) immediately in aqueous medium pH 4 at room temperature (27±1°C). The complex showed maximum absorption wavelength at 410 nm. Beer's law is obeyed in the concentration range of 1.116-12.276 µg.mL⁻¹ with apparent molar absorptivity (0.3072 x 10⁴ L mol⁻¹ cm⁻¹) and Sandell's sensitivity (0.018 µg/cm²/0.001 absorbance units). The tolerance limit of various foreign ions was reported. Proposed method was successfully applied in determination of Fe (III) in synthetic mixture.

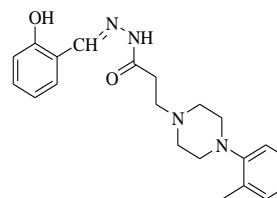
Key Words: Spectrophotometry, Fe (III)-HTP complex, Validation, synthetic sample

Introduction

Iron plays important roles in both biological and environmental media (1-2). Due to its importance in the context of clinical diagnosis, intoxication, environmental pollution monitoring (3-5) many methods such as spectrophotometry (6-7), atomic absorption spectrometry (8-9), inductively coupled plasma-mass spectrometry (10), cathodic stripping voltammetry (11), fluorimetry (12) and ion chromatography (13-14) have been proposed for determination of iron species in natural samples. Among the most widely applied methods are those based on spectrophotometry, because of their experimental rapidity, simplicity and wide applications. Spectrophotometric techniques involve the use of ligands that selectively bind to iron, or a particular redox state of iron, to produce a colored complex with a high molar absorptivity. Iron selective ligands such as thiocyanate (15-16) or di (2-pyridyl)-N, N-di[(8-quinolyl) amino] methane (17-19) were among the first selective reagents to be used for the determination of iron. In most of these methods Fe (II) is involved in reaction with an appropriate ligand and color-generation (20), Fe (III) is then determined by subtraction the concentration of Fe (II) from total iron, which is determined either by reduction to Fe (II) or by conventional nonselective methods (21-22).

The differential approach, however, often yields highly imprecise values for Fe (III) when the Fe (II) concentration is higher than that of Fe (III) (23). In addition, most above-

mentioned methods lack sufficient sensitivity for iron determination at micro molar or sub-micro molar levels. Therefore, ferrozine has been widely used for spectrophotometric determination of Fe (II), due to a sufficiently low detection limit and low blank values (4, 24). A potential problem with the classical ferrozine method is incomplete reduction of organic complex Fe (III) (25). This is probably why different reducing agents (mostly ascorbic acid and hydroxylamine hydrochloride) are used to optimize the reduction condition (7, 26). Several studies have also demonstrated that Fe (III) in solution can also react with ferrozine, which interferes with the ferrous complex (7, 27). Increasing interest has therefore focused to develop new methods for determination of Fe (III). This study presents a simple and rapid spectrophotometric method via complexation with HTP for determination of Fe (III) in synthetic mixture.



N'-(2-hydroxybenzylidene)-3-(4-o-tolylpiperazin-1-yl) propanehydrazide (HTP)

Figure 1: Chemical structure of ligand

Materials and Methods

Apparatus

All spectral and absorbance measurements were carried out on a Shimadzu UV-Visible 1601 spectrophotometer (Shimadzu Corporation, Japan) with 1 cm matched

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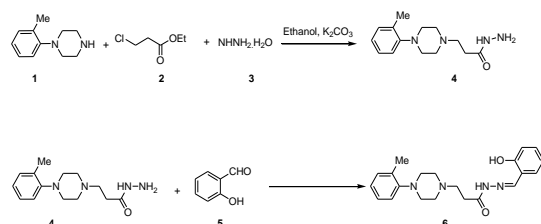
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quartz cells. The pH of buffer solutions was monitored by using Systronic digital pH meter (India). An electronic micro balance (Sartorius MC 5, Germany) and Afcost electronic balance (Mumbai, India) were used for weighing the solid materials.

