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A simple spectrophotometric method for the determination of iron(II) aqueous solutions

M. Jamaluddin AHMED, Uttam Kumer ROY

Laboratory of Analytical Chemistry, Department of Chemistry
University of Chittagong, Chittagong-BANGLADESH
e-mail: pmjahmed55@gmail.com

An ultra-sensitive and highly selective non-extractive spectrophotometric method is presented for the rapid determination of iron(II) at trace levels using $2'$, 3, $4'$, 5, 7-Pentahydroxyflavone (morin) as a new spectrophotometric reagent in slightly acidic solution (0.0001-0.0002 M H_2SO_4). The reaction is instantaneous and absorbance remains stable for over 24 h (λ_{max} = 415 nm). The molar absorptivity was found to be 6.85 $\times$ 10^4 L mol^{-1} cm^{-1}. Linear calibration graphs were obtained for 0.01-10 μg mL^{-1} of Fe^{II}. The stoichiometric composition of the chelate is 1:2 (Fe:morin). Large excesses of over 50 cations, anions, and complexing agents (e.g., tartrate, oxalate, citrate, phosphate, thio-urea, SCN$^-$) do not interfere in the determination. The method was successfully used in the determination of iron in several standard reference materials (alloys and steels) as well as in some environmental waters (portable and polluted), biological samples (human blood and urine), food samples (arum, apple, egg, etc.), soil samples, and some complex synthetic mixtures. The method has high precision and accuracy [s = $\pm$ 0.001 for 0.5 μg L^{-1} (n = 5)] over 24 h; 415 nm was used.

Key Words: Spectrophotometry; iron determination; morin; alloys; steels; environmental; biological samples; soil samples.

Introduction

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.[1] The industrial uses of Fe and its compounds are numerous.[2] It is the major constituent in steel making. Several Fe oxide forms find use as paint pigments, polishing compounds, magnetic inks, and coatings for magnetic tapes. The soluble salts are variously used as dyeing mordant, catalysts, pigments, fertilizer, feeds, and disinfectants, and in tanning, soil

conditioning, and treatment of sewage and industrial wastes.[3] Divalent Fe is a cofactor in heme enzymes such as catalase and cytochrome C, and in non-heme enzymes such as aldolase and tryptophan oxygenase.[2] In humans iron is an essential component involved in oxygen transport.[4,5] It is also essential for the regulation of cell growth, and differentiation of iron limits oxygen delivery to cells,[6] resulting in fatigue, poor work performance, and decreased immunity.[4] On the other hand, excess amounts of iron can result in toxicity and even death.[7] Toxicology considerations are important in terms of iron deficiency (anemia) and accidental acute exposure and chronic iron overload due to idiopathic hemochromatosis or as a consequence of excess dietary iron or frequent blood transfusions. The immediate cause of death from the inorganic compounds of Fe in animals is respiratory failure. Clinical signs preceding death are anorexia oligodipsia, oliguria, alkalosis, diarrhea, loss of body weight, hypothermia, and alternating irritability and depression. In human poisonings, symptoms of iron intoxication include vomiting, cirrhosis of the liver, hemochromatosis, diarrhea, lethargy, coma, irritability, seizures, and abdominal pain.[3] All these findings cause great concern regarding public health, demanding accurate determination of this metal ion at trace and ultra-trace levels.

Spectrophotometry is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis. Morin has been reported as a spectrophotometric reagent for aluminum,[8] but has not been used previously for the spectrophotometric determination of iron. This paper reports on its use in a very sensitive, highly selective spectrophotometric method for the trace determination of iron. The method possesses distinct advantages over existing methods[9−19] with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH/acidity range, thermal stability, accuracy, precision, and ease of operation. A comparison between existing methods[9−19] and the present method is shown in Table 1. The present method is also superior to even recently developed spectrophotometric methods[20−26] using morin with respect to sensitivity, selectivity, range of determination, simplicity, rapidity, pH/acidity range, and ease of operation. The method is based on the reaction of non-absorbing morin in slightly acidic solution (0.0001-0.0002 M H_2SO_4) with iron(II) to produce a highly absorbent light green chelate product, followed by a direct measurement of the absorbance in an aqueous solution. With suitable masking, the reaction can be made highly selective and the reagent blank solution does not show any absorbance.

Experimental

Apparatus

A Shimadzu (Kyoto, Japan) (Model-1601) double-beam UV-VIS spectrophotometer and a Jenway (UK) (Model-3010) pH meter with combined electrodes were used for the measurement of absorbance and pH, respectively. A Hitachi polarized Zeeman (Model-Z-5000) atomic absorption spectrometer equipped with a microcomputer controlled air acetylene flame was used for comparison of the results.

Reagents and solutions

All chemicals used were of analytical-reagent grade or the highest purity available. Doubly distilled deionized water was used throughout this study. Triply distilled ethanol (from lime) was also used. Glass vessels were cleaned by soaking in acidified solutions of $KMnO_4$ or $K_2Cr_2O_7$, followed by washing with concentrated

Table 1. Review of reagents of existing methods for spectrophotometric determination of iron.

