Spectrophotometric Assay of Iron (II) in Pharmaceutical Formulation Using Alizarin Red Sulphonate Reagent

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الخلاصة

تم تطوير طريقة طيفية سهلة وسريعة وحساسة لتقدير كميات نزرة من الحديد الثنائي في المحلول المائي. تعتمد الطريقة على تفاعل الحديد الثنائي مع كاشف اليزارين احمر السلفونات (ARS) وتكوين معقد كيليتي بني اللون في المحلول المائي يمتلك اقصى امتصاص عند طول موجي مقداره 566 نانوميتر وبامتصاصية مولارية 7.8×10^3 لتر. مول$^{-1}$. سم$^{-1}$ وان قانون بير ينطبق ضمن مدى التراكيز (0.5-5) مايكروغرام.مللتر$^{-1}$. لقد كان معدل نسبة الاسترجاع 100.06% في حين كان الانحراف القياسي النسبي اقل من 1%. وطبقت الطريقة بنجاح في تقدير الحديد في حا لته النقية وفي المستحضرات الصيدلانية (اقراص وكبسولات) وتم مقارنة النتائج مع الطريقة القياسية المعتمدة في دستور الادوية البريطاني.

Abstract

A rapid, sensitive and simple spectrophotometric method was developed for the determination of iron (II). The method was based on the reaction of iron (II) with alizarin red sulphonate reagent to form a brown chelating complex in an aqueous solution. The absorbance of the chelating complex was measured at 566 nm with a molar absorptivity of 7.8×10^3 $l.mol^{-1}.cm^{-1}$. The chelating complex conforms to Beer's law over the range (0.5-5) $\mu g.ml^{-1}$. the average recovery% was 100.06% and

precision (RSD) was found to be less than 1%. The method was successfully employed for assay of iron (II) in pharmaceutical formulations (tablets and capsules). The results have been compared with British pharmacopoeia method.

Introduction

Iron is one of the most important essential elements. Its deficiency or overload may cause health problems. Speciation of iron, occurrence of the element in two oxidation states (II, III) and equilibrium between these forms are important for biological systems using iron for metabolic processes[(1)].

Spectrophotometric method used for the determination of Iron (II) in pharmaceutical formulations by it's oxidation in the iron (II)/thiocyanate/acetone system at 480nm with a molar absorptivity of $2.10x\ 10^4$ l. $mol^{-1}.cm^{-1}$[(2)].

Spectrophotometric method was described for the determination of iron (II) with salicylic and eight mono-substituted salicylic acids in aqueous-acetone solution at pH range 2-3. Beer's law was followed in the range of (1-60)μg.ml^{-1}. The molar absorptivities of the complexes were 6796 to 47393 l. $mol^{-1}.cm^{-1}$[(3)].

Development of indirect spectrophotometric method for the determination of iron (II) in aqueous solution. The method based on the reaction of iron (III) which produces from the oxidation of iron (II) by hydrogen peroxide) with 3,5-dinitosalicylic acid (DNS) reagent at pH 2.92 to form orange coloured complex at 475nm. with a molar absorptivity 7550 l. $mol^{-1}.cm^{-1}$. This method was applied successfully for the assay of Fe (II) in various pharmaceutical preparations[(4)].

A spectrophotometric method described for the determination of iron (II) by its oxidation to iron (III) by tetrahydrofuran/water and then reacted with azide reagent to form coloured complex at 396 nm[(5)].

Iron (II) has been determined by sequential injection analysis method. The method based on the reaction of iron (II) with 1,10-phenanthroline to form a reddish orange complex which shows maximum absorption at 512 nm[(6)].

Spectrophotometric method for determination of iron (II) was described. The method based on the reaction of iron (II) (after reduction of iron (III) with ascorbic acid) with 3-mercapto-5-(2,4-dihydroxyphenylazo-1)-1,2,4-triazol (METRIAP) and 5-(5-mercapto-1,3,4triadiazolo-2-azo)-2,4-dihydroxybenzoic acid ((METIADAREZ-β) at pH 7.4 to form coloured complexes at 490, 600 nm respectively[(7)].

The applicability of derivative spectrophotometry for simultaneous determination of zinc(II), manganese(II) and iron(II) in the form of 4-(2-pyridyl azo)resorcinol (PAR) complexes was presented and discussed. Beer's law was obeyed in range 0.025–0.2 for iron ion. The method was

applied successfully for determination of mentioned ions in pharmaceutical preparation without previous separation[8].