Procedure for preparation of N'-(2-hydroxybenzylidene)-3-(4-o-tolylpiperazin-1-yl) propanehydrazide (HTP)

A mixture of Tolylpiperzine (1) (3.52 gms, 0.02 mol), ethyl 3-chloropropanoate (2) (2.72 gms, 0.02 mol) and Hydrazene hydrate (3) (1.96 gms, 0.04 mol) was taken into ethanol in presence of potassium carbonate for 6 hours at reflux temperature. After completion of the reaction, as monitored by TLC, the reaction mass was cooled to room temperature and filtered. The ethanol layer was distilled off by using vacuum to get 3-(4-O-tolylpiperazin-1-yl) propane hydrazide (4). The crude product of recrystallized from ethanol. A mixture of 4 (2.62, 0.01) and 2-hydroxybenzaldehyde (1.22 gms, 0.01 mol) in Ethanol was refluxed for 4 hrs. After completion of the reaction, as monitored by TLC, the reaction mass was cooled to room temperature and filtered. The ethanol layer was distilled off by using vacuum to get N'-(2-hydroxybenzylidene)-3-(4-o-tolylpiperazin-1-yl) propanehydrazide. The crude product of recrystallized from ethanol (28). Yield: 2.80 gms, (76.5%). The complete reaction mechanism is depicted in scheme.1.



Scheme.1

Reagents and standards

All reagents used were of analytical reagent grade. Standard Fe (III) solution (0.01 M) was prepared from (Fe (NO₃)₃·10H₂O) in 100 ml of doubled distilled water. The working solutions were prepared just before use by dilution of the standard solution with double distilled water. Acetate buffer solutions ranging from 1.0-7.0 were prepared by mixing varying volumes of 0.2 M acetic acid and 0.2 M sodium acetate. A 0.01 M HTP solution was prepared in N, N-dimethyl formamide solvent.

Procedure for the Determination of Fe (III)

Into a series of 10 mL standard volumetric flask, aliquots (0.2-2.2 mL) of 1×10⁻³ M Iron (III) aqueous solution corresponding to 1.116-12.276 µg. mL⁻¹ were pipetted. To each flask, 2.0 mL of acetate buffer (pH 4.0) and 1.0 mL of HTP reagent (1×10⁻² M) solution were added. The resulting solution diluted up to the mark with double distilled water. The contents of the flask were mixed well and the absorbance was measured at 410 nm against reagent blank prepared similarly except Iron (III). The amount of Iron (III) was obtained either from the calibration graph.

Results and Discussions

Iron (III) interacts with the HTP to form a brown colored water-soluble complex in the buffer media of (pH 1.0 – 6.0). The color reaction was instantaneous at room temperature (27±1°C) and the intensity of the color remained constant for several hours. The color reaction was utilized for the micro determination of Iron (III) in real samples.

Optimization of reaction conditions

In order to optimize the conditions for proposed spectrophotometric methods, the effect of experimental variables was studied by altering each variable in turn while keeping the others constant.

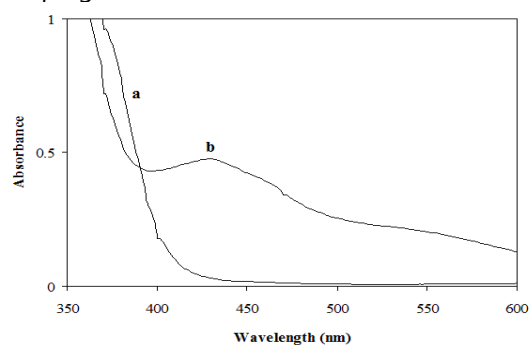


Figure 2: Absorption spectra **a)** HTP (1×10⁻³ M) against buffer pH 4 **b)** 1×10⁻⁴ M Fe (III)-HTP complex against reagent blank.

Effect of pH

Due to the pH of the aqueous solution being an important parameter for chelate formation, the influences of pH of the aqueous solution on the formation of Iron (III) - HTP complex were investigated spectrophotometrically. The effect of the pH on the formation of the Fe (III) - HTP complex was examined at 410 nm using acetate buffer solutions of different pH values (pH 1.0-6.0). The results are given in Figure 3. The complex begins to form at approximately pH 1.0, with

maximum absorbance being reached at pH 4.0 to pH 6.0. Beyond this pH range precipitate was formed. In the light of these findings, all subsequent studies were carried out at pH 4.0 acetate buffer.