Reagent	Solvent	Medium Aqueous/ Surfactant/Organic	Acidity/pH	λmax (nm)	Molar absorptivity L mol⁻¹ cm⁻¹	Beer's Law (mg L⁻¹)	Detection Limit (μg L⁻¹)	Interference	Comments	References
Leucoxylene cyanol	-	Acetate buffer medium	H_2SO_4, 2.8-4.4	615	5.6×10^4	0.15-0.9	50	Although it was not studied, suppose to be many	i. pH dependent ii. Interference was not studied iii. Detection limit was not studied	9
1,2-dihydroxy-3,4 diketo- cyclobutene (squaric acid)	Ammonium squarate	Phthalate buffer solution	2.7	515	3.95×10^3	0.5-20	30	Less selective due to much interference	i. pH dependent, less sensitive ii. Less sensitive iii. Applied to the limited samples iv. Less selective due to much interference v. Detection limit was high	10
1,2-2 methyl-3-hydroxy-4-one	Water	NH_3-NH_4Cl Buffer	8.5	450	4.4×10^3	0.1-2	65	Much	i. pH dependent, less sensitive ii. Lengthy iii. Interference was not studied	12
Thiocyanate	Water acetone mixture	Acidic aqueous medium	1-2 $HClO_4$ acid	480	2.1×10^4	0.5-2	80	NO_2, $S_2O_3^{2-}$, $C_2O_4^{2-}$, HPO_4^{2-}, Co^{2+}, Cu^{2+}, $H_2PO_4^{2-}$	i. Less selective due to much interference ii. pH dependent iii. Limited application	13
9-(4-carboxyphenyl)-2,3,7-trihydroxyl-6-flurone	-	Buffer NH_3-HAc	6.5	640	1.06×10^4	4-300	2.2	Many ions interfere	i. pH dependent ii. Lengthy iii. Less selective due to much interference	17
2',3,4',5,7-Pentahydroxyflavone (present method)	Ethanol	Aqueous	3.8 - 4	415	6.85×10^4	0.01-10	0.5	Using suitable masking, the reaction can be made highly selective	i. Ultra sensitive ii. Fairly selective iii. Aqueous reaction medium iv. Less toxic solvent used	

HNO_3, and were rinsed several times with high-purity deionized water. Stock solutions and environmental water samples (1000 mL each) were kept in polypropylene bottles containing 1 mL of concentrated HNO_3. More rigorous contamination control was applied when the iron levels in specimens were low.

Morin solution (5.05×10^{-3} M)

Morin solution was prepared by dissolving the requisite amount of morin (BDH Chemicals) in a known volume of triply distilled ethanol. More dilute solutions of the reagent were prepared as required.

Iron(II) standard solution (1.79×10^{-2} M)

A 100 mL amount of stock solution (1.00 mg mL^{-1}) of divalent iron was prepared by dissolving 497 mg of purified-grade (Merck pro analysis grade) $FeSO_4 \cdot 7H_2O$ in deionized water. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with deionized water as and when required. Concentrations were checked using the standard potassium dichromate solution.[27]

Iron(III) standard solution

A 100 mL amount of stock solution (1.00 mg mL^{-1}) of trivalent iron was prepared by dissolving 290 mg of ferric chloride (Aldrich A.C.S. grade) in doubly distilled deionized water containing 1-2 mL of nitric acid (1+1). Concentrations were checked using standard potassium dichromate solution. More dilute standard solutions were prepared from this stock solution as and when required.

Potassium permanganate solution

A 1.00% (w/v) potassium permanganate (Merck) solution was prepared by dissolving in deionized water. Aliquots of this solution were standardized with oxalic acid.

Standard potassium dichromate solution

A 100 mL amount of standard stock solution (0.1 M) was prepared by dissolving 1.4711 g of finely powdered $K_2Cr_2O_7$ (Merck) in 100 mL of deionized water.

Sodium azide solution

Sodium azide solution (2.5% w/v) (Fluka purity > 99%) was freshly prepared by dissolving 2.5 g in 100 mL of deionized water.

Tartrate solution

A 100 mL stock solution of tartrate (0.01% w/v) was prepared by dissolving 10 mg of ACS-grade (99%) potassium sodium tartrate tetrahydrate in (100 mL) deionized water.

Aqueous ammonia solution

A 100 mL solution of aqueous ammonia was prepared by diluting 10 mL of concentrated NH_3 (28%-30%, ACS grade) into 100 mL with de-ionized water. The solution was stored in a polypropylene bottle.

EDTA solution

A 100 mL stock solution of EDTA (0.01% w/v) was prepared by dissolving 10 mg of A.C.S.-grade ($\geq$ 99%) ethylenediaminetetraacetic acid as disodium salt dihydrate in (100 mL) deionized water.

Other solutions

Solutions of a large number of inorganic ions and complexing agents were prepared from their Analar grade or equivalent grade water-soluble salts (or the oxides and carbonates in hydrochloric acid).[27]

Procedure

A volume of 0.1-1.0 mL of a neutral aqueous solution containing 0.1-100 μg of iron(II) in a 10-mL calibrated flask was mixed with a 1:40-1:95-fold molar excess of the morin reagent solution (preferably 1 mL of 5.05×10^{-3} M) followed by the addition of 1-2 mL (preferably 1 mL) of 0.001 M sulfuric acid. After 1 min, 4 mL of ethanol was added and the mixture was diluted to the mark with deionized water. The absorbance was measured at 415 nm against a corresponding reagent blank. The iron content in an unknown sample was determined using a concurrently prepared calibration graph.

Results and discussion

## Factors Affecting the Absorbance

Absorption spectra

The absorption spectra of the Fe(II)-morin system in 0.001 M H_2SO_4 medium were recorded using the spectrophotometer. The absorption spectra of the Fe(II)-morin were a symmetric curve with maximum absorbance at 415 nm and the average molar absorption coefficient of 6.85×10^4 L mol^{-1} cm^{-1} is shown in Figure 1. The reagent blank exhibited negligible absorbance despite having a wavelength in the same region. The reaction mechanism of the present method was as reported earlier.[28]

