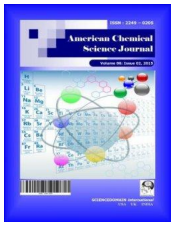

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A Simple Spectrophotometric Method for the Trace Determination of Iron in Some Real, Environmental, Biological, Food, Pharmaceutical and Soil Samples Using 2-aminophenol

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Authors' contributions

This work was carried out in collaboration between all authors. Author MJA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MTI, MJH and MFI managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A very simple, ultra-sensitive and highly selective non-extractive spectrophotometric method is presented for the rapid determination of iron (III) at trace levels using ortho aminophenol or 2-aminophenol (OAP) as a new spectrophotometric reagent (λ_{max} = 402 nm) in slightly acidic (0.0005-0.0015 M H_2SO_4) aqueous solution. The reaction is instantaneous and absorbance remains stable for over 24 h. The average molar absorption coefficient and Sandell's sensitivity were found to be 6.65×105 L mol^{-1} cm^{-1} and 5 ng cm^{-2}, respectively. Linear calibration graphs were obtained for 0.01-6 mg L^{-1} of iron with a correlation co-efficient value 0.9998 for Fe-OAP complex. The stoichiometric

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composition of the chelate is 1:3 (Fe: OAP). The detection limit and quantification of limit of the reaction system were found 1 µg L^{-1} and 10 µg L^{-1}, respectively. 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 developed 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 (bean, meat, banana, tomato, egg etc.), soil samples, pharmaceutical samples (tablets, capsules etc.), some solution containing both iron (II) and iron (III) and complex synthetic mixtures. The results of the proposed method for biological and food samples were comparable with AAS and were found to be in excellent agreement. The method has high precision and accuracy (s = ±0.01 for 0.5 mg L^{-1}).

Keywords: Spectrophotometry; iron determination; 2-aminophenol; alloys; steels; environmental; biological samples; soil samples; food and pharmaceutical samples.

1. INTRODUCTION

Iron plays a dual role in human biochemistry as in trace amounts; it is an essential nutrient, while large amounts are toxic and carcinogenic [1]. The essentiality and toxicity of iron depend on its oxidation states or the forms in which it was supplied. Iron is the most abundant transition metal in the living system and serves more biological roles than any other metal. Although iron is required for a number of vital functions, the main role of iron is to carry oxygen to the tissues where it is needed. Iron is also essential for the proper functioning of numerous enzymes involved in DNA synthesis, energy metabolism and protection against microbes and free radicals [2]. Iron salts are widely used in industrial materials [3], paint products, fertilizers, feeds and disinfectants [4]. They are important building components in biological systems [5]. The total iron content in an adult body is approximately 4 g, i.e.70 m mol, of which about two thirds is in hemoglobin. Iron stores, mainly spleen, liver, and bone marrow, contain about one-quarter of the body's iron; the remainder is in myoglobin and other hemoproteins. Only 0.1% of the total body iron is in plasma where almost all is bound to a transport protein—transferrin. Iron deficiency affects about 30% of the world population and is one of the main deficiency disorders in Europe [6]. Iron deficiency is characterized by anaemia, stunted growth, fatigue lowered resistance to infection, anorexia and death [7]. Iron is involved in oxygen transport from the lungs to tissues by hemoglobin and in oxygen storage in myoglobin; divalent Fe is a cofactor in heme enzyme such as catalase and cytochrome and in nonheme enzymes such as aldolase and tryptophan oxygenase [8]. On the other hand, excess amount of iron can result in toxicity and even death [9]. 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. In human poisonings, symptoms of iron intoxication include vomiting, cirrhosis of liver, hemochromatosis, diarrhea, lethargy, coma, irritability, seizures and abnormal pain [10]. 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. Ortho aminophenol or 2-aminophenol (OAP) has not previously been used 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 [11-19] 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 11-19 and the present method are shown in Table 1. The method is based on the reaction of nearly non-absorbent OAP in slightly acidic (0.0005-0.0015 M H_2SO_4) aqueous solution with iron (III) to produce a highly absorbent orange 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 show negligible absorbance.

2. EXPERIMENTAL SECTION

2.1 Apparatus

A Shimadzu (Kyoto, Japan) (Model-1800) double-beam UV-VIS spectrophotometer and a

Jenway (England, UK) (Model-3010) pH meter measurements of absorbance and pH, respectively. A Thermo Fisher company (Model-iCE-3000) Atomic Absorption Spectrophotometer equipped with a microcomputer controlled nitrous oxide-acetylene flame was used for comparison with combined electrodes were used for the of the results. Infrared spectrum was recorded with FTIR Spectrophotometer, Shimadju (Model-IR Prestige 21, Detector-DTGS KBr) in the range 7500-350 cm^{-1}.