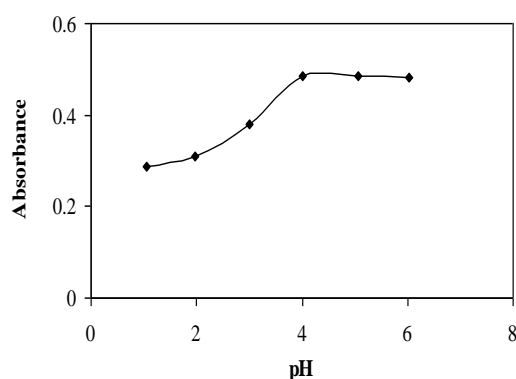


Figure 3. Effect of pH on absorbance $[\text{Fe (III)}] = 1 \times 10^{-4} \text{ M}$; $[\text{HTP}] = 1 \times 10^{-3} \text{ M}$

Effect of reagent concentration

The experimental observations pertaining to the effect of reagent concentration on the color reaction reveal that an optimum of 10-fold reagent concentration was required for the complexation reaction. Hence a 10-fold excess of the reagent concentration was selected for the further studies. However, it was found that presence of the excess of the reagent does not alter the absorbance of the reaction mixture. The effect of surfactants on the absorbance of complex was studied. Unfortunately, the surfactants did not enhance the complex absorbance. So, further studies were carried out without addition of surfactant.

Characteristics of the complex

Stoichiometry of the reaction was obtained by Job's method of continuous variation (29). Keeping the total volume constant, equimolar solutions of Iron (III) and HTP were taken in different volume ratios in 10 mL volumetric flasks. The general experimental procedure was followed and a graph was plotted between the absorbance and mole fraction of the metal. The plot (Figure 4) reveals stoichiometry between metal and ligand is 1:1 ratio. The formed complex was stable for 10 h.

Effect of interference ions

The selectivity of the proposed method was examined by studying the effect of diverse ions on the absorbance of the experimental solution containing fixed amount of Iron (III). The results are presented in Table 1.

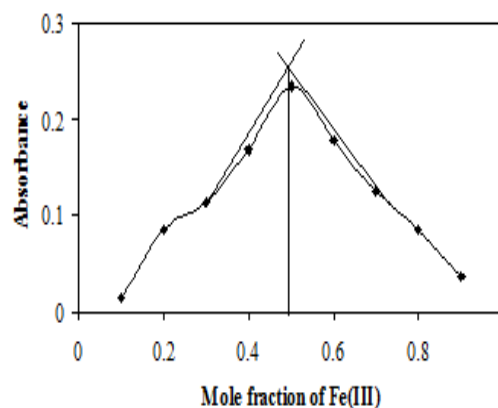


Figure 4: Job's method of continuous variation $[\text{Fe (III)}] = [\text{HTP}] = 1 \times 10^{-3} \text{ M}$; $\lambda_{\text{max}} = 410 \text{ nm}$; $\text{pH} = 4$.

Table 1: Effect metal ions on the determination of $5.58 (\mu\text{g mL}^{-1}) \text{ Fe (III)}$

Metal ion	Tolerance limit ($\mu\text{g mL}^{-1}$)
Iodide	706
Tetraborate	550
Citrate	385
Thiourea	900
Tartrate	875
Thiosulphate	433
Oxalate	150
Chloride	780
Fluoride	210
Ba^{+2}	60
Sn^{+2}	56
Bi^{+2}	55
Se^{+4}	70
Fe^{+2}	63
Mo^{+6}	24
Pd^{+2}	76
Zn^{+2}	50
Cd^{+2}	38
Mn^{+2}	46
Mg^{+2}	64

It can be seen from the table that large number of common ions did not interfere in the proposed method.

Validation

Linear was conducted between absorbance and concentration of Iron (III) in the proposed method under the optimized experimental conditions. Regression analysis for the results was carried out using least-square method (Figure 5). Beer's law plots were linear with good correlation coefficients as shown Table 2. The limits of detection (LOD) and limits of quantification (LOQ) were determined (30) using the formula: $\text{LOD or LOQ} = kS_a/b$, where $k = 3.3$ for LOD and 10 for LOQ, S_a is the standard deviation of the intercept, and b is the slope.