Effect of solvent

Because morin was insoluble in water, a suitable organic solvent was used for the system. Of the various solvents (acetone, isobutyl alcohol, ethanol, and 1, 4-dioxane) studied, ethanol was found to be the best solvent for the system. No absorbance was observed in the organic phase with the exception of n-butanol. In 50 $\pm$ 2% (v/v) ethanolic medium, however, maximum absorbance was observed; hence, a 50% ethanol solution was used in the determination procedure. It is shown in Figure 2 (other parameters were kept constant 1 mg L^{-1} of Fe^{2+}, acidity 0.001 M H_2SO_4, and reagent concentration 1:50-fold).
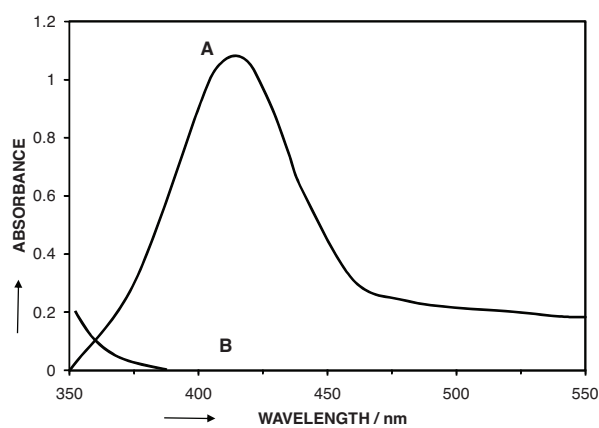
Figure 1. A and B absorption spectra of Fe^{II} – morin (1.5 mg L^{-1}) and the reagent blank ($\lambda_{max} = 415$ nm) in aqueous solutions.
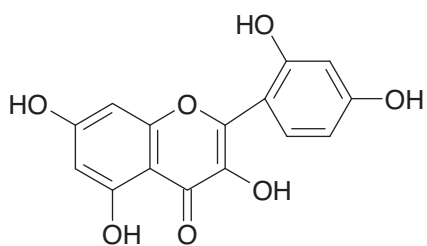
$2'$, 3, $4'$, 5, 7-Pentahydroxyflavone (morin).
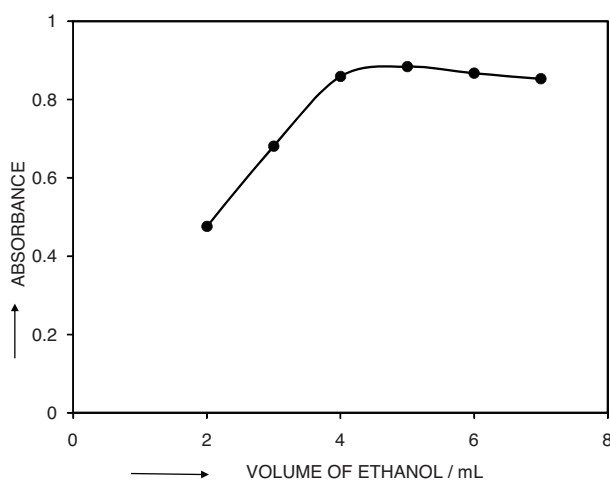
Figure 2. Effect of ethanol on the absorbance of the Fe^{II} – morin (1.0 mg L^{-1}) system.
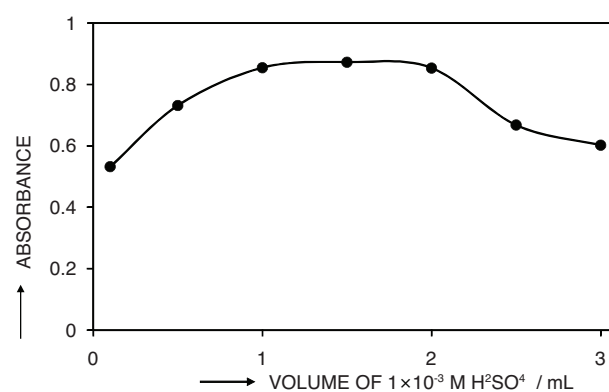
Figure 3. Effect of acidity on the absorbance of the Fe^{II} – morin (1.0 mg L^{-1}) system.

Effect of acidity

Of the various acids (nitric, hydrochloric, sulfuric, and phosphoric) studied, sulfuric acid was found to be the best for the system. The absorbance was maximum and constant when the 10 mL of solution (1 μg mL^{-1}) contained 1-2 mL of 0.001 M H$_2$SO$_4$ at room temperature. Outside this range of acidity, the absorbance decreased (Figure 3) (other parameters were kept constant 1 mg L^{-1} of Fe^{2+}, 50% ethanol, and reagent concentration 1:50-fold). For all subsequent measurements, 1 mL of 0.001 M H$_2$SO$_4$ was added.
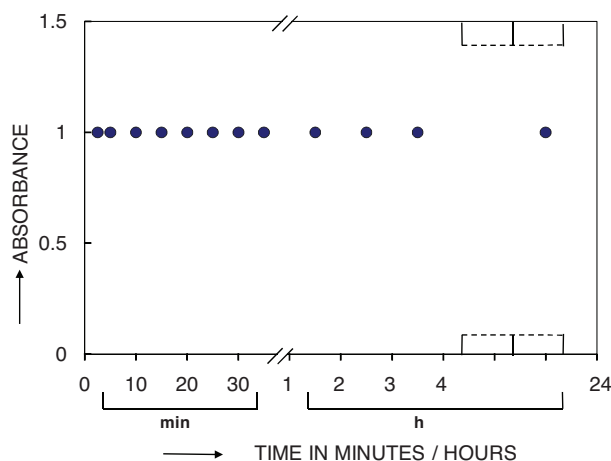
Effect of time

The reaction was very fast. Constant maximum absorbance was obtained within a few seconds after the dilution to volume and remained strictly unaltered for over 24 h (Figure 4) (other parameters were kept constant 1 mg L^{-1} of Fe^{2+}, 50% ethanol, acidity 0.001 M H$_2$SO$_4$, and reagent concentration 1:50-fold). A longer period of time was not studied.

Effect of temperature

Effect of various temperatures (10-90 $^\circ$C) on the iron(II)-morin system was studied. The iron(II)-morin system attained maximum and constant absorbance at room temperature (25 $\pm$ 5 $^\circ$C).