Table 1. Summary of review on the existing spectrophotometric methods for the determination of iron

Reagent	λ_{max} (nm)	€($Lmol^{-1}$ cm^{-1})	Beers law (mg L^{-1})	Interfer-ence	Remarks	Reference
Pentacyanoamine ferroate and ferrozine	535	2.5×10^{3}	0.02-2.0	Many	i) Solvent extractive hence lengthy and time consuming ii) Less selective due to much interference iii) Less sensitive iv) pH dependent v) Toxic organic solvent used	[11]
2-[2(3,5 dibromopyridyl)azo]5diethylaminobezic acid	624	1.08×10^{4}	0.05-5.0	Cu, Co, Ni, V	i) Solvent extractive hence lengthy and time consuming ii) Less selective due to much interference iii) Less sensitive iv) Toxic organic solvent used Application was limited	[12]
Erriochrome cyanine R	560	5.36×10^{4}	0.1-40	Many	i) Solvent extractive hence lengthy and time consuming ii) Less selective to much interference iii) Toxic organic solvent was used iv) Limited application	[13]
N-Octylacetamide	480	2.60×10^{4}	0.06-50	Many	i) Solvent extractive hence lengthy and time consuming ii) Less sensitive iii) Less selective due to much interference iv) Toxic organic solvent was used	[14]
Batho	490	3.5×10^{3}	0.8-10	Many	i) Solvent extractive	[15]

Reagent	λ_{max} (nm)	€(Lmol^{-1} cm^{-1})	Beers law (mg L^{-1})	Interfer-ence	Remarks	Reference
phenanthroline					hence lengthy and time consuming ii) Less selective due to much interference	
3-(2-pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,2,4-triazine	562	2.8×10^4	1-1.1	PO_4, NO_3, NO_2, Co, Cu, CN, Ni	i) Solvent extractive ii) Lengthy and time consuming iii) Less selective due to much interference iv) Limited application	[16]
Thiocyanate	480	2.1×10^4	0.5-2	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) Less sensitive iv) Limited application	[17]
1-(-2pyridylazo)-2-napthol (PAN)	550	5.8×10^3	0.3-5.0	Many	i) Solvent extractive hence lengthy and time consuming ii) pH dependent iii) Less selective due to much interference iv) Less sensitive	[18]
9-(4-carboxyphenyl)-2,3,7-trihydroxyl-6-flurone	640	1.06×10^3	4-300	Many ions interfere	i) Less sensitive ii) Solvent extractive hence lengthy &time consuming. iii) Less selective due to much interference iv) Toxic organic solvent	[19]
2-aminophenol	402	6.65×10^5	0.01-6	Using suitable masking , the reaction can be highly selective	i) Ultrasensitive ii) Highly selective iii) Completely aqueous reaction medium iv) No toxic solvent was used v) Simple and rapid determination vi) Application in various real, biological, soil, food and pharmaceutical samples vii) Complex stable more than 24	**Present method**

Reagent	λ_{max} (nm)	€($Lmol^{-1}$ cm^{-1})	Beers law (mg L^{-1})	Interfer-ence	Remarks	Reference
					hours	

2.2 Reagents and Solutions

All chemicals used were of analytical-reagent grade or the highest purity available. Deionised water was used throughout the study. Triply distilled ethanol (from lime), which is non absorbent under ultraviolet radiation, was also used. High purity water was obtained by passing tap water through cellulose absorbent and to mixed-bed ion exchange columns, followed by distillation in a corning AG-11 unit. The A1 level in the high-purity water was found to be below the spectrophotometric detection limit (3s of the blank) of 1 µg L^{-1}. Glass vessels were cleaned by soaking in acidified solutions of $KMnO_4$ or $K_2Cr_2O_7$, followed by washing with concentrated 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 bottle containing 1 mL of concentrated HNO_3. More rigorous contamination control was applied when the iron levels in specimens were low.

2.3 2-Aminophenol (OAP) Solution

The reagent solution was prepared by dissolving the requisite amount of 2-aminophenol (BDH chemicals, proanalysis grade, 99% pure), in a known volume of distilled de-ionized water. More dilute solutions of the reagent were prepared as and when required.

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

A 100 mL amount of stock solution (1 mg mL^{-1}) of divalent iron was prepared by dissolving 497 mg of ferrous sulfate ($FeSO_4.7H_2O$) purified-grade (Merck pro analysis grade) 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 [20].

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

A 100 mL amount of stock solution (1 mg mL^{-1}) of trivalent iron was prepared by dissolving 490 mg of Ferric chloride ($FeCl_3.6H_2O$) (Aldrich A.C.S. grade) in doubly distilled de-ionized water. Aliquots of this solution were standardized with potassium dichromate solution [20]. More dilute standard solutions were prepared by appropriate dilution of aliquots from this stock solution with de-ionized water as and when required.

2.3.3 Potassium permanganate solution

A 1% potassium permanganate (Merck) solution was prepared by dissolving in de-ionized water. Aliquots of this solution were standardized with oxalic acid.

2.3.4 Potassium dichromate solution

A 100 mL amount of stock solution (0.1 N) was prepared by dissolving 500 mg of finely powdered $K_2Cr_2O_7$ (Merck) in 100 mL de-ionized water.

2.3.5 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.

2.3.6 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 tetra hydrate in (100 mL) de-ionized water.

2.3.7 Aqueous ammonia solution

A 100 mL solution of aqueous ammonia was prepared by diluting 10 mL concentrated NH_4OH (28-30%, ACS grade) to 100 mL with de-ionized water. The solution was stored in a polypropylene bottle.

2.3.8 EDTA solution

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

2.3.9 Other solutions

Solutions of a large number of inorganic ions and complexing agents were prepared from their analytical grade or equivalent grade water-

soluble salts (or the oxides and carbonates in hydrochloric acid).

2.4 General Procedure

A volume of 0.1-1.0 mL of a neutral aqueous solution containing 0.1-60 μg of iron (III) in a 10-mL calibrated flask was mixed with a 1:220-1:800 fold molar excess of the 2-aminophenol (OAP) reagent solution (preferably 1 mL of 4.5×10^{-3} M) followed by the addition of 0.5-1.5 mL (preferably 1 mL) of 0.01 M sulphuric acid. After one minute the mixture was diluted to the volume with deionized water. The absorbance was measured at 402 nm against a corresponding reagent blank. The iron content in an unknown sample was extrapolated from a concurrently prepared calibration graph.