The complexing reagent 2 – thiophenaldehyde – 4 – phenyl – 3 - thiosemicarbazone (TAPT) was examined for high performance liquid chromatographic (HPLC) separations of cobalt(II), copper(II) and iron(II) as metal chelates on a Microsorb C-18, 5-μm column UV detection was at 254 nm with detection limits within 0.5–2.5 $\mu g.ml^{-1}$ in the final solution. The method was applied for the determination of copper, cobalt and iron in pharmaceutical preparation[9].

The reactions of bis(salicycladehyde)tetramethylethylenediimine (H_2SA_2Ten) with cobalt(II), cobalt(III), iron(II) and iron(III) were studied. They were completely separated on a 3-μm Microsorb ODS column with spectrophotometric detection at 270 nm. The detection limits were in the range 0.25–1.0 $\mu g.ml^{-1}$. The method was applied to the determination of cobalt and iron in pharmaceutical preparations[10].

The use of azo dye, namely 2-mercapto-5-(2,4-dihydroxy-5-carboxyphenylazo-1)-1,3,4-tiadiazole (METIDAREZ-β) was proposed for the spectrophotometric determination of Fe(II) in the pharmaceutical multivitamin preparations[11].

In this work a spectrophotometric method has been developed for determination of iron (II) based on chelating reaction with alizarin red sulphonate reagent in neutral aqueous solution and applied successfully in pharmaceutical applications (tablets and capsules).

EXPERIMENTAL

Apparatus

A computerized Shimadzu UV-1650 a digital double beam spectrophotometer with 1-cm matched quartz cells was used for all spectral and absorbance measurements.

Reagents

All chemicals used were of the highest purity available.

Alizarin red sulphonate (ARS) $1x10^{-3}$ M solution:

This solution was prepared by dissolving 0.0856 g of ARS in absolute ethanol in 250 ml volumetric flask. This solution was kept in brown bottle and it was stable for at least one month.

Ferrous sulphate solution(1000 $\mu g.ml^{-1}$)

This solution was prepared by dissolving 0.4964 gm of $FeSO_4.7H_2O$ in 100 ml boiling distilled water (this solution was standardized by titration with 0.01 N potassium permanganate in acidic medium).From this solution 100 $\mu g.ml^{-1}$ was prepared by dilution with distilled water[12].

Procedure for calibration

To a series of 10 ml calibrated flasks, transfer an increasing volumes of 100 $\mu g.ml^{-1}$ of iron (II) solution to cover the concentration

range (0.5-5) μg. $ml^{-1,}$ followed by addition of 2.5 ml of $1x10^{-3}$ M ARS, the solution then diluted to the mark with distilled water and absorbance measured at 566 nm after 20 minutes at room temperature against the reagent blank.

Analysis of tablets

Ten tablets weighed accurately (each tablet contains 200 mg dried ferrous sulphate which is equivalent to 64 mg elemental iron) crushed, mixed well then a weight equivalent to one tablet was dissolved in 100 ml distilled water, 0.5 gm of charcoal was added, the solution was heated for 10 minutes for colour removal then the solution was filtered and the filtrate was transferred to 500 ml volumetric flask and completed to the mark with distilled water to obtain 128 μg. ml^{-1}.From this solution 100 $\mu g.ml^{-1}$ was prepared by dilution and followed the procedure for calibration to determine iron (II) in tablets.

Analysis of capsules

Ten capsules were weighed accurately (each capsule contains 150 mg dried ferrous sulphate equivalent to 47 mg elemental iron), weight equivalent to one capsule was dissolved in 100 ml boiled distilled water, 0.5 gm of charcoal was added, the solution was heated for 10 minutes for colour removal then filtered and the filtrate was transferred to 250 ml volumetric flask and completed to the mark with distilled water to obtain 188 μg. ml^{-1}. From this solution 100 μg. ml^{-1} solution was prepared by dilution and followed the procedure for calibration to determine iron (II) in capsules.

Results and discussion

Preliminary investigation

When a solution of iron (II) and alizarin red sulphonate reagent were mixed in a neutral aqueous solution a brown solution was observed with maximum absorption at 566 nm in contrast to the reagent blank which shows a maximum absorption at 420 nm Figure (1).