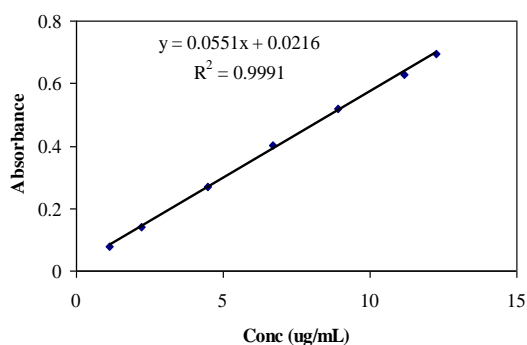


Figure 5: Analytical determination of Iron (III) with HTP (1×10^{-3} M) and pH = 4 at 410 nm wavelength.

Table 2: Optical and regression characteristics of the proposed method

Parameter	Proposed method
λ_{\max} (nm)	410
Beers law limit ($\mu\text{g mL}^{-1}$)	1.116-12.276
Molar absorptivity (L/mol. cm)	0.3072×10^4
Sandell's sensitivity ($\mu\text{g cm}^{-2}$)	0.018
Régression équation ($Y = a + bC$)	
Slope (b)	0.0551
Intercept (a)	0.0216
Regression coefficient (r^2)	0.9991
Standard deviation of slope (S_b)	7×10^{-4}
Standard deviation of intercept (S_a)	0.0014
Detection limit LOD ($\mu\text{g mL}^{-1}$)	0.09
Quantification limit LOQ ($\mu\text{g mL}^{-1}$)	0.26

The precision and accuracy of the proposed methods were determined at three different concentrations of Fe (III). At each concentration, six replicate determinations were made. The relative standard deviation as precision and percentage relative error (RE %) as accuracy of the suggested methods was calculated. The results presented in Table 3, reveal that precision and accuracy of the proposed methods are fairly high as indicated by the low values of % RSD and % RE.

Table 3: Precision and accuracy of the proposed method

Fe (III) taken ($\mu\text{g mL}^{-1}$)	Fe (III) found \pm SD ($\mu\text{g mL}^{-1}$)	% RSD	% RE
2.232	2.241 \pm 0.039	1.74	0.40
8.928	8.939 \pm 0.072	0.81	0.12
12.276	12.278 \pm 0.084	0.68	0.02

Applications

The proposed method was applied for the determination of Fe (III) in synthetic mixture (31) and real samples such as tap water and cow milk.

Synthetic mixture of iron (III) was prepared by taking 200 mg of ferric sulphate with 13.9 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 192.3 mg $3\text{CdSO} \cdot 8\text{H}_2\text{O}$, 90.0 mg

$\text{Pb}(\text{NO}_3)_2$, 23.01 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 222.18 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 84.5 mg $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in 100 ml standard volumetric flask and diluted up to the mark with distilled water. Iron (III) was determined by developed method.

Tap water samples were obtained from Saifabad campus Hyderabad. To determine Fe (III), 10 mL of water sample was spiked with solution of $10 \mu\text{g mL}^{-1}$ Fe (III) and was analyzed by the developed method.

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, and the resulting solution was spiked with solution of $10 \mu\text{g mL}^{-1}$ Fe (III). The solution was then analyzed according to the given procedure. Results are shown in Table 4 (a) and Table 4 (b).

Table 4 (a): Determination of Iron (III) in synthetic mixer

Amount of Iron (III) (mg)	Amount of Iron (III) found (mg)	Recovery (%)	% RE
200	199.4	99.7	-0.3

Table 4 (b): Determination of Iron (III) in water and Cow milk samples

Sample	Amount of Fe (III) added ($\mu\text{g mL}^{-1}$)	Amount of Fe (III) found ($\mu\text{g mL}^{-1}$)	Recovery (%)	%RE
Tap water	10	10.01	100.1	0.1
Cow milk	10	9.93	99.3	-0.7

Conclusions

The proposed method is simple, rapid, and selective. The performance of the method described here allows the determination of iron (III) in synthetic mixer, tap water and cow milk. The stoichiometry of complex was determined (1: 1 for Fe (III): ligand). There was no interference from Fe (II) at the detection of Fe (III) at concentration ratio = ~ 11 . As a result, this ligand can be used in speciation analysis. The proposed method has avoided the use of extraction and heating of reaction mixture. The proposed method is an alternate for determination of Iron (III) in quality control samples like tap water and cow milk.

