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EXTRACTIVE SPECTROPHOTOMETRIC DETERMINATION OF IRON (II) USING AN ION ASSOCIATION COMPLEX FORMATION

Alaa S. Amin*, Mamdouh M. Metwally, Magda M. El-Henawee, and Wafaa S. Hassan
*Chemistry Department, Faculty of Science, Benha University, Benha
Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

ABSTRACT

Spectrophotometric determination of micro amount of iron has been developed using a solvent extraction of an ion association complex of ferrous ion. The method is dependent on the formation of an ion associate complex of iron(II) with ethylene diamine disuccinic acid as a primary ligand and cetyl trimethyl ammonium bromide (CTMA) as counter ion, which is extracted into 1,2-dichloroethane (DCE). The complex is formed at pH = 5.0-7.0 and the iron concentration can be determined by measuring the absorbance directly in the organic phase. The apparent molar absorptiviy is 1.73×10^5 L mol^{-1} cm^{-1} and Sandell sensitivity is 0.32 mg cm^{-1}, free from interferences. The proposed method was applied to the determination of iron in water, fruit juice, wine, food minerals and pharmaceutical formulations.

INTRODUCTION

Iron is an important element in environment, industry, medicine and in biology. It is present in a variety of rock and soil minerals in oxidation states 2 and 3[1]. Both iron(II) and iron(III) play a major role in the biosphere, being involved in oxygen transport and electron transfer and in enzymes including hydroxylases, peroxidases and dismutases[2]. For the human body, iron is a key element in nutritional and has a central role in energy metabolism[3]. On the other hand, the toxicity of iron[4], and in particular iron overload[5], have aroused considerable interest in recent years. Moreover iron plays an essential role in photosynthesis[6]. The reported spectrophotometric methods for the determination of iron, based on complexation reactions with various organic reagents such as 4-(pyridylazo) resorcinol (PAR)[7], chrome azural S,[8,9] chromol blue G[10] pyrocatechol violet[11,12], bromo-pyrogallol red[13,14], Eriochrome cyanine R[15], 4,7-diphenyl-1-10-phenanthroline[16], haematoxylin[17], 2-bromo-4,5-dihydroxy-azo-benz-ene-4-sulphonate[18] and thiocyanate[19] in micellar media, are good examples of sensitive but rather non-selective methods. Also most of those methods require a preliminary step to separate iron from complex matrix. On the other hand, morin has been widely used for the spectrophotometric determination of metal ions[20,21]. However, the sensitivity of the iron-morin system is low ($\varepsilon = 9.68 \times 10^3$ L mol^{-1} cm^{-1} at 400 nm)[22]. Hence, there is a search for a simple spectrophotometric determination of iron in natural samples. In recent years, systems containing a metal ion, an organic chromophore reagent and a surfactant have been used widely and effectively in spectrophotometric determination of trace amount of metal ions[23-26] in these system, the presence of the surface produces bathochromic and hyperchromic shifts in the absorption spectra and facilities determination of metal ions in aqueous solution. In the present paper, a sensitive, selective and accurate spectrophotometric method for determination of iron with ethylene diamine disuccinic acid is presented.

EXPERIMENTAL

Apparatus:

The absorbance measurements were performed on a Perkin-Elmer Model Lambda 3B double beam UV-visible spectrophotometer fitted with matched 10-mm quartz cells. The pH measurements were carried out with an Orion research Model 601 A/digital ionalyzer pH-meter equipped with a combined glass-calomel electrode system.

Chemicals :

All chemical used were of analytical-reagent grade unless indicated otherwise.