2.5 Sample Collection and Preservation

2.5.1 Environmental samples

Water samples were collected in polyethylene bottles from different places of Bangladesh. After collection, conc. HNO_3 (1 mL per Liter) was added as preservative.

2.5.2 Blood and urine

Blood and urine samples were collected in polythene bottles from effected persons of Chittagong Medical College Hospital, Bangladesh. Immediately after collection they were stored in ice and later, in the laboratory, at -20°C.

2.5.3 Soil samples

Soil samples were collected from different location of Bangladesh. Samples were dried in air and homogenized with a mortar.

2.5.4 Food samples

Food samples (banana, meat, tomato, bean, egg, arum and lentil) were collected from local market of Chittagong. After collection the samples were stored in refrigerator for preservation. Samples (arum, lentil, bean) were used as dry condition and homogenized with mortar.

2.5.5 Pharmaceutical samples

Pharmaceutical samples (tablets and capsules) of different companies were collected from local pharmacy of Chittagong, Bangladesh. Samples were homogenized with a mortar.

2.6 Procedure for Applications

2.6.1 Determination of iron in 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. [21]. To it, 10 mL of concentrated HNO_3, 1 mL of concentrated H_2SO_4 and 1-2 mL of 1% $KMnO_4$ were added to oxidize Fe (II) to Fe (III), excess of $KMnO_4$ was removed by addition of 1-2 mL freshly prepared 2.5% sodium azide solution and carefully covering the flask with a watch glass until the brisk reaction subsided. The solution was heated to drive off excess azide solution 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)ºC. After suitable dilution with de-ionized water, the contents of the Erlenmeyer flask were warmed to dissolve the soluble salts. The solution was then cooled and neutralized with a dilute NH_4OH solution. The resulting solution was filtered, if necessary, through a 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 de-ionized 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 under the general procedure, using tartrate and 1,5-diphenylcarbazide as masking agents. Based on five replicate analyses, average iron concentration determined by spectrophotometric method was in close agreement with the certified values (Table 5). The results are shown in Table 5.

2.6.2 Determination of iron in environmental water samples

Each filtered (with Whatman No. 40) environmental water sample (1000 mL) evaporated nearly to dryness with a mixture of 2 mL concentrated H_2SO_4 and 10 mL of concentrated HNO_3 in a fume cupboard and 1-2 mL of $KMnO_4$, following a method recommended

by Greenberg et al. [22] Excess of $KMnO_4$ was removed by 2.5% freshly prepared azide solution and was heated with 10 mL of deionized water in order to remove excess azide solution and dissolved the salts. The solution was then cooled and neutralized with dilute NH_4OH 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 pre-concentrated water sample was pipetted into a 10-mL calibrated flask and the iron content was determined as described under the general procedure, using mixture of tartrate and 1,5-diphenylcarbazide as masking agents. The analyses of environmental water samples for iron from various sources are shown in Table 6.

Most spectrophotometric method for the determination of iron in natural and sea-water require pre-concentration of iron [22]. The concentration of iron in natural and sea-water is a few μg L^{-1} in Japan [16]. The mean concentration of iron found in UK drinking water is less than 1 mgL^{-1} (Av. 200 μg L^{-1}) [23].

2.6.3 Determination of iron in biological samples

Human blood (2-5 mL) and urine (20-30 mL) was collected in polythene bottles from the affected persons. Immediately after collection, they were stored in a salt-ice mixture and later, at the laboratory, were kept at -20ºC. The samples were taken into a 100 mL micro-Kjeldahl flask. 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 [24]. 1 mL volume of concentrated sulfuric acid and 1-2 mL of 1% $KMnO_4$ were added carefully and excess of $KMnO_4$ was removed by 2.5% freshly prepared sodium azide solution followed by the addition of 0.5 mL of 70% $HClO_4$ and heating was continued for at least ½ hr to remove excess azide solution and then cooled. The solution of flask then neutralized with dilute NH_4OH solution in the presence of 1-2 mL of a 0.01% (w/v) tartrate. 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 under the general procedure using tartrate and 1,5-diphenylcarbazide as masking agents. 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 high iron concentration with Cu and Zn. Occurrence of such high iron contents are also reported in liver cirrhosis patients from some developed countries [25].

2.6.4 Determination of iron in food samples

An air dried food sample bean (2 gm), chicken (2 gm), banana (2 gm), tomato (5 gm), arum (1 gm), lentil (1 gm), egg (1 g) 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 a method recommended by Stahr [24]. 1 mL volume of concentrated sulfuric acid and 1-2 mL of 1% $KMnO_4$ were added carefully and excess of $KMnO_4$ was removed by 2.5% of freshly prepared sodium azide solution followed by the addition of 0.5 mL of 70% $HClO_4$ and heating was continued for at least ½ hr to remove excess azide solution and then cooled. The solution of flask then neutralized with dilute NH_4OH solution. The resultant solution was then transferred quantitatively into a 25-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 under the general procedure using tartrate and 1,5-diphenylcarbazide as masking agents. High value of iron for banana *(Musa acuminata*) is probably due to the involvement of high iron concentration in the soil. The results are shown in Table 8.

2.6.5 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 oxidizing agent (1% $KMnO_4$), following the method recommended by Hesse [26]. Excess of $KMnO_4$ was removed by 2.5% freshly prepared sodium azide solution and heating was continued for at least half an hour to

remove excess azide solution and then cooled. 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. 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. The iron content was determined as described under the general procedure using tartrate and 1,5-diphenylcarbazide as masking agents. The iron content was then determined by the above procedure and quantified from a calibration graph prepared concurrently. The results are shown in Table 9.