Effect of reagent concentration

Different molar excesses of morin were added to a fixed metal ion concentration and absorbencies were measured according to the standard procedure. It was observed that at 1 μg mL^{-1} Fe(II) metal, the analyte to reagent molar ratios of 1:40-1:95 produced a constant absorbance of the Fe-chelate (Figure 5) (other parameters were kept constant 1 mg L^{-1} of Fe^{2+}, 50% ethanol, and acidity 0.001 M H$_2$SO$_4$). For the different Fe concentrations (0.1 and 0.5 μg mL^{-1}) an identical effect of varying the reagent concentration was noted. For all subsequent measurements, 1 mL of 5.05 $\times$ 10^{-3} M morin reagent was added.

Figure 4. Effect of time on the absorbance of the Fe^{II} – morin (1.0 mg L^{-1}) system.
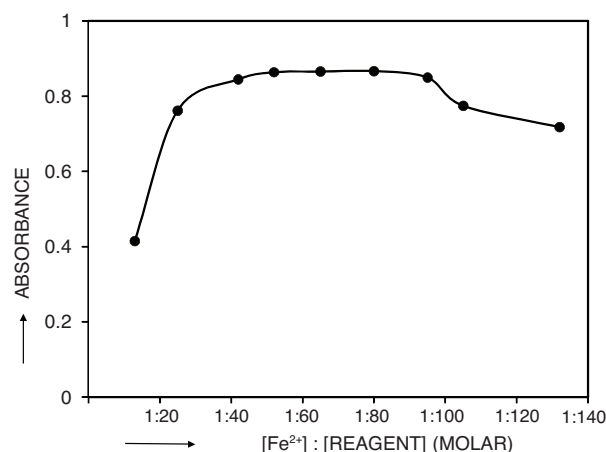
Figure 5. Effect of reagent (morin:Fe^{II}) molar concentration ratio (1.0 mg L^{-1}) on the absorbance of the Fe^{II} – morin (1.0 mg L^{-1}) system.

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Effect of metal concentration (Beer's law)

The well-known equation for spectrophotometric analysis in very dilute solutions is derived from Beer's law. The effect of metal concentration was studied over 0.01- 0.1μ, 0.1-1.0, and 1-50 μg mL^{-1} for convenience of measurement. The absorbance was linear for 0.01-10 μg mL^{-1} of Fe(II) at 415 nm. The molar absorptivity[29] was found to be 6.85 $\times$ 10^4 L mol^{-1} cm^{-1}. Of the 3 calibration graphs, next 2 are straight-line graphs passing through the origin (Figures 6 and 7) and 1 showing the limit of the linearity is given in Figure 8.

The selected analytical parameters obtained with the optimization experiments are summarized in Table 2.
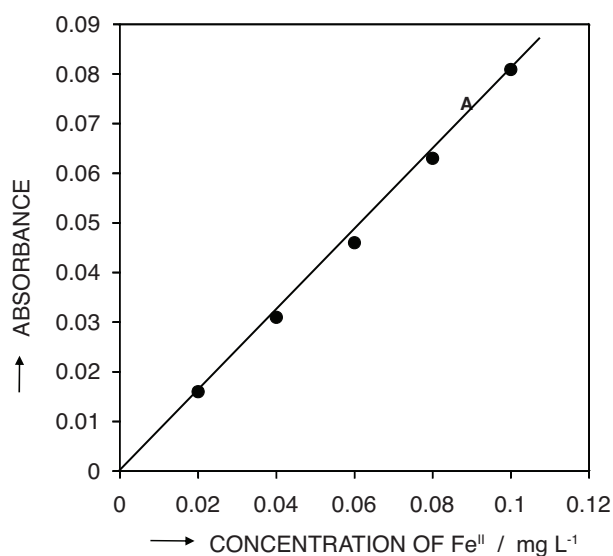
Figure 6. Calibration graph A, 0.01-0.1 mg L^{-1} of iron(II).
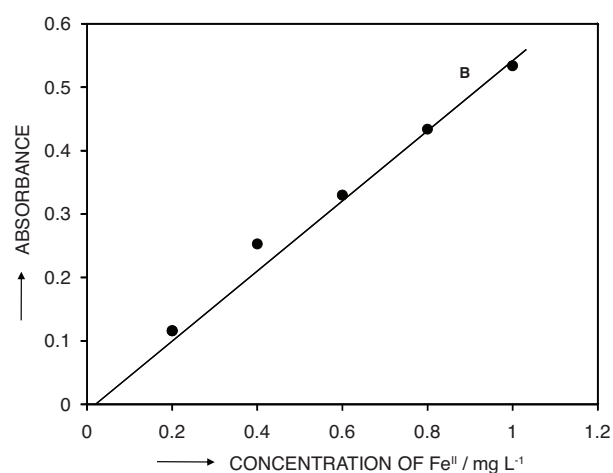
Figure 7. Calibration graph B, 0.1-1.0 mg L^{-1} of iron(II).

Figure 8. Calibration graph C, 1.0-10.0 mg L^{-1} of iron(II).

Table 2. Selected analytical parameters obtained with the optimization experiments.

