EXTRACTIVE SPECTROPHOTOMETRIC DETERMINATION OF IRON (III) WITH 5-(*P*-HYDROXYBENZYLIDENE)-THIAZOLIDINEDIONE-2,4

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ABSTRACT

5-(p-hydroxybenzylidene)-Thiazolidinedione-2,4 (L) as a photometric reagent for the extractive spectrophotometric determination of iron(III) is presented in this paper. The reagent L gave instantaneous and stable red colour with iron (III) in the pH range 3.8 to 5.2. The Beer's law was applicable in the range of 0.05 - 3.2µg/ml at 535 nm. The Limit of Detection (LOD) is found to be 12 ng/mL. The stoichiometry of the complex is established as 1:2 (Fe: L) by equilibrium shift method. The standard deviation and the coefficient of variance are presented. The results of the prescribed procedure applied for the determination of the micro amounts of Fe (III) in pharmaceutical, food and in plant samples are presented.

***Keywords*:** Iron, spectrophotometric determination, 5-(p-hydroxybenzylidene)-Thiazolidinedione-2,4

1.INTRODUCTION

In living organisms, iron is an essential trace mineral that catalyzes the exchange processes oxygen. Iron deficiency is manifested as a disease organism (chlorosis in plants and anemia in animals). Excess too bad: iron compounds are deposited in the tissues of the eyes and the lungs, causing them to siderosis [1].

Iron is an absolute requirement for most forms of life, including humans and most bacterials species. Plants and animals all use iron, and it can be found in a wide variety of food sources. Fe(II) is a cofactor in heme enzymes such as catalase and cytochrome C, and in non-heme enzymes such as aldolase and tryptophan oxygenase. In humans iron is an essential component involved in oxygen transport. It is also essential for the regulation of cell growth, and differentiation of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity [2].

Iron is an essential trace element that make up the sea water, and can be counted among the biogenic elements. On the other hand, its high content in some waters is an indicator of water pollution due to human factors [3].

Reagents containing OH-groups and nitrogen donor atoms are considered most suitable for the determination of Fe (III). Techniques for the photometric determination of iron (III) in the form of MLC these reagents in the presence of various third components have high sensitivity and selectivity [4].

For spectrophotometric determination of iron in various samples suggested leucoxylene cyanol [5], 1,2-dihydroxy-3,4-diketocyclobutene (squaric acid) [6], thiocyanate [7], 9-(4-carboxypheny1)-2,3,7-trihydroxy1-6-flurone[8], 2^1,3,4^1,5,7-Pentahydroxyflavone[9], 4-nitrocatechol and 2,3,5-triphenyl-2H-tetrazolium chloride [10], 4-nitrocatechol and 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide [11].

The real work is devoted to studying of reaction of a complex formation of iron (III) with 5-(p-hydroxybenzylidene)-Thiazolidinedione-2,4 (L) .

2. MATERIALS AND METHODS

2.1. Instruments

The absorbance of the extracts was measured using a Shimadzu UV1240 spectrophotometer (Japan) and KFK 2 photocolorimeter (USSR). Glass cells with optical path of 10 or 5 mm were used. pH of aqueous phase was measured using an I-120.2 potentiometer with a glass electrode.

Muffle furnace was used for dissolution of the samples. IR spectra were recorded on a spectrophotometer "Specord M80" (Germany). ^{1}H-NMR spectra were recorded on "Bruker" Fourier Transform (300,18 MHz) in C_6D_6.

2.2. Reagents and Solutions

Standard Fe(III) solution (1.00 mg mL^{-1}) was prepared by dissolving a weighed amount of ($Fe(NO_3)_3 \cdot 10H_2O$) in 100 ml of doubled distilled deionised water containing 1-2 mL of nitric acid (1:1). Concentrations were checked using standard potassium permanganate solution[12]. The working solutions were prepared just before use by dilution of the standard solution with redistilled deionised water.

Solutions of L in chloroform (0.01M) were used. The extractant was purified chloroform.