2.6.6 Determination of iron in pharmaceutical samples

Finished pharmaceutical samples (Fe containing tablet and capsule) were quantitatively taken in a beaker and digested following a method by Ahmed et al. [27]. Add 10 mL of concentrated nitric acid and heated to dryness and then added 10 mL of 20% (v/v) of H_2SO_4 and 1-2 drops of perchloric acid. The volume was reduced to 2-5 mL and then cooled to room temperature. The solution was then heated in presence of 1-2 mL of 0.1% (w/v) $KMnO_4$ to oxidize Fe (II) to Fe (III) and excess of $KMnO_4$ was removed by 2.5% freshly prepared sodium azide solution. The resulting solution was then neutralized with dilute NH_4OH and filtered and quantitatively transferred to a 25 mL calibrated flask and made up to the mark with deionized water.

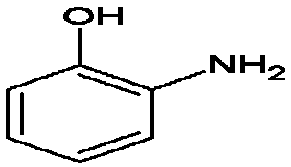
An suitable aliquot (1-2 mL) of the final solution was pippetted into a 10 mL calibrated flask and then iron content was determined as described under the general general procedure using tartrate and 1,5-diphenylcarbazide as masking agents. The results of some pharmaceutical analyses are in excellent agreement with the reported value. The analyses of pharmaceutical samples from several pharmaceutical companies for iron are given in Table 10.

3. RESULTS AND DISCUSSION

3.1 Factors Affecting the Absorbance Absorption Spectra

3.1.1 Absorption spectra

The absorption spectra of the Fe(III)-OAP system in 0.01 M H_2SO_4 medium was recorded using the spectrophotometer. The absorption spectra of the Fe(III)-OAP is a symmetric curve with maximum absorbance at 402 nm and the average molar absorption coefficient of 6.65×10^5 L $mol^{-1}cm^{-1}$ was shown in Fig. 1. The reagent blank exhibited negligible absorbance despite having wave length in the same region. The reaction mechanism of the present method was as reported earlier [28]. The structure of 2-aminophenol are shown in Scheme 1.



Scheme 1.

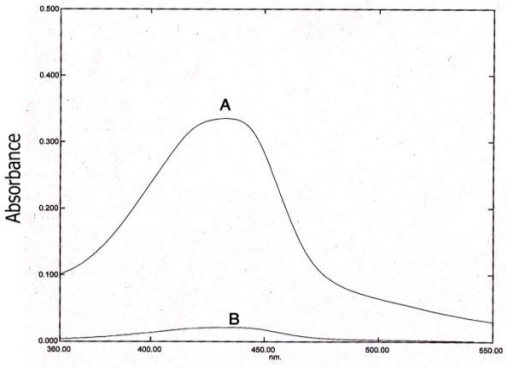


Fig. 1. A and B absorption spectra of Fe (III)-OAP system and the reagent blank (λ_{max} = 402 nm) in aqueous solutions, respectively

3.2 Effect of Acidity

Of the various acids (nitric, sulfuric, hydrochloric, phosphoric) studied, sulfuric acid was found to be the best acid for the system. The absorbance was maximum and constant when the 10 mL of solution (1 mg L-1) contained 0.5-1.5 mL of 0.01 M sulfuric acid at room temperature (25±5ºC). Outside this range of acidity, the absorbance decreased (Fig. 2). For all subsequent measurements 1 mL of 0.01 M sulfuric acid was added.

3.3 Effect of Time

The reaction was very fast and instantaneous. Constant maximum absorbance was obtained

within few seconds after dilution to volume and remained strictly unaltered for over 24 hours. A longer period of time was not studied.

3.4 Effect of Temperature

Effect of various temperatures (20-60ºC) on Fe (III)-OAP system was studied. The Fe (III)-OAP system attained maximum and constant absorbance at room temperature (25±5ºC).

3.5 Effect of Reagent Concentration

Different molar excess of 2-aminophenol (OAP) were added to a fixed metal ion concentration and absorbance were measured according to the standard procedure. It was observed that at 1 mg L^{-1} Fe (III) metal, the reagent molar ratios of 1:220-1:800 produced a constant absorbance of the Fe-chelate (Fig. 3). For all the subsequent measurements 1 mL of 4.5×10^{-3} M OAP reagent was added.

3.6 Effect of Metal Concentration

The well-known equation for spectrophotometric analysis in very dilute solutions derived from Beer's law. The effect of metal concentration was studied over 0.01-100 mg L^{-1} distributed in four different sets (0.01-0.1, 0.1-1, 1-10, 10-100 mg L^{-1}) for convenience of measurement. The absorbance was linear for 0.01-6 mg L^{-1} of iron at 402 nm. The molar absorptivity and Sandell's sensitivity [29] were found to be 6.65×10^{5} L $mol^{-1}cm^{-1}$ and 5 ng cm^{-2} of iron (III), respectively. Of the four calibration curve, the first three pass through the origin and the fourth (Fig. 4) one shows the deviation from linearity. The selected analytical parameters obtained with the optimization experiments are summarized in Table 1.