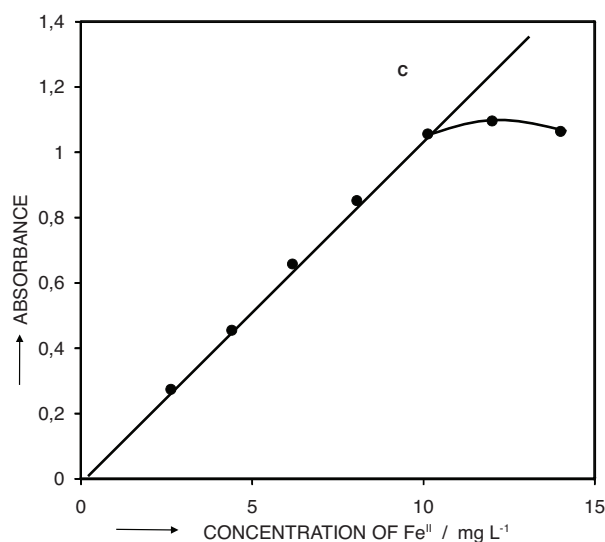| Parameters	Studied range	Selected value
Wavelength/λ_{max} (nm)	200-800	415
Acidity/M H_2SO_4	0.00001-0.003	0.0001-0.0002 (preferably, 0.0001)
pH	4.58-3.76	3.80-4.00 (preferably, 3.90)
Time/h	0-72	1 min-24 h (preferably, 5 min)
Solvent/ % Ethanol	10-100	40-60 (preferably, 50)
Temperature/°C	10-90	25 ± 5
Reagent (fold molar excess, Fe:morin)	1:5-1:132	1:40-1:95 (preferably, 1:50)
Molar absorptivity L mol^{-1} cm^{-1}	-	6.85×10^4
Linear range/mg L^{-1}	0.001-100	0.01-1.0
Detection limit/μg L^{-1}	-	0.5
Regression coefficient	-	0.9992

Effect of foreign ions

The effect of over 50 anions, cations, and complexing agents on the determination of only 1 μg mL^{-1} of Fe(II) was studied. The criterion for interference[30] was an absorbance value varying by more than 5% from the expected value for Fe(II) alone. The results are summarized in Table 3. As can be seen, a large number of ions have no significant effect on the determination of iron. The most serious interference were from V(V) and Al(III) ions. Interference from these ions is due to complex formation with morin. The greater tolerance limits (absorbance value varying by more than 5% from the accepted value for Fe(II) alone) for these ions can be achieved by using several masking methods. In order to eliminate interference of V(V) and Al(III), H_2O_2 and EDTA were used as masking agents, respectively. During the interference studies, if a precipitate was formed, it was removed by centrifugation. The amount mentioned is not the tolerance limit but the actual amount studied. However, for those ions whose tolerance limit has been studied, their tolerance ratios are given in Table 3.

Composition of the absorbing complex

Job's method[31] of continuous variation and the molar ratio[32] method were applied to ascertain the stoichiometric composition of the complex. A Fe-morin (1:2) complex was indicated by both methods.

Precision and accuracy

The precision of the present method was evaluated by determining different concentrations of iron (each analyzed n = 5 times). The relative standard deviation (n = 5) was 2%-0% for 0.1-100 μg of iron(II) in 10 mL, indicating that this method is highly precise and reproducible. The detection limit (3 s of the blank) was found to be 0.5 μg L^{-1}. The method was tested by analyzing several synthetic mixtures containing iron(II) and diverse ions (Table 4). The results for total iron were in good agreement with certified values (Table 5). The reliability of our Fe-chelate procedure was tested by recovery studies. The average percentage recovery obtained for addition

of iron(II) spike to some environmental water samples was quantitative as shown in Table 6. The results of biological analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS (Table 7). The results of soil samples (analyzed) by the spectrophotometric method were highly reproducible (Table 8). The results of food samples analyzed by the spectrophotometric method were in good agreement with the expected values (Table 9). The results of speciation of iron(II) and iron(III) in mixtures were highly reproducible (Table 10). Hence, the precision and accuracy of the method were excellent.

Table 3. Table of tolerance limits of foreign ions.

Species x	Tolerance ratio [Species (x) / Fe (w/w)]	Species x	Tolerance ratio [Species (x) / Fe (w/w)]
Acetate	100	Lead(II)	50^{++}
Aluminum	20^{+++}	Magnesium(II)	100
Ammonium(I)	1000	Manganese(II)	50
Arsenic(III)	50	Mercury(II)	50
Arsenic(V)	50	Molybdenum(V)	50^{+}
Ascorbic acid	500	Nickel(II)	50
Azide	100	Nitrate	1000
Barium	100	Oxalate	50
Beryllium(II)	50	Phosphate	1000
Bromide	1000	Potassium	1000
Cadmium	50	Selenium(IV)	100
Calcium(II)	50	Selenium(VI)	50
Cesium (II)	50^{++}	Silver	50
Chloride	1000	Sodium	50
Chromium(III)	100	Strontium	100
Chromium(VI)	50	Tartrate	500
Citrate	50	Tellurium	50
Copper(II)	50	Thiocyanate	50
Cyanide	100	Tin(II)	50^{+}
EDTA	100	Tungsten(VI)	100
Fluoride	100	Vanadium(V)	50^{+}
Iodide	100	Zinc(II)	50
Iron(III)	100^{+++}		

Tolerance limit was defined as ratio that causes less than 5% interference
$^{+}$With 10 mgL^{-1} H$_2$O$_2$.
$^{++}$With 50 mgL^{-1} tartrate.
$^{+++}$With 50 mgL^{-1} EDTA.

Table 4. Determination of iron in some synthetic mixtures.

Sample	Sample Composition before adding Fe species, mg L^{-1}	Iron (II) / mg L^{-1}		
		Added	Found[a]	Recovery $\pm$ s[b] (%)
A	"Distilled water". Fe(II) solution is added to this.	0.50	0.49	98 $\pm$ 1
		1.00	1.00	100 $\pm$ 0
B	Ca^{2+}(25) + Ag$^+$(25) + EDTA(50)	0.50	0.50	100 $\pm$ 0
		1.00	0.99	99 $\pm$ 1
C	Sample B before iron addition + Na$^+$(25) + Se(IV)(25)	0.50	0.49	98 $\pm$ 1
		1.00	0.1.02	102 $\pm$ 1
D	Sample C before iron addition + Hg^{2+}(25) + Cd^{2+}(25) + Tartrate (50)	0.50	0.52	104 $\pm$ 1
		1.00	1.03	106 $\pm$ 2
E	Sample D before iron addition + As^{3+}(25) + Mn^{2+}(25)	0.50	0.54	108 $\pm$ 2
		1.00	1.09	109 $\pm$ 2

[a]Average of 5 analyses of each sample

[b]The measure of precision is the standard deviation.