Acetate buffers of pH 4, 5 and 6 were prepared by mixing 0.05 M solutions of CH_3COONa and acetic acid. 4. Phosphate buffers of pH 7 and 8 were prepared by mixing 0.05 M solutions of KH_2PO_4 and 0.01 M NaOH.

The stock solution of various metal ions and anions were prepared by dissolving the appropriate metal salts in distilled water or with suitable dilute acids and making up to a known volume.

2.3. General Procedure

2.3. 1. Procedure for the extraction

Aliquots of Fe(IIİ) solution, L solution (up to 2.0 mL) and buffer solution (pH ranging from 3.0 to 9) were introduced into 100-mL separatory funnels. The resulting solutions were diluted with distilled water to a total volume of 25 mL. Then 3.0 mL of chloroform was added(the volume of the organic phase was 5 mL). and the funnels were shaken for a defined period of time (up to 5 min). When the equilibrium was reached, the organic layer was separated from the aqueous layer. A portion of the organic extract was filtered through a filter paper into a cell and the absorbance was read against a blank.

2. 3. 2.Determination of Fe (II) in Pharmaceutical Samples

0.5 - 1.0 gm sample of pharmaceutical product was dissolved in boiling with 10 ml of aqua-regia. The resulting solution was evaporated to dryness and the residue was dissolved in 10 ml of 1N HCl filter, if required and solution was diluted to 100 ml with doubly distilled water. The working solution was prepared by appropriate dilution of stock solution. To an aliquot of this solution 1ml was analyzed for Fe(III) by the procedure as described earlier

2. 3. 3. Determination of iron in food samples

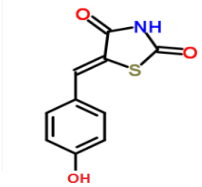
An air-dried food sample (25-50 g, egg-1 piece). incinerated in a muffle furnace at a temperature 550-750^o S. 2 g of the resulting ash is dissolved in a mixture of 15 ml of HCl and 5 ml of HNO_3. To completely remove the HNO_3 sample 2-3 was treated with 3-4 ml of HCl. The solution was concentrated to 2 mL and diluted with distilled water, filtered into a volumetric flask of 100 ml.

3. RESULTS AND DISCUSSION

L were synthesized according to the procedure [13]. L was recrystallised using aqueous ethanol. Its solution (0.01 M) was prepared in chloroform. Structure of ligand was confirmed by using NMR and IR spectra.

In weakly acid and neutral media (pH 3.0-9.0) The reagents L with Fe(III) forms red coloured complexes, which extracted into organic phase. The extraction of Fe(III) forms an aqueous

phase by L in chloroform is studied over a wide range of experimental condition. The results of various studies are discussed below.



5-(p-hydroxybenzylidene)-Thiazolidinedione-2,4

3. 1. Extraction as a function of pH

The extraction of Iron with L has been studied over the pH range 1-9 and was observed that percentage extraction of Fe(III) is maximum at pH 3,8-5,2. The effect of pH on the intensity of the color reaction is shown in the Fig. 1. Hence further analytical investigations were carried out in media of pH 4.5. At pH 9 complex practically not extractable in connection with the hydrolysis of iron(III) ion.