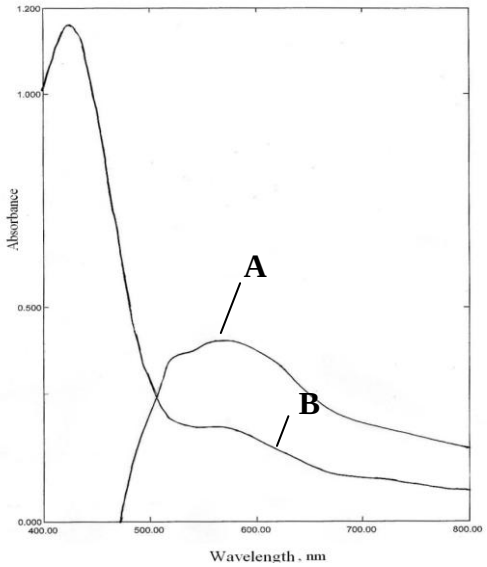


Figure (1): **Absorption spectra of A-3 μg. ml^{-1} iron (II) – ARS (1×10^{-3} M) product versus reagent blank and B-Reagent blank versus distilled water**

Optimization of conditions

Since ARS behaves as an acid base indicator so neither acid nor base could be used in the reaction medium[13].

Effect of temperature and reaction time

The reaction time was determined by the colour development at room temperature and in thermostatically controlled water-bath adjusted at 40 and 50 °C. The absorbance was measured at 10 minutes intervals against reagent blank. It was observed that the absorbance was reached maximum after 20 minutes at room temperature (RT) and remains constant more than 2 hrs. RT and reaction time 20 min were chosen for colour development (Table 1).

Table (1): Effect of temperature and reaction time

Temp. (°C)	Absorbance									
	Time (min)									
	10	20	30	40	50	60	90	100	120	150
0	0.289	0.291	0.300	0.295	0.311	0.310	0.292	0.298	0.298	0.295
RT	0.292	0.334	0.334	0.334	0.334	0.334	0.334	0.332	0.333	0.315
40	0.291	0.311	0.310	0.309	0.309	0.310	0.308	0.308	0.307	0.307
50	0.290	0.298	0.297	0.298	0.298	0.298	0.296	0.294	0.295	0.294

Effect of ARS concentration

The effect of different ARS concentrations on the absorbance of solution containing 3 μg. ml^{-1} iron (II) was studied, it is evident that the absorbance increases with increasing ARS concentration and reached maximum on using 2.5 ml of $1x10^{-3}$ M ARS. Therefore, this concentration was used in all subsequent work (Table 2).

Table (2): Effect of ARS concentration

ARS solution 1×10^{-3} M (ml)	1.5	2	2.5	3	3.5
Absorbance	0.281	0.334	0.420	0.366	0.332

Effect of surfactant

The effect of different types of surfactants were used for the improvement of the absorption intensity, but none of them improve the absorption intensity therefore they were excluded from this study (Table 3).

Table (3): Effect of surfactant

Surfactant	Absorbance/ml			
	0.5	1	2	3
Cetyltrimethyl ammonium bromide (0.1%)	0.417	0.415	0.415	0.413
Sodium dodecyl sulphate (0.1%)	0.418	0.416	0.417	0.412
Triton x-100 (1%)	0.417	0.413	0.415	0.413
Without surfactant	0.420			

Calibration graph

Under the optimum conditions, a linear relationship between the absorbance and the concentration of iron (II) was observed cover the concentration range (0.5-5) μg. ml^{-1} (Fig.2) with correlation coefficient of 0.9998. A negative deviation from Beer's law was observed at higher concentrations of iron (II). The molar absorptivity was $7.8x10^3$ l. mol^{-1}. cm^{-1}.