Table 5. Determination of iron in certified reference materials.

Certified Reference Materials (Composition, %)	Iron, %		
	Certified value	Found* (n = 5)	R.S.D. %
BAS-10 g, High tensile brass (Cu=60.8, Fe=1.56, Pb=0.23, Ni=0.16, Sn=0.21, Al=3.34, Zn=32.0 and Mn=0.12)	1.56	1.52	2.0
YSBC 19716* High tensile steel (Fe=34.26, Zn=36.24, Si=0.38, Cd=1.2, Sb=48.57, S=0.95 and F=0.32)	34.26	34.05	0.6
GSBD 33001-94* High tensile steel (Fe=9.53, Si=14.64, Al=9.29, Ca=1.04, Mg=21.49 and Cr=32.79)	9.53	9.55	1.5
BY 0110-1* High tensile steel (Zn=42.98, Si=19.89, Fe=4.13, Pb=0.351, Sn=0.06, Cd=0.04, As=0.024, Sn=0.06 and Cu=0.14)	4.13	4.06	1.8

Applications for determination of total iron

The proposed method was successfully applied for the determination of iron(II) in a series of synthetic mixtures of various compositions (Table 4) and also in a number of real samples, e.g., several Certified Reference Materials

(CRMs) (Table 5). The method was also extended to the determination of iron in a number of environmental, biological, soil, and food samples. In view of the unknown composition of environmental water samples, the same equivalent portions of such samples were analyzed for iron content; the recoveries in both the "spiked" (added to the samples before the mineralization or dissolution) and the "unspiked" samples are in good agreement (Table 6). The results of biological analyses by spectrophotometric method were found to be in excellent agreement with those obtained by AAS (Table 7). The results of soil samples analyzed by the spectrophotometric method are shown in Table 8. The results of food samples analyzed by the spectrophotometric method are shown in Table 9. The results of speciation of iron(II) and iron(III) in mixtures are shown in Table 10.

Table 6. Determination of iron in some environmental water samples.

Sample		Iron / μg L^{-1}		Recovery ± s (%)	s_r^b (%)
		Added	Found [a]		
Tap water		0	138.0		
		100	240.0	100.8 ± 1	0.31
		500	645.0	101 ± 1	0.29
Well water		0	66.0		
		100	170.0	102 ±1	0.45
		500	565.0	99.8 ± 1	0.26
River water	Karnaphuly (upper)	0	45.0		
		100	145.0	100 ± 0	0.00
		500	550.0	100.9 ± 1	0.42
	Karnaphuly (lower)	0	48.0		
		100	150.0	101 ± 1	0.22
		500	550.0	100 ± 1	0.16
	Padma (upper)	0	40.0		
		100	140.0	100 ± 0	0.00
		500	545.0	100.9 ± 1	0.25
	Padma (lower)	0	42.0		
		100	145.0	102 ± 1	0.23
		500	544.0	100.3 ±1	0.21
Sea water	Bay of Bengal (upper)	0	5.0		
		100	105.0	100 ± 0.0	0.00
		500	510.0	100.9 ± 1	0.27
	Bay of Bengal (lower)	0	6.0		
		100	105.0	99 ± 1	0.35
		500	506.0	100 ± 0	0.00
Drain water	TSP Complex[c]	0	386.0		
		100	490.0	100.8 ± 1	0.45
		500	885.0	99.9 ± 1	0.29
	Eastern Refinery[d]	0	150.0		
		100	250.0	100 ± 0	0.00
		500	655.0	100.7 ± 1	0.28
	BSRM[e]	0	505.0		
		100	605.0	100 ± 0	0.00
		500	1010.0	100.5 ± 1	0.47

[a] average of 5 replicate determinations.
[b] The measure precision is the relative standard deviation (s_r)
[c] T.S.P. Complex, Patenga, Chitagong
[d] Eastern Refinery, Chittagong, Bangladesh.
[e] Bangladesh Steel Re-rolling Mills Ltd, Chittagong, Bangladesh.

Table 7. Concentration of iron in blood and urine samples.

| Serial No. | Sample | Iron / mg L^{-1} | | | | Sample source[a] |
| | | AAS (n = 5) | | Proposed method n = 5 | | |
		Found	RSD,%	Found	RSD,%	
1	Blood	1.00	1.0	1.05	1.0	Normal adult (Male)
	Urine	0.24	1.2	0.25	1.3	
2	Blood	0.55	1.5	0.53	1.5	Anemia patient (Female)
	Urine	0.13	1.8	0.14	2.0	
3	Blood	1.40	1.3	1.45	1.0	Kidney disease patient (Male)
	Urine	0.37	1.8	0.40	1.5	
4	Blood	4.80	1.3	4.85	1.2	Liver cirrhosis patient (Male)
	Urine	1.10	2.0	1.05	1.8	

[a]Samples were from Chittagong Medical College Hospital, Chittagong.