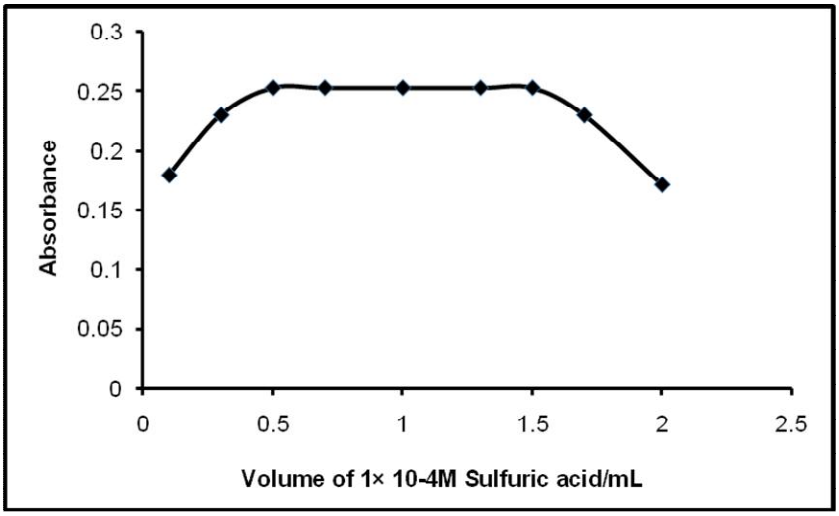


Fig. 2. Effect of acidity on the absorbance of Fe (III)-OAP system

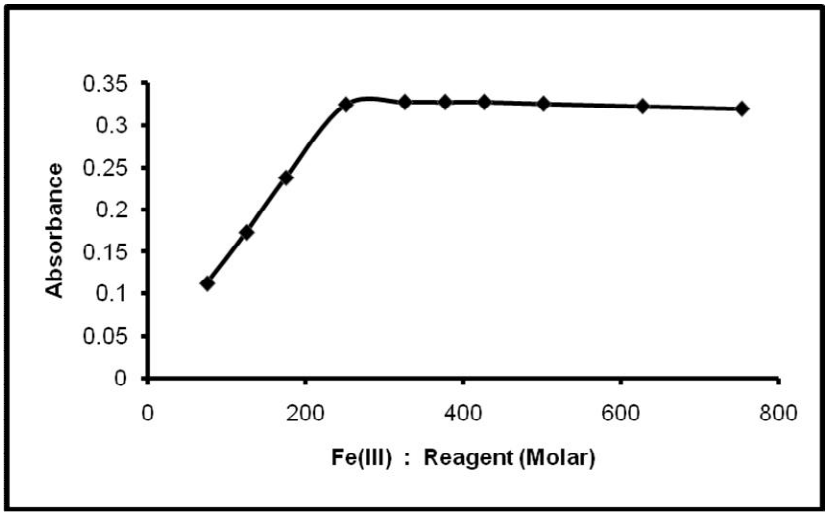


Fig. 3. Effect of reagent on the absorbance of Fe (III)-OAP system

3.7 Effect of Foreign Ions

The effect of over 50 anions, cations and complexing agents on the determination of only 1 mg L^{-1} of Fe (III) was studied. The criterion for an interference [30] was an absorbance value varying by more than 5% from the expected value for Fe (III) 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 Cr (VI) ions. In order to eliminate interference of V (V) and Cr (VI) ions, tartrate and 1,5-diphenylcarbazide used as masking agent, 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.

3.8 Composition of the Absorbent Complex

Job's method [31] of continuous variation method was applied to ascertain the stoichiometric composition of the complex under the optimum conditions (Table 2). A Fe (III)-OAP (1:3) complex was indicated by this method (Fig. 5).

3.9 Precision and Accuracy

The precision of the present method was evaluated by determining different concentrations of iron (each analyzed at least five times). The relative standard deviation (n = 5) was 0-2% for 0.1-60 µg of iron (III) in 10-mL, indicating that this method is highly precise and reproducible. The detection limit (3s of the blank) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for iron (III) were found to be 1 µg mL^{-1} and 5 ng cm^{-2}, respectively. The method was tested by analyzing several synthetic mixtures containing iron (III) 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 (III) 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).

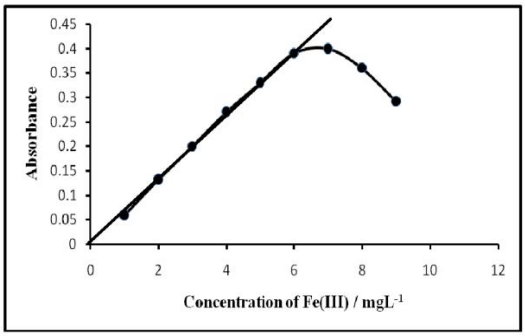


Fig. 4. Calibration graph: 1-6 mg L^{-1} of iron (III)

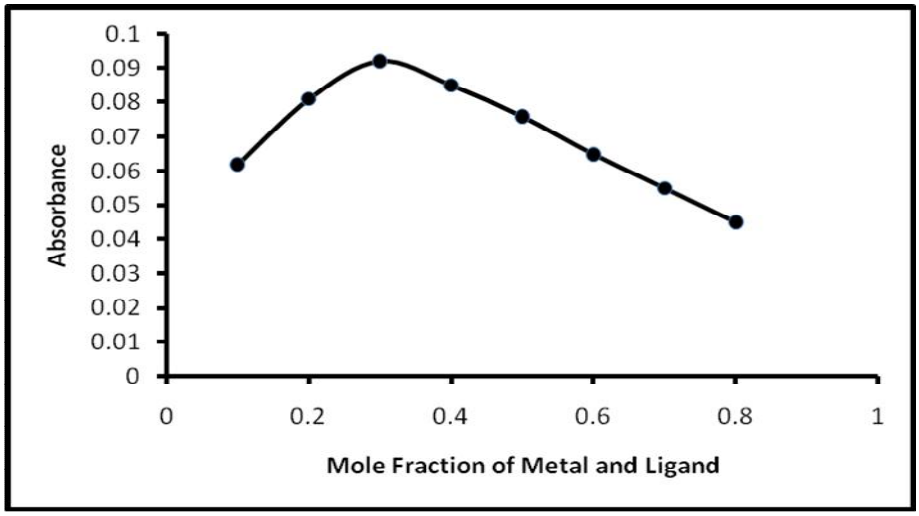


Fig. 5. Job's method for the determination the composition of Fe (III): OAP complex