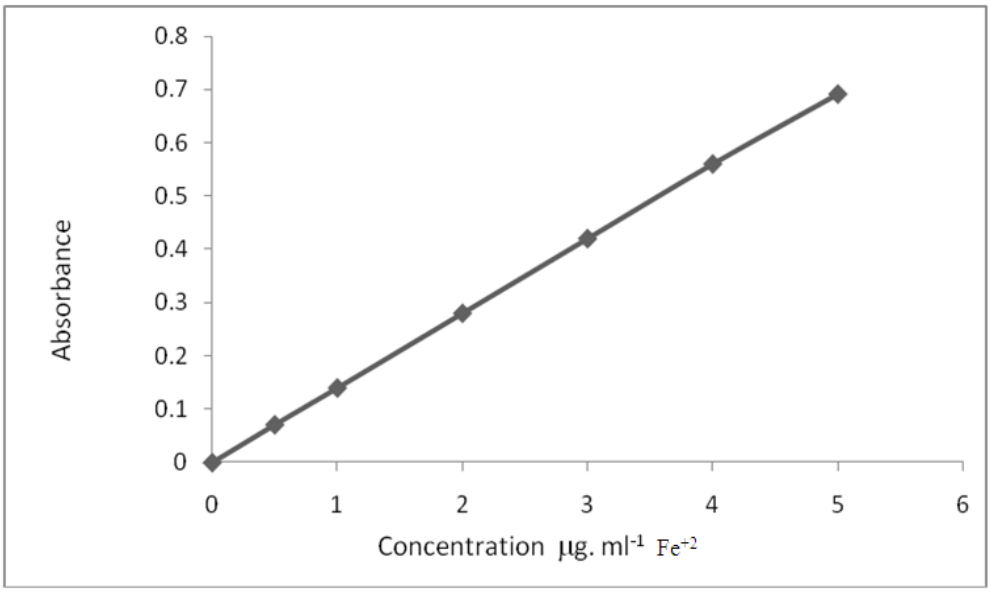


Figure (2): Calibration graph of iron (II)

Accuracy and precision

To determine the accuracy and precision of the method, iron (II) was determined at three different concentrations. The results in table (4),showed that the accuracy (average recovery%) was 100.06% and the precision (RSD) < 1%.

Table (4): Accuracy and precision of the present proposed method

Amount of iron (II) taken μg. ml^{-1}	Recovery*%	Relative standard deviation* (RSD%)
2	100.36	0.94
3	99.76	0.56
4	100.06	0.54

* Average for six determinations

Effect of interferences

To check the selectivity of the method, 3 μg. ml^{-1} of iron (II) was determined using the recommended procedure in presence of foreign compounds usually present in pharmaceutical formulations. The results didn't show any interfering effect on the present method which indicate that the method is selective (Table 5).

Table (5): Effect of interferences

Foreign compounds	Fold excess	Recovery %
$ZnSO_4.7H_2O$	10	100.0
	20	101.5
	30	102.2
Folic acid	10	101.1
	15	102.0
	30	102.3
Glucose	10	100.0
	20	99.9
	50	97.6
Starch	10	98.5
	15	98.6
	20	98.7
Glycerol	10	100.0
	20	101.2
	30	101.5
Acacia	10	101.1
	20	101.6
	50	102.0
lactose	10	101.2
	15	101.7
	30	102.3

Nature of product and reaction mechanism

The stochiometry of the reaction between iron(II) and ARS was investigated using Job's method[14]. The results obtained show that 1:2 iron (II): ARS ratio was formed (Fig.3).

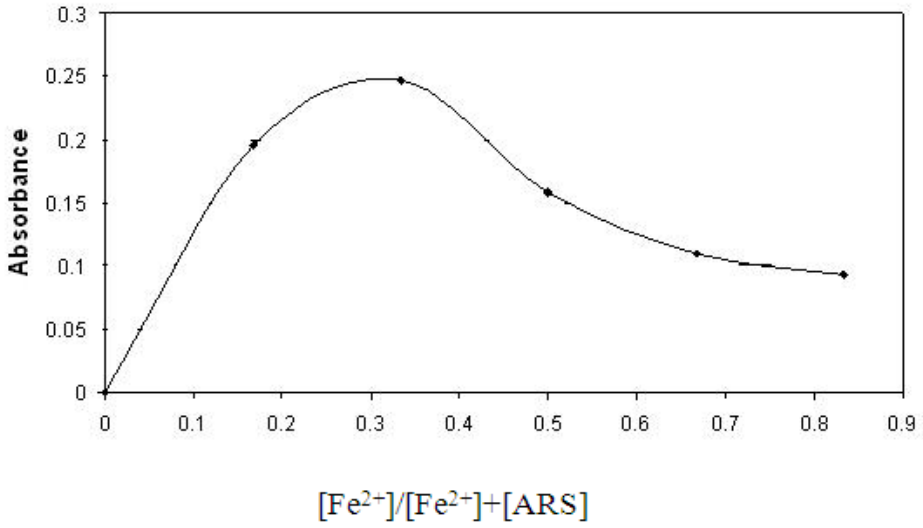
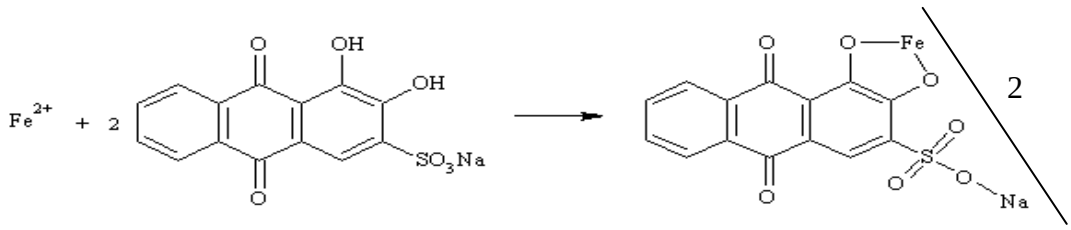