Table 8. Determination of iron in some surface soils.[a,b]

Serial No.	Iron (mg kg^{-1})[a]	Sample source
S$_1$	36.8 ± 1[b]	Agriculture soil (Tikapara, Rajshahi, Bangladesh)
S$_2$	17 ± 2	Marine soil (Bay of Bengal, Chittagong, Bangladesh)
S$_3$	30.0 ± 2	River soil (River Padma, Rajshahi, Bangladesh)
S$_4$	31.5 ± 2	Eustrain soil (Junction of Bay of Bengal and River Karnaphuly, Chittagong, Bangladesh)
S$_5$	48.8 ± 2	Industrial soil (Bangladesh Steel Re-rolling Mills Ltd., Chittagong, Bangladesh)

[a]Average of 5 analyses of each sample

[b]Measure of precision is the standard deviation

[c]Composition of the soil samples: C, N, P, K, Na, Ca, Mg, Cu, Fe, Pb, NO$_3$, NO$_2$, Zn, SO$_4$, Mn, Mo, Co, etc.

Table 9. Determination of iron in some food samples.

Serial No.	Sample	Iron/mg kg^{-1} Found[a] ± s	Sample source
1	*Arum discorides* (Arum)	1.2 ± 0.1	Chittagong Market
2	*Malus domestica* (Apple)	2.5 ± 0.1	Chittagong Market
3	*Psidium guajava* (Guava)	3.7 ± 0.1	Chittagong University Campus
4	Egg	0.06 ± 0.1	Chittagong Market

Table 10. Determination of iron(II) and iron(III) in mixtures.

Serial No.	Fe(II) : Fe(III)	Fe, taken (mg L^{-1})		Fe, found (mg L^{-1})		Error (mg L^{-1})	
		Fe(II)	Fe(III)	Fe(II)	Fe(III)	Fe(II)	Fe(III)
1	1:1	1.00	1.00	0.99	0.98	0.01	0.02
2	1:1	1.00	1.00	1.00	1.00	0.00	0.00
3	1:1	1.00	1.00	0.98	0.99	0.02	0.01
Mean error: Fe(II)= ± 0.01; Fe(III) = ± 0.01							
Standard deviation: Fe(II) = ± 0.005; Fe(III) = ± 0.006							
1	1:2	1.00	2.00	0.99	1.99	0.01	0.01
2	1:2	1.00	2.00	0.98	1.98	0.02	0.02
3	1:2	1.00	2.00	0.99	1.98	0.01	0.02
Mean error: Fe(II)= ± 0.013; Fe(III) = ± 0.016							
Standard deviation: Fe(II) = ± 0.006; Fe(III) = ± 0.0058							
1	1:3	1.00	3.00	0.98	2.98	0.02	0.02
2	1:3	1.00	3.00	0.98	2.99	0.02	0.01
3	1:3	1.00	3.00	0.99	2.98	0.01	0.02
Mean error: Fe(II)= ± 0.016; Fe(III) = ± 0.0016							
Standard deviation: Fe(II) = ± 0.0058; Fe(III) = ± 0.0058							

Determination of iron in synthetic mixtures

Several synthetic mixtures of varying compositions containing iron and diverse ions of known concentrations were determined by the present method using tartrate and EDTA (0.01% w/v) as masking agent and the results were found to be highly reproducible. The results are shown in Table 4. Accurate recoveries were achieved in all solutions.

Determination of iron in brass, alloys and steels (Certified reference materials)

A 0.1 g amount of a brass or alloy or steel sample containing 1.56%-34.26% of iron was weighed accurately and placed in a 50 mL Erlenmeyer flask following a method recommended by Parker et al.[33] To it, 10 mL of concentrated HNO_3 and 1 mL of concentrated H_2SO_4 were added, carefully covering the flask with a watch glass until the brisk reaction subsided. The solution was heated and simmered gently after the addition of 5 mL of concentrated HNO_3 until all carbides were decomposed. The solution was carefully evaporated to dense white fumes to drive off the oxides of nitrogen and then cooled to room temperature (25 ± 5 °C). After suitable dilution with deionized water, the contents of the Erlenmeyer flask were warmed to dissolve the soluble salts. Then the content of the flask was reduced to iron(II) by using freshly prepared sodium azide solution and excess of the azide solution was removed by boiling. The solution was then cooled and neutralized with a dilute NH_3 solution. The resulting solution was filtered, if necessary, through Whatman No. 40 filter paper into a 25-mL calibrated flask. The residue was washed with a small volume of hot (1 + 99) H_2SO_4, followed by water, and the volume was made up to the mark with deionized water.

A suitable aliquot (1-2 mL) of the above solution was taken into a 10-mL calibrated flask and the iron content was determined as described above, using tartrate (0.01% w/v) and EDTA (0.01 w/v) mixture as masking agent. Based on 5 replicate analyses, the average iron concentration determined by spectrophotometric method was in close agreement with the certified values. The results are shown in Table 5.

Determination of iron in environmental waters

Each filtered (with Whatman No. 40) environmental water sample (1000 mL) evaporated nearly to dryness with a mixture of 3 mL of concentrated H_2SO_4 and 10 mL of concentrated HNO_3 in a fume cupboard, following a method recommended by Greenberg et al.[34] and was heated with 10 mL of deionized water in order to dissolve the salts. The content of the flask was reduced to iron(II) using sodium azide. The solution was then cooled and neutralized with dilute NH_4OH solution in the presence of 1-2 mL of 0.01% (w/v) tartrate or EDTA solution. The resulting solution was then filtered (if necessary) and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with deionized water.

An aliquot (1-2 mL) of this preconcentrated water sample was pipetted into a 10-mL calibrated flask and the iron content was determined as described above, using a mixture of tartrate and EDTA as masking agent. The analyses of environmental water samples for iron from various sources are shown in Table 6.

