Method validation on iron determination by spectrophotometric method in aqueous medium

Kazi Moriom Rahman[a], Bristy Biswas[a], Tahuran Neger[a], Nahid Sharmin[a] & Lutfor Rahman[a,*]

[a]Institute of Glass and Ceramic Research and Testing (IGCRT), Bangladesh Council of Scientific and Industrial Research (BCSIR),
Dr.Qudrat-I-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh

Email: lutforju33@yahoo.com

This paper deals with the validation of a method for the determination of iron in spectrophotometric method in aqueous medium. The method is based on complex formation of iron with thioglycolic acid in alkaline medium in presence of a masking agent to produce a red purple chelate that has an absorption maximum at 535 nm wavelength. Beer-Lambert law is obeyed and linear calibration curves are obtained for the concentration range of iron from 0.1 mg/L to 30 mg/L. The reaction is found to be spontaneous in alkaline medium. The limit of detection and limit of quantification for the developed method are 0.0108 and 0.0345, respectively. Effect of different parameters like molar ratio of iron to different reagents and interferences, effect of time and effect of temperature of this method of determination have been studied. It is found that this method is moderately sensitive and has been successfully applied for the determination of iron(III) in different fields like ceramic materials, clay, sand, glass, stone, soil, water, and any inorganic iron containing compound or alloys. A comparison report is made for Chevron gas field waste material and Certified Reference Material of iron, which is done by atomic absorption and UV-visible spectroscopy techniques and found to be comparable.

Keywords: Determination of iron, Spectrophotometric method, Method validation, Limit of detection, Limit of quantification

Iron present in the nature in the form of +2 and +3 oxidation state. Since Fe^{2+} and Fe^{3+} ions have chromophoric properties, many methods utilize reagents without chromophoric groups[1]. The basis in the spectrophotometric determination deals with the formation of chelate complexes with metal ions. These complexes may be water soluble or organic solvent soluble or insoluble in both. They may be either anionic or cationic. There are many well-known spectrophotometric methods for the determination of iron(II) and iron(III). Among them thiocyanate method[1, 2], 1, 10-phenanthroline method and 2, 2'-bipyridal method[3-7], bathophenanthroline extraction method[8-10], sulfosalicylic acid method[11, 12] and thioglycolic acid method[13] are usually used to determination of iron. However, thioglycolic acid (TGA) method is more convenient from other method because it is relatively easy, extraction is not required and has minimum interferences (only Al^{3+} and Cr^{3+} ions) which are easily masked by a suitable masking agent to make the method highly selective[14]. The primary theme of this paper is to find out an easy, single step spectrophotometric determination method for iron, which can be applied in any inorganic field. The objective of this study is to validate a method to determine iron by UV-visible spectrophotometric method without extraction of iron. To the best of our knowledge, no such work has so far been reported.

Materials and Methods

Reagents

Certified Reference Material (CRM) for iron and aluminium were collected from Sigma-Aldrich. Analytical grade (Merck, Germany) ammonia, TGA, tartaric acid (TA), potassium dichromate were collected from local market. The assay of used chemicals is given in Table 1. Deionized water, which is non-absorbent under ultraviolet radiation and certified glass apparatus were used throughout the study.

Apparatus

i) A Hitachi UV-visible Double Beam Spectrophotometer (Model-UH 5300) connected with a microcomputer (Model: HP 19US) ii) Atomic absorption spectrophotometer (AAS) (Model No.: AA7000, Shimadzu, Japan) iii) Electric Balance (Model No.: ATX224, SHIMADZU).

Experimental procedure

Requisite amount of iron from 10 ppm CRM solution were allowed to react in a calibrated 10 ml

volumetric flask with 1.1 M TGA in alkaline medium in presence of 0.67 M masking agent to produce chelate complex. The absorbance of the red purple chelate complex was measured at 535 nm against respective reagent blank. Five replicas of each experiment or analyses were carried out and their mean, mean deviation (MD) and standard deviation (SD) were calculated according to the standard statistical procedure[15].

Reaction Mechanism

A complex reaction takes place[13] during chelating of iron with TGA in alkaline medium and is represented in Scheme 1:

Results and Discussion

Absorption spectra

The absorption spectra of TA, TGA, reagent blank and after chelating with iron were recorded using the spectrophotometer and shown in the Fig. 1 a-d, respectively. It is found from these curves that TA, TGA and reagent blank exhibit negligible absorbance at 535 nm whereas chelate complex of iron shows the maximum absorbance at that region.