Working standard solutions :

1. Standard iron(II) solutions (RIEDEL-DE HÄÉN AG SEELZE-HANNOVER). A 500 µg mL^{-1} solution was prepared by dissolving 3.511 g of ammonium iron(II) sulphate hexahydrate in 5.0 mL of 10.0 M sulphuric acid and diluting to volume in a one liter flask. The solution was standardized complexometrically[27]. A 10 µg mL^{-1} solution was prepared by diluting this solution, and other ranges of iron concentrations were prepared by appropriate dilution. All those solutions were stored in iron-free glass bottles.
2. Ethylene diamine disuccinic acid (Aldrich product) solution. An approximately 2×10^{-3} M solution was prepared by dissolving 0.070 g of the compound in a few millilitre ofethanol and then diluted to the mark in 250 mL calibrated flask with ethanol.
3. 10% Hydroxylammonium chloride solution (RIEDEL-DE HAEN AG SEELZE-HANNOVER) prepared by dissolving 50 g of the salt in 500 mL of distilled water.
4. Serial buffer solutions of pH =2-12, were prepared using the previously recommended method[28].
5. Stock surfactant solution, A 1×10^{-2} M of the following agents were prepared, cetylpyridinium chloride (CPC), sodium dodecyi sulphate (SDS), polyoxyethylenedocylether (PO10), hexadecyl-trimethyl-ammonium bromide (HTAB) and cetyl-

trimethylammonium bromide (CTMA) (Aldrich), methyltrioctylammonium chloride (MTOA), Triton X-100 and Tween-80 (sigma).

6. Foreign ion solutions. Solutions of diverse ions for interference studies were prepared by dissolving the amount of each compound need to give 10-1000 μg mL^{-1} concentrations of the ion concerned.

General procedure:

An aliquot of sample solution containing not more than 160 μg of iron, 3.0 mL of the buffer solution, pH 6, 5 mL of hydroxylammonium chloride solution and 5.0 mL of 2×10^{-3} M ethylene diamine disuccinic acid solution and 2.0 mL of 10^{-2} M CTMA. The total volume was adjusted to 50 mL with water, mixed well and sited a side for 3.0 min. Then 5.0 mL of 1,2-di-chloroethane was added and shaken for another 3.0 min., then the organic layer was separated. The absorbance of the orange extracted layer was measured at 514 nm against a reagent blank prepared under the same conditions.

Analytical applications:

1. Analysis of Nile river water:

River water was filtered immediately after sampling, acidified and boiled for 10 min. Aliquots of the solution were used to determine iron as recommended above.

2. Analysis of fruit juice:

The juice was filtered and acidified with a few drops of hydrochloric acid. An aliquot of juice containing 5.0-100 μg of iron was analyzed using the general procedure described above. The absorbance was measured against a blank prepared by a similar treatment of the same volume of the juice.

3. Analysis of vegetables and fruits:

The following method was applied to determine iron in spinach and lentils. About 20 g of spinach or lentils, after drying at 100°C, was calcined and treated by heating with 10 mL of concentrated hydrochloric acid. The solution was filtered and carefully poured into 100 mL calibrated flask. Aliquots of the solutions were used for the above recommended procedure.

4. Analysis of magnesites:

About 0.5 g of sample was treated with 10 ml of concentrated perchloric acid by heating. The solution was cooled and diluted in order to obtain the required concentration of iron in the stock solution. To an aliquot of the solution, the above general procedure was applied.

5. Analysis of portland cement:

About 0.25 g of sample was mixed with 0.5 g of ammonium chloride, heating in a water bath for 30 min, 20 mL of hot water was added and the solution was filtered and carefully washed into a 250 mL calibrated flask. Aliquots of the solution were used to estimate iron by applying the above recommended procedure.

Analysis of pharmaceutical formulations:

A weighed amount of the sample was transferred into a conical flask and heated gently with a mixture of concentrated nitric and sulphuric acid (10+1) until charring commenced. Dropwise addition of concentrated nitric acid and boiling were continued until either a colourless or a pale yellow solution was obtained. This solution was diluted to an appropriate volume. A blank digestion was conducted in the same way. An aliquot of the solution was taken and iron was determined by applying the general procedure described above.

RESULTS AND DISCUSSION

Spectral characteristics:

The absorption spectra of the ethylenediamine discuccinic acid, binary complex of iron-EDDS and iron-EDDS, CTMA ion-associate were measured (Fig. 1). EDDS showed a band at 404 nm, whereas that for binary complex at 488 nm using reagent as blank. The ion-associate shows an absorption band at 514 nm using a blank similarly prepared without iron. It can be observed that the EDDS do not absorb at 514 nm when the iron concentration was increased, a significant change was observed in the absorbance measured at 514 nm.