Figure (3): Job's method for iron (II)-ARS product

Therefore, the formation of the product may be occur as follows



The stability constant of the product was estimated and found to be $4.25x10^{10}$ $l^2.mol^{-2}$.

Analytical applications

Five types of pharmaceutical formulations containing iron (II) have been analyzed and they gave a good accuracy (Table 6). The proposed method was compared successfully with British pharmacopoeia standard method [15] (Table 7), since the T-test at three degree of freedom and 95% confidence limit showed that there was no significant differences between the proposed method and the standard method.

(t-exp.=0.33, t-tabulated= 3.18).

Table (6): Application of the proposed method for determination of iron (II) in pharmaceutical formulations

Pharmaceutical preparation of and company	Wt of tablet (mg)	Certified value of iron (II) (mg)	Amount of iron (II) present ($\mu g.ml^{-1}$)	Recovery* %	Drug content found (mg)
Ferrous sulphate tablets Ajanta pharma limited, India	200	64	2 3 4	100.71 99.29 100.36	64.45 63.55 64.23

Ferrous sulphate folic acid tablets Holden medical BV, Lelystad. Netherlands	200	65	2 3 4	100.35 100.95 99.46	65.22 65.62 64.65
Ferrous sulphate folic acid capsules EIPCO / EGYPT	150	47	2 3 4	99.28 99.05 100.54	46.66 46.55 47.25
Folicron Folic acid + iron Julphar, Gulf pharmaceutical industrial, Ras Al Khaimah, U.A.E	650	47	2 3 4	98.57 99.28 100.18	46.33 46.66 47.08
FEFOL-Z Ferrous sulphate, folic acid and zinc sulphate Manufactured by Avenzor S.A.R Licenced by Glaxosmithkline, U.K	650	47	2 3 4	100.71 99.52 99.82	47.33 46.77 46.92

Table (7): Comparison of the proposed method with standard method for the determination of iron (II) in pharmaceutical formulations

Iron (II) pharmaceutical formulation	Present method		British pharmacopoeia	
	Recovery* %	Drug content found mg	Recovery* %	Drug content found mg
Ferrous sulphate tablets	100.71	64.45	99.86	63.91
	99.29	63.55	100.45	64.29
	100.36	64.23	99.91	63.94
Ferrous sulphate folic acid tablets	100.35	65.22	100.51	65.33
	100.95	65.62	99.97	64.98
	99.46	64.65	99.86	64.91
Ferrous sulphate folic acid capsules	99.28	46.66	100.97	47.46
	99.05	46.55	99.91	46.96
	100.54	47.25	99.87	46.8
Folicron	98.57	46.33	99.12	46.62
	99.28	46.66	100.23	47.10
	100.18	47.08	100.68	47.32
FEFOL-Z	100.71	47.33	99.95	46.98
	99.52	46.77	100.14	47.07
	99.82	46.92	100.25	47.12

*Average of six determinations

Comparison of methods

The results obtained by application of the present method and literature method to the determination of iron (II) in pharmaceutical preparations were given in table (8).

Table (8): Comparison of methods

Analytical parameter	Present method	Literature method[4]
λ_{max} (nm)	566	475
Temp (°C)	R.T	R.T
pH	-	2.92 (phthalate buffer)
Development time (min)	20	5.0
Period time (min)	120	180
Molar absorptivity l. mol^{-1}. cm^{-1}	7800	7550
Linear range (µg. ml^{-1})	0.5-5	0.05-6.0
Average recovery (%)	100.06	99.87
RSD (%)	<1	<1
Analytical application	Tablets, Capsules	Tablets

It is evident from the table that the results compared favorably between the two methods in all analytical parameters but the present method does not require buffer solution.

Conclusion

The reported method is simple and sensitive. The product formed is stable for at least 120 min., thus permitting quantitative analysis to be carried with good reproducibility, also the reported method does not require neither buffer solution nor solvent extraction.

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