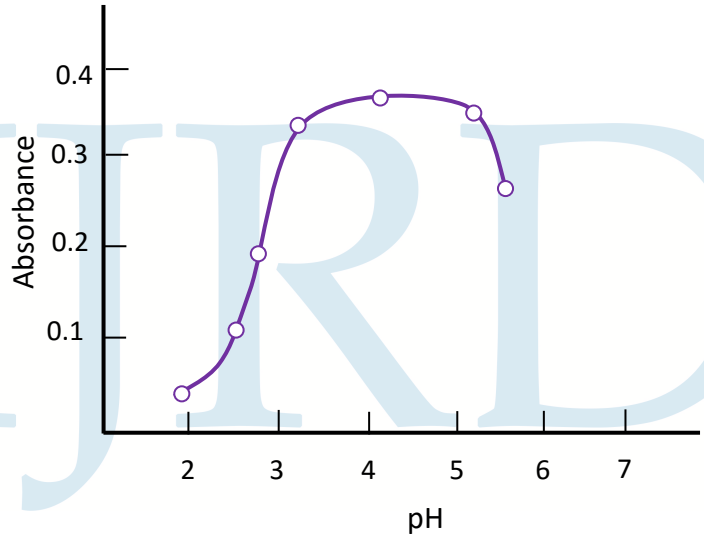


Figure 1. Absorbance of complexes as a function of the pH of the aqueous phase
C_{Fe} = 3.57×10^{-5} M; C_L = 8.0×10^{-4} M, λ=540 nm, l = 0,5 см

3. 2. Absorption spectrum

The absorption spectrum of Fe(III)-L in chloroform shows the maximum absorption at 535 nm. The absorption due to reagent at this wavelength is nearly negligible. Hence the absorption measurements were carried out at 540 nm.

Contrast of reactions was high: initial reagent - are colourless, and complex – are intensively painted (fig. 2). Molar coefficients of absorption make 3.29 • 10^4 dm ^{3} · mol^{-1}· cm^{-1}.

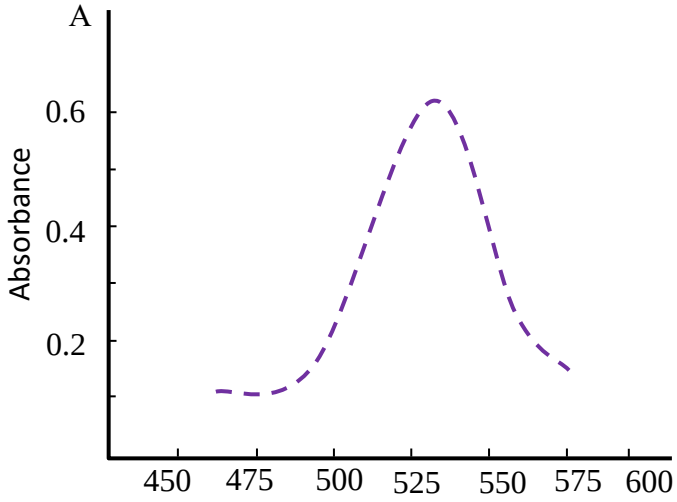


Figure 2. Absorption of complexes
$C_{Fe} = 3.57\times10^{-5}$ M; $C_L = 8.0\times10^{-4}$ M; l = 1 см

3. 3. Influence of diluents

The suitability of diluents was investigated using organic solvents such as chloroform,ethyl acetate, isoamyl alcohol, xylene, hexane, diethyl ether, toluene, *n*-butanol, carbon tetrachloride, nitrobenzene, etc. Within compounds of one class extraction ability decreases with increasing number of carbons in the solvent molecule. The best solvents are chloroform, dichloroethane and carbon tetrachloride. The extraction of Iron (III) was quantitative with L in chloroform. Hence, chloroform was used for further extraction studies as it gave better and quicker phase separation. After a single extraction with chloroform, 97.5 % of iron was extracted as an complex (in a case the dichloroethane and carbontetrachloride was removed 95.2 % of iron). After separation of the two phases, Fe(III) in each phase was determined by salisylic acid [14].

3. 4. Effect of salting out agents and of temperature

The presence of 0.1 M salts of various alkali and alkaline metals does not show any effect over the absorbance value of Fe(II)-L complexes extract. Therefore, no salting out agent was required during the extraction.

Effect of various temperatures (10-80° C) on the Fe(III)-L system was studied. The Fe(III)-L system attained maximum and constant absorbance at room temperature (25° C).

3. 5. Effect of reagent concentration

Various volumes of 0.01 mol l^{-1} reagent solution were added to the sample solution containing 50 µg of Iron at respective pH values. The absorbance remained nearly constant when the volume of the reagent solution used was more than 2 ml. Therefore, 2.0 ml of 0.01 mol l^{-1} reagent L was chosen for the quantitative determination of the metal. For the formation and extraction of of complex Fe-L are provided by 8.0×10^{-4} M L. It was found that the presence of excess of the reagent solution does not alter the absorbance of the color reaction.