Influence of TGA

The effect of molar ratio of iron to TGA in the formation of chelate complex with respect to absorbance is shown in Table 2. Here five different complex formations were carried out with five

Table 1 — Assay of used chemicals

Name of Chemicals	Assay report	Origin
CRM for iron	1001 ± 4 ppm	Sigma-Aldrich
CRM for aluminium	1000 ± 4 ppm	Sigma-Aldrich
Ammonia	25.0%	Merck, Germany
Thioglycolic acid	80.0%	Merck, Germany
Tartaric acid	99.5%	Merck, Germany
Potassium dichormate	99.7%	Merck, Germany
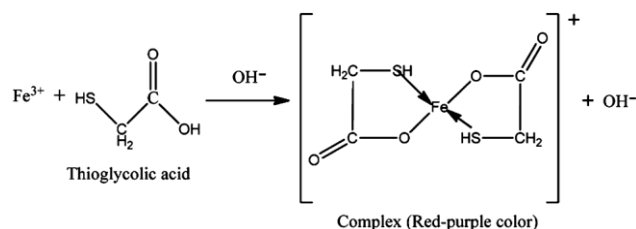
Scheme 1 — A Schematic representation of complex reaction during chelation of iron with TGA in alkaline medium.
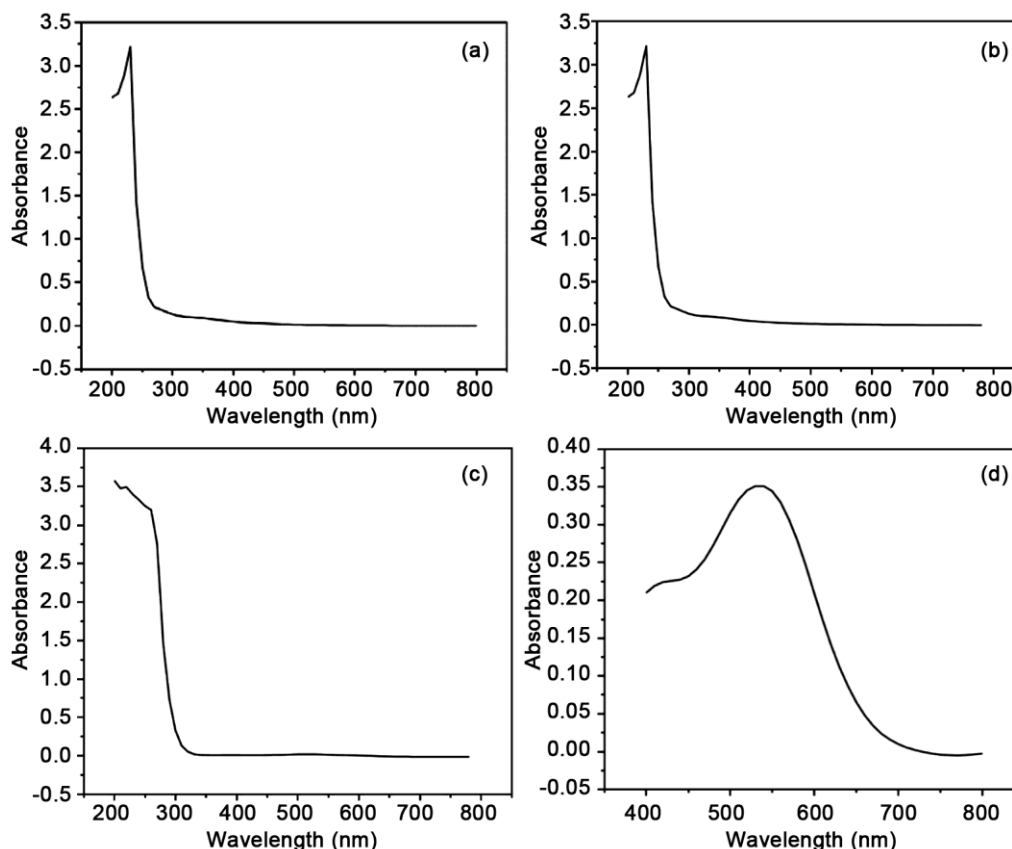
Fig. 1 — Absorption spectra of (a) 0.67 M TA, (b) TGA, (c) reagent blank and (d) chelate complex of iron.

different molar ratio of iron to TGA. It is found that the absorbance value increases with the increase of molar ratio of iron to TGA up to 0.01:12.37 and then declines. It indicates that 0.01: 12.37 molar ratio of iron to TGA is optimal for chelate formation of iron.

Influence of TA

Table 3 depicts the effect of molar ratio of iron to TA with respect to absorbance in the formation of chelate complex. Here five different complex formations were done with five different molar ratio of iron to TA. It is found that the absorbance readings of this series have no consistence. The values are neither increases nor decreases with the increase of molar ratio which indicate that tartaric acid itself has no effect in the formation of chelate complex. It is needed for masking interferences ions (Al^{+3} and Cr^{+3}) only, whose individual studies were carried out later. For further study, we choose the middle one i.e. 0.01:7.47 iron to TA molar ratio for chelating iron to complete this paper.