Composition and characteristics of the complex:

The ion-associate isolated from the aqueous solution was analysed and some of its properties were studied. The molar ratio of EDDS to iron was found to be 2:1. Also, the Yoe and Jones molar ratio method was used to determine the composition of the ion-associate, and a 1:2 molar ratio of iron. EDDS complex to CTMA was found. From these results, the composition of the ion-associate complex is assumed to be $Fe(EDDS)_2(CTMA)_2$. The isolated complex is stable for more than 24 hours in aqueous solution.

$$Fe^{2+} + 2EDDS \longrightarrow [Fe(EDDS)_2]^{2+}$$

$$[Fe(EDDS)_2]^{2+} + 2\,CTMA \longrightarrow [Fe(EDDS)_2][CTMA]_2$$

Choice of extractant:

It was found that the complex can be extracted into chloroform, 1,2-dichloromethane (DCM) and 1,2-dichloroethane (DCE). The absorbance values for DCM and DCE solutions were higher than those for chloroform solutions. DCE was selected where it's less volatile than DCM.

Effect of reagent concentration:

The effect of the concentration of demidone on the formation and extraction of the complex was examined by measurement of the absorbance at various EDDS concentration. The result shows that using 5.0 mL of 2×10^{-3} M EDDS a constant and maximum absorbance is obtained for 100 μg of iron. An excess of reagent have no effect on the extraction rate. So this concentration was selected for all further studies.

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Choice of surfactants:

Maximum absorbance and its apparent molar absorptivity of the binary complex is measured at 488 nm and have value of 335×10^3 L mol^{-1} cm^{-1}, respectively. Various cationic and non-ionic surfactants (CPC, SDS, P010, HTAB, CTMA, MTOA, Triton X-100 and Tween - 80) are tested. The highest and constant absorbance values are obtained on using CTMA and for that reason, all mentioned work has been carried out in this medium. Moreover the amount of CTMA was tested and results shows that a CTMA concentration between 3×10^{-4} and 5×10^{-4} M is the optimum concentration to form the highly intense orange colour of the ion-associate complex. Therefore the medium concentration 4×10^{-4} M in the final assay solution is used in the recommended procedure. Concentrations outside this range give lower absorbance for the ion- associate complex. Concentrations > 10^{-3} M produce a stable emulsion on shaking and the two phases do not separate.

Effect of pH:

The effect of pH on the formation and extraction of the ion-association complex was examined at 514 nm with a sample containing 2.0 µg mL^{-1} iron, and the other conditions kept constant. The absorbance was a maximum at pH 5.0 to 7.0. At pH < 5.0, the formation of the ion-associate was incomplete owing to protonation of the EDDS therefore pH 6.0 was chosen as optimum pH.

Other reaction conditions:

At the volume of organic phase is small compared with that of the aqueous phase, it was essential to study the effect of the aqueous phase volume on the extraction. The absorbance of the organic phase was found to be constant for aqueous/organic phase volume ratio ranging from 1 to 10. A repeated extracting of an aqueous solution shows no absorbance indicating that a single extraction is sufficient to obtain over-all extraction efficiency of 99.7%.

The minimum shaking time for complete extraction of the complex into DCE was found to be 3.0 min. at room temperature and no change was observed when the shaking time was varied form 2.0 to 10 min.

Beer's law/ sensitivity and precision:

A straight line passing through the origin point, which corresponds to 0.01-0.32 µg ml^{-1}, in the organic phase, and corresponds to 0.1-3.2 µg ml^{-1}, in the aqueous phase when V_{aq}/V_{org} = 10. The optimum concentration range of iron that can be measured accurately, as evaluated from Ringbom plot is 0.02-0.29 µg ml^{-1}. The apparent molar absorptivity was calculated to be 1.73×10^5 L mol^{-1} cm^{-1}, whereas Sandell sensitivity was found to be 0.32 µg cm^{-1}. The standard deviation (S_D) for ten independent measurements of the reagent blank absorbance was 0.0025. The slope of the calibration graph (S) was 3.10 ml/µg. The theoretical limits of detection and quantification (C = KSD/S), with K = 3 and K = 10, respectively[29,30], were found to be 2.4 µg ml^{-1} and 8.01 µg mL^{-1}. Accordingly to this criterion, the region of non- detection is < 2.4 µg mL^{-1}, detection region 2.4-8.01 ng mL^{-1}, region of quantification > 8.01 µg mL^{-1}. The relative standard deviation for 1.5 µg mL^{-1} iron was 1.12 % (ten independent determinations).