Most spectrophotometric methods for the determination of iron in natural and sea-water require preconcentration of iron.[34] Preconcentration is a very cheap and easy method. The concentration of iron in natural and sea-water is a few $\mu g\ L^{-1}$ in Japan.[35] The mean concentration of iron found in UK drinking water is less than 1 mg L^{-1} (av. 200 $\mu g\ L^{-1}$).[36]

Determination of iron in biological samples

Human blood (2-5 mL) and urine (20-30 mL) were collected in polyethylene bottles from the affected persons (diseases occur due Fe poisoning such as anemia, liver cirrhosis, kidney diseases). Immediately after collection, they were stored in a salt-ice mixture and later, at the laboratory, were kept at -20 °C. The samples were taken into a 100 mL micro-Kjeldahl flask. A glass bead and 10 mL of concentrated nitric acid were added and the flask was placed on the digester under gentle heating. When the initial brisk reaction was over, the solution was removed and cooled following a method recommended by Stahr.[37] Then 1 mL of concentrated sulfuric acid was added carefully, followed by the addition of 2 mL of concentrated HF, and heating was continued for at least 30 min, followed by cooling. The content of the flask was reduced to iron(II) using sodium azide solution and excess azide was removed by boiling and then the content was filtered. The solution of flask was then neutralized with dilute NH_4OH solution in the presence of 1-2 mL of a 0.01% (w/v) tartrate or EDTA solution. The resultant solution was then transferred quantitatively into a 10-mL calibrated flask and made up to the mark with deionized water.

A suitable aliquot (1-2-mL) of the final solution was pipetted into a 10-mL calibrated flask and the iron content was determined as described above using tartrate or EDTA as masking agent. The results of biological analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS. The results are shown in Table 7.

The abnormally high value for the liver cirrhosis patient is probably due to the involvement of a high iron

concentration with Cu and Zn. Occurrence of such high iron contents is also reported in liver cirrhosis patients from some developed countries.[2]

Determination of iron in soil samples

An air dried homogenized soil sample (100 g) was weighed accurately and placed in a 100-mL micro-Kjeldahl flask. The sample was digested in the presence of a reducing agent (2.5% freshly prepared azide solution), following the method recommended by Hesse.[38] The content of the flask was filtered through a Whatman No. 40 filter paper into a 25-mL calibrated flask and neutralized with dilute NH_4OH solution in the presence of 1-2 mL of a 0.01% (w/v) tartrate or EDTA solution. Then the solution of the flask was made up to the mark with deionized water.

Suitable aliquots (1-2 mL) were transferred into a 10-mL calibrated flask and a calculated amount of 0.001 M H_2SO_4 needed to give a final acidity of 0.0001-0.0002 M H_2SO_4 was added followed by 1-2 mL of a 0.01% (w/v) mixture of tartrate and EDTA solution as masking agent. The iron content was then determined by the above procedure and quantified from a calibration graph prepared concurrently. The results are shown in Table 8.

Determination of iron in food samples

An air-dried food sample (arum (25 g), apple (50 g), guava (50 g), and egg (1 piece)) was taken in a 100-mL micro-Kjeldahl flask. A glass bead and 10 mL of concentrated nitric acid were added and the flask was placed on the digester under gentle heating. When the initial brisk reaction was over, the solution was removed and cooled following the method recommended by Stahr.[37] Then 1 mL volume of concentrated sulfuric acid was added carefully, followed by the addition of 2 mL of concentrated HF, and heating was continued for at least 30 min and then cooling. The content of the flask was reduced to iron(II) using sodium azide solution and excess azide was removed by boiling and then the content was filtered. The solution of the flask was then neutralized with dilute NH_4OH in the presence of 1-2 mL of a 0.01% (w/v) tartrate or EDTA solution. The resultant solution was then transferred quantitatively into a 50-mL calibrated flask and made up to the mark with deionized water.

A suitable aliquot (1-2 mL) of the final solution was pipetted into a 10-mL calibrated flask and the iron content was determined as described above using tartrate or EDTA as masking agent. The high value of iron for psidium guajava (guava) is probably due to the involvement of a high iron concentration in the soil. The results are shown in Table 9.

Determination of iron(II) and iron(III) in mixtures

Suitable aliquots (1-2 mL) of iron (II + III) mixtures (preferably 1:1, 1:2, 1:3) were taken in a 25-mL conical flask. A few drops of freshly prepared sodium azide solution was added to reduce the trivalent iron to divalent. A 5-mL volume of water was added to the mixtures, which were then heated on a steam bath for 10-15 min with occasional gentle shaking to remove excess azide and then cooling to room temperature. The reaction mixture was transferred quantitatively into a 10-mL calibrated flask. Then the total iron (II+III) content was determined according to the general procedure with the help of the calibration graph.

An equal aliquot of the above iron (II + III) mixture was taken into a 25-mL beaker. Then 2 mL of 0.01% (w/v) EDTA was added to mask iron(III). Then the content of the beaker was transferred into a 10-mL calibrated flask, and its iron(II) content was determined according to the general procedure. The iron concentration was calculated in μg L^{-1} or mg L^{-1} with the aid of a calibration graph. This gives a measure of iron originally present as iron(II) in the mixture. The value of the iron(III) concentration was calculated by subtracting the concentration of iron(II) from the corresponding total iron concentration. The results were found to be highly reproducible. The results of a set of determination are given in Table 10.

Conclusions

A new, simple, sensitive, selective, and inexpensive method with the Fe(II)-morin complex was developed for the determination of iron in some industrial, biological, soil, and environmental samples, for continuous monitoring to establish the trace levels of iron in different sample matrices. It offers also a very efficient procedure for speciation analysis. Although many sophisticated techniques such as pulse polarography, HPLC, AAS, ICP-AES, and ICP-MS are available for the determination of iron at trace levels in numerous complex materials, factors such as the low cost of the instrument, easy handling, lack of requirement for consumables, and almost no maintenance have caused spectrophotometry to remain a popular technique, particularly in laboratories in developing countries with limited budgets. The sensitivity in terms of molar absorptivity and precision in terms of relative standard deviation of the present method are very reliable for the determination of iron in real samples down to ng g^{-1} levels in aqueous medium at room temperature (25 $\pm$ 5 $^\circ$C).

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