Table 2. Selected analytical parameters obtained with the optimization experiments

Parameters	Studied range	Selected value
Wavelength/λ_{max} (nm)	200-800	402
Acidity/M H_2SO_4	0.0001 - 0.0025	0.0005 - 0.0015 (preferably, 0.001)
pH	5 – 2.5	3.5 – 2.8 (preferably, 3)
Time/h	0 - 48	1 min – 24 h (preferably, 1 min)
Temperature/°C	10-80	20-60 (preferably 25±5)
Reagent (fold molar excess, M:R)	1:1-1:1000	1:220– 1:800 (preferably, 1:300)
Average molar absorption Co-efficient / L mol^{-1} cm^{-1}	5.1×10^5-8.2×10^5	6.65×10^5
Linear range/mg L^{-1}	0.001-100	0.01 – 6
Detection limit/µg L^{-1}	0.01-10	1
Sandell's sensitivity/ng cm^{-2}	1 - 100	5
Reproducibility (% RSD)	0-10	0-2%
Regression co-efficient	0.9976-0.9998	0.9988

Table 3. Tolerance limits with foreign ions[a], tolerance ratio [species(x)/Fe(w/w)]

Species x	Tolerance ratio x/Fe (w/w)	Species x	Tolerance ratio x/Fe (w/w)
Aluminum	100	Lead	100
Ammonium	100	Lithium	100
Antimony	100	Magnesium	100
Arsenic (III)	50	Manganese (II)	100
Arsenic (V)	100	Manganese (VII)	100
Ascorbic acid	50	Mercury (II)	50
Azide	100	Molybdenum (VI)	50
Barium	50	Nickel	100
Beryllium	100	Nitrate	100
Bismuth (III)	100	Oxalate	100
Bromide	100	Potassium	100
Cadmium (II)	100	Phosphate	50
Calcium (II)	100	Selenium (IV)	50
Carbonate	100	Selenium (VI)	100

Species x	Tolerance ratio x/Fe (w/w)	Species x	Tolerance ratio x/Fe (w/w)
Cesium	100	Silver	50
Chloride	50	Sodium	100
Chromium (III)	100	Strontium	100
Chromium(VI)	50[c]	Sulfate	100
Citrate	50	Tellurium (IV)	200
Cobalt (II)	50	Tartrate	100
Cobalt (III)	50	Thiourea	100
Cyanide	20	Tin (II)	50
EDTA	100	Tin (IV)	100
Fluoride	100	Titanium (IV)	100
Iodide	100	Thiocyanate	100
Iron (II)	50	Tungsten	50
Vanadium	50[b]	Zinc	100

[a]Tolerance limit was defined as ratio that causes less than ±5 percent interference; [b]With 100 mgL^{-1} tartrate; [c]With 100 mgL^{-1} diphenylcarbazide

4. APPLICATIONS

The proposed method was successfully applied to the determination of iron (III) 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, food and pharmaceutical samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such sample 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 food samples by the spectrophotometric method are shown in Table 8. The results of soil analyses by the spectrophotometric method are shown in Table 9. The results of pharmaceutical samples by the spectrophotometric method are shown in Table 10. The results of speciation of iron (II) and iron (III) in mixtures are shown in Table 11.

4.1 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 1,5-diphenylcarbazide as masking agent [32,33]. The results were found to be highly reproducible. The results are shown in Table 4. Accurate recoveries were achieved in all solutions.

Table 4. Determination of iron in some synthetic mixtures

Sample	Composition of mixtures (mg L^{-1})	Iron (III)/mg L^{-1}		
		Added	Found[a] (n=5)	Recovery ±s[b] (%)
A	Fe^{3+}	0.50	0.49	98±1.0
		1.00	1.00	100±0.0
B	As in A +	0.50	0.50	100±0.0
	Na(25)+Mg(25)+Se^{6+}(25)+Cd(25)	1.00	0.99	99±1.0
C	As in B+	0.50	0.49	98±1.0
	Ba(25)+Al(25)+Cr^{3+}(25)	1.00	1.02	102±1.0
D	As in C + Zn(25)+ K(25)+Bi^{3+}(25)	0.50	0.52	104±1.3
		1.00	1.03	103±1.1
E	As in D + Ti(25)+ Sr(25)	0.50	0.54	108±1.2
		1.00	1.08	108±1.3

[a]Average of five analyses of each sample; [b]The measure of precision is the standard deviation (s)

Table 5. Determination of iron in certified reference materials

Certified reference materials (Composition, %)	Iron, %		
	Certified value	Found[a] (n=5)	RSD[b] %
BAS-10g : 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	1.53	2.0
GSBD-33001-94[a] : High tensile steel (Fe= 9.53, Si= 14.64, Al= 9.29, Ca= 1.04, Mg= 21.49 and Cr= 32.79)	9.53	9.54	1.6
YSBC-19716[a]: 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	34.10	0.8
BY0110-1[a] : High tensile steel (Zn= 42.98, Si= 19.89, Fe= 4.13, Pb= 0.351, Sn= 0.06, Cd=0.04, As= 0.024 and Cu= 0.14	4.13	4.08	1.8
GSBD33001.4-94[a] : High tensile steel Fe= 12.56, Si= 3.56, Al= 13.12, Ca= 0.17, Mg= 9.87, Cu= 50.95	12.56	12.49	1.5