Effect of foreign ions:

The effect of more than 50 ions on the determination of iron as ion-associate complex was studied. The tolerance limits was taken as the amount that caused ±3.0% error in the absorbance. The alkali and alkaline-earth metal ions and most common anions are tolerated even when present in large amounts. For the other metal ions tested, the tolerance levels that is reported in Table (1) when applied reveals that sufficient EDDS and CTMA are present.

Amongst the masking and complexing agents examined, only EDTA and cyanide interfered. EDTA interferes at all levels, and when cyanide is present, the full colour takes one hour o develop.

Analytical application:

To confirm the usefulness of the proposed method it was applied to the determination of iron in Nile river water, fruit juice, wine, foods, minerals and pharmaceutical formulations. The results (Table 2) were in good agreement with those obtained with atomic absorption spectrometry or 1,10-phenanthroline methods. In order to validate the proposed procedure, we applied a regression analysis to the two sets of results according to the model.

$$Y = a + bX$$

Where Y is the result produced by the proposed method and X is the result obtained by AAS or phenanthroline method. The regression procedure used taken into account the errors associated with variables X and Y this leads to the equation.

$$Y = (2.95 \times 10^{-2} \pm 0.012) + (0.9930 \pm 0.01)\, X$$

$$Y = (3.18 \times 10^{-2} \pm 0.015) + (0.9918 \pm 0.014)\, X$$

With a correlation coefficient of 0.9996 and 0.9992 compared with AAS and phenanthroline methods, respectively.

Alternatively, a significant test was applied to compare the accuracy and precision of the proposed method with both AAS and phenanthroline methods. The t- and F-values calculated were less than the critical value in all instances (for five degree of freedom and 95% confidence level). Hence there is no significant difference between them.

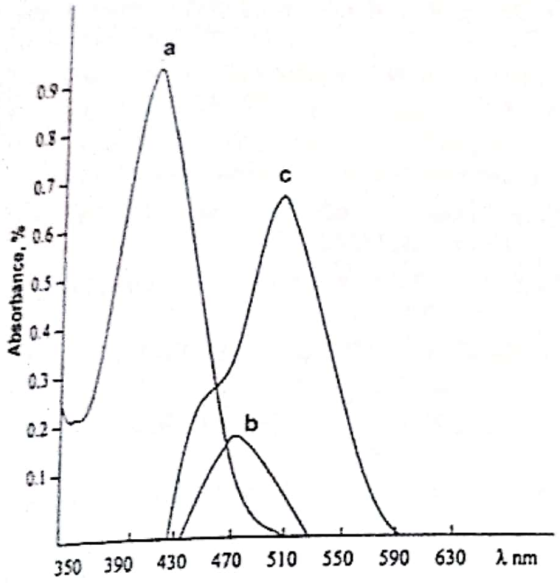

Fig. (1): Absorption spectra for a) 1×10^{-4} M reagent, b) 2.0 μg ml^{-1} Fe^{2+} complexed with 1×10^{-4} M reagent c) b+2.0 mL 10^{-2} M, CTMA.

Once the proposed method had been validated, it was applied to determine iron in the tested samples.

Table (1): The effect of diverse ions on the sensitivity, selectivity and stability of the ion associate complex.

Cation added	Tolerance limit*, μg/100 ml	Anion added	Tolerance limit*, μg/100 ml
As^{3+}, Ti^{4+}, Pd^{2+}, Zn^{2+}	1400	Tartrate ascorbate, malonate	220
Ag^{+}, Sn^{2+}, Sn^{4+}, W^{4+}, Mo^{2+}	850	$S_2O_3^{2-}$, Thioura, benzoate	100
Cr^{3+}, Gd^{+3}, Sm^{3+}, La^{3+}	400	Thioglycollate, NO_2^{-1}	30
SO^{2+}, V^{5+}, Zi^{4+}, Pr^{3+}	180	F^-, SCN^-	12
Uo_2^{2+}, Th^{4+}	100	EDTA	> 0.0001
Co^{2+}, Ni^{2+}, Cu^{2+}	40	CN^{-1}	> 0.0001

* Maximum tested.