3. 6. Effect of equilibration time and stability of the complex

The study of change in absorbance with variation in equilibrium time for extraction of extraction of the complex into organic solvent shows that equilibration time of 5 min is sufficient for the quantitative extraction of Iron. The study of stability of colour of the Fe(III)-L complex with

respect to time shows that the absorbance due to extracted species is stable up to 48 hours, after which slight decrease in absorbance is observed. Throughout the experimental work, for practical convenience, the measurements have been carried out within one hour of extraction of Iron. Complex of Fe(III) with L not decompose over a month after extraction.

3. 7. Stoichiometry of the Complexes and the Mechanism of Complexation

The stoichiometry of the complex have established an equilibrium shift method and confirmed the methods of relative yield, Asmus straight line and the intersection curves [15]. The data shown in Fig. 3 shows that the composition ratio Fe: L = 1: 2.

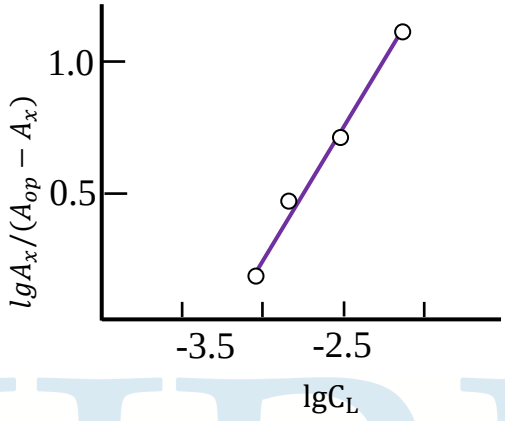


Figure 3. Determination of the ratio of components by equilibrium shift method for Fe -L
C_L = 8.0×10^{-4} M; pH=5, 540 nm, l=1см.

The Infrared spectrum of the ligand (L) was compared with the spectra of its Fe(III)-L complex. Were observed bands at 1593-1448 cm^{-1} (C=C), 1745 cm^{-1} (C=O), 3028 cm^{-1} (Ar) [16,17,18].

New bands were observed between 400 - 600 cm^{-1} region in the complex, which were absent in the spectrum of ligand. The bands between 440 cm^{-1} were assigned to stretching frequencies of υ(Fe-O) and the band between 573 cm^{-1} have been assigned to the stretching frequencies υ(Fe-N) respectively.

The iron content in the complexes was determined after their decomposition aqua regia photometrically using phenantroline. The purity of the compound was checked by the elemental analysis. Elemental analysis individually complexes are given in Table 1.

Table. 1. Elemental analysis of ligand L and complex Fe-L

Compound	%	C	H	N	Fe
L	Found	54.48	3.25	6.47	-
	Calculated	54.29	3.19	6.33	-
Fe- L	Found	46.83	2.62	5.67	10.95
	Calculated	46.69	2.53	5.45	10.89

Thermogravimetric study of the complex Fe-L shown that thermal decomposition of the complex takes place in two stages: at 80-130^{o}C water evaporates (weight loss – 3.35%) at 410-470^{o}C-decomposed L (weight loss -82.90%). The final product of the termolysis of the complex is Fe_2O_3.

Given the molar ratio of components in the complexes, the complexing form of central ions, monomeric complexes in the organic phase, IR spectroscopic data, thermogravimetric studies and chemical analysis, it can be assumed that the Fe (III) with L forms mixed ligand complexes (Figure 4).

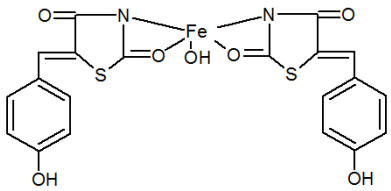


Figure 4. Structure of complexes Fe-L

Constant of stability of complex are determined by method of crossing of curves [30]. It was found using the Nazarenko method that Fe(III) in the complexes was present in the form of $FeOH^{2+}$. The number of protons replaced by iron in one L molecule appeared to be one [19,20].