[a] CRMs obtained from Beijing NCS analytical Instrument Co. China; [b] The measures of precision is the relative standard deviation (RSD)

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	140.0		
		100	240.0	100±0.0	0.00
		500	645.0	100.8±0.5	0.31
Well water		0	39.0		
		100	140.0	100.7±1.0	0.29
		500	535.0	99.2±1.2	0.31
Rain water		0	10.5		
		100	112.0	101.0±0.8	0.45
		500	515.0	100.8±1.0	0.36
River water	Karnaphuly (upper)	0	63.0		
		100	165.0	98±1.5	0.22
		500	670.0	101.2±1.0	0.19
	Karnaphuly (lower)	0	68.0		
		100	168.0	100±0.00	0.00
		500	670.0	100.3±0.5	0.32
	Halda (upper)	0	45.0		
		100	150.0	103±1.4	0.42
		500	645.0	100±0.0	0.00
	Halda (lower)	0	50.0		
		100	150.0	100±0.0	0.00
		500	655.0	100.7±1.5	0.25
Sea water	Bay of Bengal (upper)	0	12.0		
		100	115.0	102.6±0.9	0.23
		500	510.0	99.6±0.5	0.29
	Bay of Bengal (lower)	0	15.0		
		100	112.0	97.4±0.6	0.21
		500	515.0	100±0.0	0.00
Drain water	KSRM[c]	0	575.0		
		100	680.0	100.7±0.8	0.35
		500	1080.0	100.5±1.0	0.45

PHP[d]	0	235.0		
	100	340.0	101.5±0.9	0.28
	500	545.0	101.8±1.0	0.37
BSRM[e]	0	585.0		
	100	690.0	100.7±0.8	
	500	1092.0	100.6±1.0	0.31
				0.18
KPM[f]	0	135.0		
	100	240.0	102.0±0.5	0.35
	500	645.0	101.6±1.2	0.48
Elite paint[g]	0	265.0		
	100	370.0	101.4±1.5	0.46
	500	775.0	101.3±1.8	0.52

[a]average of the five replicate determination; [b] the measure precision is the relative standard deviation (s_r); [c] Kabir Steel Re-rolling Mills, Kumira, Sitakunda, Chitagong; [d] PHP Foat Glass Industries, Chittagong, Bangladesh; [e] Bangladesh Steel Re-rolling Mills Ltd, Chittagong, Bangladesh; [f]Karnafully Paper Mills, Chandragona, Rangamati; [g]Elite Paint Ltd. Nasirabad, Chittagong

4.2 Determination of Iron (Ii) and Iron (Iii) Speciation in Mixtures

Suitable aliquots (1-2 mL) of iron (III+II) mixtures (preferably 1:1, 1:5, 1:10) were taken in a 25-mL conical flask. A few drops of 0.05 M H_2SO_4 and 1-3 mL of 1% (w/v) $KMnO_4$ solution were added to oxidize Fe (II) to Fe (III). 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 and then cooled to room temperature. Then 3-4 drops of a freshly prepared sodium azide solution (2.5%, w/v) was added to remove excess $KMnO_4$ solution and heated gently with the further addition of 2-3 mL water, if necessary, for 5 minutes to drive off the excess azide solution and cooled to room temperature. The reaction mixture was neutralized with dilute NH_4OH and transferred quantitatively into a 10-mL calibrated flask [34,35]. Then the total iron (III+II) content was determined according to the general procedure with the help of the calibration graph.

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[b], %	Found	RSD[b], %	
1	Blood	1.2	1.0	1.25	1.2	Normal Adult (Male)
	Urine	0.25	1.5	0.29	1.5	
2	Blood	0.62	1.2	0.65	1.0	Anemia patient (Male)
	Urine	0.16	1.8	0.17	1.9	
3	Blood	1.75	1.5	1.80	1.5	Diabetes patient
	Urine	0.45	1.8	0.48	2.0	(Male)
4	Blood	4.91	1.5	4.87	1.3	Liver cirrhosis patient
	Urine	1.25	2.5	1.27	2.0	(Male)
5	Blood	1.1	1.0	1.2	1.0	Pregnant woman
	Urine	28.0	1.3	31.0	1.5	
6	Blood	2.0	1.3	2.10	1.0	Adolescent patient
	Urine	0.55	1.8	0.58	1.5	(Male)

[a]Samples were from Chittagong Medical College Hospital, Chittagong, [b] the measures of precision is the relative standard deviation (RSD)

Table 8. Determination of iron in some food samples

Sample[a]	Iron/μg g^{-1}			
	AAS (n = 5)		Proposed method	
	Found	RSD[b] (%)	Found	RSD[b] (%)

Bean (*Phaseolus vulgaris*)	1.02	1.0	1.08	1.2
Chicken meat (*Gallus gallus domesticus*)	0.77	1.0	0.82	1.0
Banana *(Musa acuminata*)	17.8	2.0	18.5	2.1
Tomato (*Solanum lycopersicum*)	14.5	1.8	15.0	2.0
Arum (*Arum dioscoridis*)	11.4	1.5	11.8	1.6
Lentil (*Lens Culinaris)*	0.99	1.0	1.2	1.0
Egg *(Gallus domesticas)*	1.33	1.5	1.4	1.8

[a]Samples were from local market, Chittagong; [B]The measure of precision is the relative standard deviation (RSD)

Table 9. Determination of iron in some surface soil

Serial No.	Iron (mg kg^{-1})[a]	RSD[b] (%)	Sample source
S_1[c]	35.5	1.5	Agriculture soil (Chittagong University Campus)
S_2	19.5	1.2	Marine soil (Bay of Bengal)
S_3	32.8	1.6	Eustrain soil (Junction of Bay of Bengal + River Karnaphully, Chittagong)
S_4	25.6	1.5	River soil (River Halda, Chittagong)
S_5	75.8	2.0	Industrial soil (Bangladesh Steel Re-rolling Mills Ltd., Chittagong, Bangladesh)

[a]Average of five analyses of each sample; [b]Measure of precision is the relative standard deviation (RSD), [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.