The sensitivity and selectivity ofethylene diamine disuccinic acid for iron(II) determination, as well as the stability of the complex have been studied and the limits for diverse ions on the determination of 2.0 mg of $iron^{(II)}$ as the ion-associated complex.

Table (2) : Determination of iron in various samples.

Sample	Proposed	AAS	o-phenan-throline	t-test	f-value
Nile river water	0.11	0.108	0.105	0.98	2.16
Red wine[a]	8.5	8.6	8.4	1.26	2.68
White wine[a]	3.3	3.25	3.4	1.57	3.13
Black wine[a]	5.60	5.60	5.50	1.17	2.48
Lentils[b]	20.1	20.25	20.0	0.93	2.05
Spinachs[c]	44.6	44.20	43.90	1.34	2.80
Apple juice[d]	0.60	0.59	0.60	1.65	3.31
Tomato juice[d]	0.75	0.77	0.74	1.42	2.93
Lemon juice[d]	0.52	0.53	0.50	1.20	2.52
Orange juice[d]	0.46	0.45	0.47	1.18	2.49
Magnesites[e]	7.55	7.60	7.50	1.52	3.06
Portland cement[f]	2.10	2.06	2.08	1.35	2.85
Rubraton-B Elixir (squibb)	152	153	-	1.11	2.30

[a] = Henkel Company (Germany)
[b] = Dahab Company (Egypt)
[c] = Montana (Egypt)
[d] = Local-Kaha (Egypt)
[e] = Natural (Egypt)
[f] = Cement Company (Egypt)

CONCLUSIONS

The sensitivity and selectivity of EDDA for iron(II) determination, as well as the stability of the complex, have been further improved by the introduction of a third component that cause sensitization of the complex through the formation of an ion-associate. This chemical sensitization results in a greater molar absorptivity and relatively longer wavelength of maximum absorbance. Moreover, as the ion association complex can be extracted into organic solvent and concentrated, the increased sensitivity makes possible iron determination at lower levels. The method was successfully applied to determine iron in natural real samples.

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تعيين الحديد (ثنائي النكافؤ) باستخدام النجمع الأيوني المعقد المكون من معقد (الأيثيلين ثنائى امين ثنائى سكسنيك) والسيتيل الثلاثى ميثيل أمونيوم بروميد وقياس المستخلص السائل بالتحليل القياسي الطيفي

علاء السيد أمين* – ممدوح محمد فكرى متولي – ماجدة محمد الحناوي – وفاء السيد حسن

*قسم الكيمياء– كلية العلوم– جامعة بنها – بنها

قسم الكيمياء التحليلية – كلية الصيدلة– جامعة الزقازيق– الزقازيق– مصر

تم تطوير وتعيين كميات صغيرة من الحديد بالتحليل الطيفي القياسي باستخدام المذيب لاستخلاص تجميع ايوني معقد من الحديدوز الأيوني. تعتمد الطريقة علي تكوين معقد أيوني تجميعي من الحديد (الثنائي النكافؤ) مع الإيثيلين ثنائى أمين ثنائى سكسنيك الحمضي والأستيل الثلاثى ميثيل الأمونيوم البروميد. ثم تم الاستخلاص إلي ٢,١–ثنائى كلورو ايثان والمعقد المتكون عند وسط أيوني ٥–٧ والتركيز للحديدوز يمكن تعيينة بقياس الامتصاص مباشرة فى وسط عضوي وقد وجد أن المعامل المعياري الامتصاصي = ١,٧٣ × ١٠$^{-٥}$ في اللتر مول$^{-١}$سم$^{-١}$ وعامل ساندل الحساس = ٠,٣٢ للنانوجرام علي السنتيمتر خالي من التداخل من أى شئ.

و قد طبقت الطريقة المقترحة بنجاح في تعيين الحديد في الماء ومستخلص الفواكه والنبيذ والغذاء والمعادن والمركبات الصيدلية.