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References

1. Suwansaksri J, Sookarun S, Wiwanitkit V, Boonchalermvichian C, Nuchprayoon I. *Lab Hematol* 2003; 9(4): 234-236.
2. Martin JH, Fitzwater SE. *Nature* 1988; 331: 341-342.
3. Willey JD, Kieber RJ, Williams KH, Crozier JS, Skrabal SA. *J Atmos Chem* 2000; 37: 185-205.
4. Goswami A, Singh AK. *Anal Bioanal Chem* 2002; 374(3): 554-560.
5. Cotton FA, Wilkinson G. *Advanced inorganic chemistry*, 3rd ed., New York: Wiley; 1998.
6. de Jong JTM, den Das J, Bathmann U, Stoll MHC, Kattner G, Nolting RF, de Baar HJW. *Anal Chim Acta* 1998; 377: 113-117.
7. Blain S, Treguer P. *Anal Chim Acta* 1995; 308:425-429.
8. Sohrin Y, Iwamoto S, Akiyama S, Fujita T, Kugii T, Obata H, Nakayama E, Goda S, Fujishima Y, Hasegawa H, Ueda K, Matsui M. *Anal Chim Acta* 1998; 363: 11-16.
9. Wu JF, Boyle EA. *Anal Chim Acta* 1998; 367: 183-187.
10. Yan XP, Hendry MJ, Kerrich R. *Anal Chem* 2000; 72: 1879-1885.
11. Croot PL, Johansson M. *Electroanalysis* 2000; 12:565-576.
12. Pulido-Tofiño P, Barrero-Moreno JM, Pérez-Conde MC. *Talanta* 2000; 51: 537-542.
13. Schnell S, Ratering S, Jansen KH. *Environ Sci Technol* 1998; 32: 1530-1536.
14. Deutsch F, Hoffmann P, Ortner HM. *J Atmos Chem* 2001; 40: 87-92.
15. Josephs HW. *J Lab Clin Med* 1954; 44(1): 63-74.
16. Kawakubo S, Naito A, Fujihara A, Iwatsuki M. *Anal Sci* 2004; 20(8): 159-1163.
17. Sullivan DJ. *J Assoc Off Anal Chem.* 1977; 60(6): 1350-1354.
18. Escobar R, Cano Pavon JM. *Analyst* 1983; 108(1288): 821-826.
19. Kovalev LM, Kruglikovskaia LI, Ianova VM, Iashina OG. *Nauchnye Doki Vyss Shkoly Biol Nauki* 1984; 12: 96-100.
20. Stookey LL. *Anal Chem* 1970; 42: 779-785.
21. Giokas DL, Paleologos EK, Karayannis MI. *Chem.* 2002; 373(4-5): 237-243.
22. Bagheri H, Gholami A, Najafi A. *Anal Chim Acta* 2000; 424: 233-238.
23. To TB, Nordstrom DK, Cunningham KM, Ball JW, McCleskey RB. *Environ Sci Technol* 1999; 33:807-815.
24. Gibbs CR. *Anal Chem* 1976; 48: 1197-2002.
25. Horak E, Hohnadel DC, Sunderman FW Jr. *Ann Clin Lab Sci* 1975; 5(4): 303-307.
26. Dawson MV, Lyle SJ. *Talanta* 1990; 37: 1189-1195.
27. Siffert C. PhD Thesis, ETH-Zurich, Switzerland; 1989.
28. Ravichandran C, Ramachandraiah C, Chandrasekhar K. B. *International Journal of Chemical and Life Sciences* 2014; 03: 1294-1300.
29. W. Likussar, D. F. Boltz, *Anal. Chem* 1971; 43: 1265-1272.
30. Validation of Analytical Procedures, ICH Harmonized Tripartite Guideline, Q2 (R1), Current Step 4 Version, Parent Guidelines 1996, Incorporated in November (2005).
31. Lutfullah, Sharma S, Rahman N, Syed Najmul H.A. *J Sci Ind Res* 2010;69: 135-141.

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