Additional experiments by the Akhmedly's method [21] showed that the complex exists in monomeric form in the organic phase (the obtained coefficient of polymerization γ was equal to 1.09.

3. 8. Effect of divalent ions and foreign ions

The influence of the presence of diverse ions on the absorbance value of Fe(III)–L complex system was studied with 50 µg Fe(III) in the presence of foreign ions. The results are summarized in Table 2. The ions which show interference in the spectrophotometric determination of Iron were overcome by using appropriate masking agents. Large amounts of alkali and alkaline-earth metals and REE do not interfere with the determination of iron.

Co(II). Ni(II), Cu(II), V(IV,V), W(VI), Mo(VI), Ti(IV), Mn(II) and etc. interfere determination of Fe(III). The interfering effect of Zn(II), Mn (II), Co(II), Ni(II), Cd(II) and Ag(I) in the determination of the iron eliminated by precipitation Fe(III) with ammonia. Among the anions studied, thiosulphate, oxalate, citrate and thiocyanate show severe interference at all levels of concentration. In order to eliminate interference of V(V) and Mo(VI), H_2O_2 and NaF were used as masking agents, respectively. When using a small amount of 0.01M solution EDTA, determination of Fe(III) do not interfere with Ti(IV), V(IV), Nb(V), Ta(V), Mo(VI).

Table 2. Influence of interfering ions on the determination of Fe(III) with L (50.0 µg Fe added)

Ion	Molar excess of the ion	Masking agent	Found Fe, µg	RSD(%)
Co(II)	25	Ascorbic asid	50.3	4
Ni(II)	25		49.7	2
Cd(II)	200		49.5	4
Bi(III)	200		50.2	2
Cu(II)	15	Sodium thiosulphate	49.5	4
Zr(IV)	50		49.8	3
W(VI)	25	Oxalic acid	49.6	5
Hg(II)	40	$Na_2S_2O_3$	50,3	5
Ti(IV)	30		49.8	3
V(IV)	40	H_2O_2	50.4	4
Mo(VI)	25	NaF	50.2	6
Mn(II)	50			
Nb(V)	60	NaF	50.2	4
Ta(V)	60	NaF	49.6	6
UO_2^{2+}	50		50.5	3
Ag(I)	25	KBr	49.5	4

Ammonium(I)	1000			
Acetate	100		50.4	5
Tartarate	300		50.3	5
Sulphate	125		49.8	4
Thiourea	25		49.7	5
Fluoride	110		50.3	5
Oxalate	50		49.8	3
Thiocyanate	30		49.6	5
Iodide	100		50.3	6
Ascorbic acid	300		49.8	3
Nitrate	1000		50.6	2
Cyanide	80		49.8	3
Citrate	50		49.6	5
EDTA	90		50.3	5
Salisil asid	10		49.8	5

The proposed method compares favourably with the existing ones (Table 3) and offers the advantages of better simplicity, rapidity, sensitivity and selectivity.

Table 3. Comparative characteristics of the procedures for determining of iron.

Reagent	pH (solvent)	λ, nm	$\varepsilon \cdot 10^{-4}$
Gallic acid + aniline[22]	4-5 (*n*-amyl alcohol))	560	0,44
Sulfosalicylic acid[14]	1,2	528	0,38
Phenanthroline [14,23]	2-9 (Isoamyl alcohol))	512	1,10
Batofenantroline[14,23]	4-7(Chloroform - ethanol)	533	2,24
L	3.8-5.2 (Chloroform)	535	3.29

3. 9. Calibration plot

A calibration plot of absorbance against varying Iron concentration and fixed L concentration gives linear and reproducible graph in the concentration range 0.05 to 3.2 ppm of Iron. This shows that the Beer's law is obeyed in this range [24].

The detection limit indicates the smallest amount of analyte which can be detected with a reasonable degree of confidence under specified conditions. The equations of the obtained straight lines and some important characteristics concerning the application of the ternary complexes for extractive-spectrophotometric determination of Fe(III) are listed in Table 4.