An equal aliquot of the above iron (III+II) mixture was taken into a 25-mL beaker. 1 mL of 0.01% (w/v) 1,10-phenanthroline was added to mask iron (II) and neutralize with dilute NH_4OH. Then, the content of the beaker was transferred into a 10-mL calibrated flask and its iron (III) content was determined according to the general procedure. The iron concentration was calculated in $\mu g\ L^{-1}$ or $mg\ L^{-1}$ with the aid of a calibration graph. This gives a measure of iron originally present as iron (III) in the mixture. The value of the iron (II) concentration was calculated by subtracting the concentration of iron (III) from the corresponding total iron concentration. The results were found to be highly reproducible. The occurrence of such reproducible results is also reported for different oxidation states of iron [31]. The results of a set of determination are given in Table 11.

The present method was compared with a reported method [27] statistically. It was found that present method is much superior that of the reported method. The results are shown in Table 12.

Table 10. Determination of iron in some pharmaceutical samples

Pharmaceutical samples	Brand name	Trade name	Iron/$\mu g g^{-1}$		RSD (%)
			Reported (claimed) value	Found (n=5)	
Tablet	Aristopharma Ltd.	Ipac plus/mg	188	185.8	2.0
	Incepta pharmaceuticals Ltd.	Alneed Gold/mg	47	48	1.5
	Beximco Pharmaceutical Ltd	Hemofix FZ/mg	48	47.8	1.8
	Beximco Pharmaceutical Ltd.	Zovia Gold/mg	18	17.5	2.0
Capsule	Square Pharmaceutical Ltd.	Zif - CI/mg	25	24.8	2.5

Table 11. Determination of iron (III) and iron (II) speciation in mixtures

Serial No.	Fe (III): Fe (II)	Fe, taken (mg L^{-1})		Fe, found (mg L^{-1})		Error (mg L^{-1})	
		Fe (III)	Fe (II)	Fe (III)	Fe (II)	Fe (III)	Fe (II)
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 (III)= ± 0.01; Fe (II) = ± 0.01; Standard deviation: Fe (III) = ± 0.005; Fe (II) = ± 0.006							
1	1:3	1.00	3.00	0.98	2.98	0.02	0.02
2	1:3	1.00	3.00	0.99	2.99	0.02	0.01
3	1:5	1.00	3.00	0.98	2.98	0.01	0.02
Mean error: Fe (III)= ±0.016; Fe (II) = ±0.016; Standard deviation: Fe (III) = ±0.0058; Fe (II) = ±0.006							
1	1:5	1.00	5.00	0.98	4.98	0.02	0.02
2	1:5	1.00	5.00	0.99	5.00	0.01	0.00
3	1:5	1.00	5.00	0.98	4.98	0.02	0.02
Mean error: Fe(III) = ± 0.0167; Fe (II) = ± 0.0016; Standard deviation: Fe(III) = ± 0.006; Fe (II) = ± 0.005							

Table 12. Statistical comparison of proposed method with reference method [31]

Serial No.	Sample	Sample sources	Proposed method, s_1^2	Reference method[31], s_2^2	F-test[a] values, s_2^2/s_1^2
1	Blood	Anaemia patient (Male)	1.0	1.5	0.44
	Urine		1.9	1.9	1.0
2	Blood	Lever cirrhosis patient	1.3	1.4	0.92
	Urine	(Male)	2.0	2.0	1.0
3	Blood	Pregnant woman	1.0	1.3	0.59
	Urine		1.5	1.6	0.87
4	Blood	Normal (Male)	1.5	1.8	0.8
	Urine		1.5	1.5	1.0
5	Agricultural soil	Chittagong University Campus	1.2	1.5	0.8
6	Marine soil	Bay of Bengal,Chittagong, Bangladesh	1.2	1.5	0.64
7	Estuarine soil	Junction of Bay of Bengal + River, Karnaphully, Chittagong	1.6	1.8	0.88
8	Industrial soil	Bangladesh Steel Re-rolling Mills Ltd., Chittagong, Bangladesh	2.0	2.0	1.0

[a]*Tabulated F-value for (5.5) degrees of freedom at P(0.98) is 5.72.* s_1*=standard, deviation of proposed method;* s_2*= standard deviation of reported method*

5. CONCLUSIONS

In this paper, a new, simple, sensitive, selective and inexpensive method with the Fe (III)-OAP complex was developed successfully for the determination of iron in some real, environmental, biological, soil, food and pharmaceutical samples for continuous monitoring to establish the trace levels of iron in difficult sample matrices. It offers also a very efficient procedure for speciation analysis. Although many sophisticated techniques such as pulse polarography, HPLC, AAS, ICP-OES 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 spectrophtometry to remain a popular technique, particularly in laboratories of 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±5ºC).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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