In conclusion the analytical parameters pertaining to the proposed method are given in Table 4.

Table 4. Optical characteristics, precision and accuracy of the spectrophotometric determination of Fe(II) with L

Parameter	Value
Color	red
The pH range of education and extraction	3.0-9.0
The pH range of maximum extraction	3.8-5.2
λmax (nm)	535

Molar absorptivity (L· mol^{-1} cm^{-1})	$3.29 \cdot 10^4$
Sandell's sensitivity (ng·cm^{-2})	1.7
R,%	97.5
The equation of calibration curves	0.032+0.27x
Correlation coefficient	0.9975
lg k_e	6.25
Stability constant (β)	9.5
Beer's law range (μg·ml^{-1})	0.05-3.2
Limit of detection (LOD): ng ·mL	12
Limit of quantification (LOQ): ng ·mL^{-1}	39

3. 10. Applications

Various commercial samples and synthetic mixtures containing Fe(III) were prepared and analyzed according to the recommended procedure and the results were compared to those obtained by standard method. The proposed methods facilitates separation of Fe(III) from in Pharmaceutical Samples, in soils, in blood and urine samples water samples, in some food samples. The results found to be in good agreement with those obtained by the standard known method [10] (Table 5).

Table 5. Determination of Fe(III) in Pharmaceutical Samples

Pharmaceutical Samples	Found, Fe(III)	RSD(%)
Dexorange	32.6 mg	4.5
Autrin capsule	98.5 mg	4.8
Fefol	149 mg	3.9
Dexorange	49.6 μg/ml	3.6
Autrin capsule	1.3 mg/ml	4.1

Average of three determinations

Table 6. Determination of iron in some food samples. (n = 6, P = 0.95)

Методика	Found, Fe(III)	S	RSD(%)	$\overline{X} \pm \frac{t_P \cdot S}{\sqrt{n}}$
strawberries mg/kg				
Sulfosalicylate	11.2	0.470	4.2	11.2±0.50
DMP+BPhen	11.4	0.410	3.6	11.4±0.43
DMP+BPhen	11.3	0.282	2.5	11.3±0.30
DMP+BPhen	11.4	0.364	3.2	11.4±0.38
briar (mg/kg)				
Sulfosalicylate	13	0.405	3.2	13±0.430
DMEP+BPhen	15	0.420	2.8	14±0.441
wild strawberry (мg/кg)				
Sulfosalicylate	12	0.348	2.9	12±0.366
DMEP+Phen	11	0.495	4.5	11±0.519
beef ((mg/ml)				
Sulfosalicylate	2.92	0.134	4.5	2.92±0.14
Sulfosalicylate	2.95	0.127	4.3	2.95±0.13
DMMP+Dip	2.94	0.103	3.5	2.91±0.11
DMEP+Dip	2.93	0.120	3.9	2.92±0.13
cow's milk (mg/ml)				
Sulfosalicylate	3.88	0.163	4.2	3.88±0.17
DMMP+Dip	3.94	0.123	3.3	3.93±0.14
DMEP+Phen	3.91	0.135	3.4	3.94±0.14

Egg(µg/kg)				
Sulfosalicylate	70	1.75	2.5	70±1.83
DMEP+Phen	68	1.56	2.3	68±1.64

4. CONCLUSION

The results obtained show that L in chloroform can be effectively used for quantitative extraction from aqueous media. The reagent L gave instantaneous and stable red colour with iron (III) in the pH range 3.8 to 5.2. The Beer's law was applicable in the range of 0.05 - 3.2µg/ml at 535 nm. The proposed method is found to be quantitative as compared to other standard methods. The equilibrium time required is 5 min and the complex is stable for 48 hrs. The results show good agreement with the standard method. The method is very fast, accurate and precise. The proposed method shows good selectivity.

A new, simple, sensitive, selective, and inexpensive method with the Fe(III)-L complexes was developed for the determination of iron in some industrial, biological, soil, and environmental samples.

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