Effect of TA on interference ions in the determination of iron

It is stated earlier that only Al^{3+} and Cr^{3+} affect this method to determine the iron. The effect of interference ion and complexing agent in this determination method with 5ppm iron was studied and depicted in the Table 4 and Table 5 for Al^{3+} and Cr^{3+}, respectively. It can be said from the Table 4 and Table 5 that the additive ions have no significant effect on the determination of iron in presence of tartaric acid. Without TA, the absorbance readings of the complex prepared with the molar ratio of 0.01:0.0124 iron to aluminium and that of 0.01:0.0032 iron to chromium are found to be slightly higher than those with TA which indicate that aluminium and chromium have positive effect on absorbance by this method. Al^{3+} ion easily forms water soluble Al-tartrate complex with tartaric acid[16,17] and Cr^{3+} ion forms inert complexes with tartaric acid as tartrate[18]. Though the tolerance limits for these ions was not evaluated in this study.

Influence of ammonia

Influence of ammonia with respect to absorbance in the formation of chelate complex was studied and is shown in Table 6. Here five different complex formations were done with five different molar ratio of iron to ammonia. It is found that the absorbance value increases with the increase of molar ratio of iron to ammonia. Therefore, it proves that molar ratio of iron to ammonia is a dependable factor to determine

iron by this method. The reason for the influence is that, with the addition of ammonia, TGA is converted to ammonium thioglycolate and it reaches to

Table 2 — Influence of TGA on the absorbance

Molar ratio of iron to TGA	Mean absorbance of 5 replica $\pm$ MD	SD
0.01: 7.39	0.3236 $\pm$ 0.0024	0.0036
0.01: 9.90	0.3291 $\pm$ 0.0044	0.0053
0.01: 12.37	0.3373 $\pm$ 0.0024	0.0018
0.01: 14.84	0.3325 $\pm$ 0.0024	0.0028
0.01: 17.32	0.3282 $\pm$ 0.0012	0.0017

Conditions: Amount of iron: 5ml from 10 ppm CRM solution, Amount of 0.67 M TA:1 ml, Amount of 6.62 M ammonia: 3 ml, T 26 °C, t below 5 min

Table 3 — Influence of TA on the absorbance

Molar ratio of iron to TA	Mean absorbance of 5 replica $\pm$ MD	SD
0.01: 4.48	0.3212 $\pm$ 0.0042	0.0066
0.01: 5.98	0.3176 $\pm$ 0.0035	0.0043
0.01: 7.47	0.3203 $\pm$ 0.0009	0.0012
0.01: 8.97	0.3274 $\pm$ 0.0016	0.0022
0.01: 10.46	0.3177 $\pm$ 0.0043	0.0052

Conditions: Amount of iron: 5 ml from 10 ppm CRM solution, molar ratio of iron to TGA 0.01:12.37, Amount of 6.62 M ammonia: 3 ml, T 26 °C, t below 5 min

Table 4 — Influence of Al^{3+} ions in the determination of iron

Molar ratio of iron to Al^{3+} with TA	Molar ratio of iron to Al^{3+} without TA	Mean absorbance of 5 replica $\pm$ MD	SD
0.01: 0.0041	Not Done	0.3404 $\pm$ 0.0002	0.0002
0.01: 0.0082	Not Done	0.3407 $\pm$ 0.0002	0.0003
0.01: 0.0124		**0.3410 $\pm$ 0.0002**	**0.0002**
	0.01: 0.0124	**0.3452 $\pm$ 0.0002**	**0.0003**
0.01: 0.0166	Not Done	0.3408 $\pm$ 0.0002	0.0003
0.01: 0.0207	Not Done	0.3408 $\pm$ 0.0002	0.0002

Conditions: Molar ratio of iron:TGA:TA::0.01:12.37:7.47, Amount of 6.62 M ammonia: 3 ml, T 26 °C, t below 5 min

Table 5 — Influence of Cr^{3+} ions in the determination of iron

Molar ratio of iron to Cr^{3+} with TA	Molar ratio of iron to Cr^{3+} without TA	Mean absorbance of 5 replica $\pm$ MD	SD
0.01: 0.001	Not Done	0.3690 $\pm$ 0.0001	0.0002
0.01: 0.0022	Not Done	0.3692 $\pm$ 0.0001	0.0001
0.01: 0.0032		**0.3691 $\pm$ 0.0002**	**0.0002**
	0.01: 0.0032	**0.3723 $\pm$ 0.0002**	**0.0003**
0.01: 0.0043	Not Done	0.3688 $\pm$ 0.0001	0.0002
0.01: 0.0053	Not Done	0.3689 $\pm$ 0.0002	0.0002

Conditions: Molar ratio of iron:TGA:TA::0.01:12.37:7.47, Amount of 6.62 M ammonia: 3 ml, T 26 °C, t below 5 min

equilibrium after the addition of certain amount of ammonia. The more basic is the ligand, more easily it can donate electron pairs to the central metal ion and hence more easily it can form complex with greater stability. So, thioglycolate ion is a stronger ligand than thioglycolic acid due to the presence of negatively charged carboxylate oxygen. Hence, the metal-thioglycolate complex formation occurs more rapidly[19].

Influence of pH

As absorbance value increases with the increase of molar ratio of iron to ammonia, hence the effect of pH on the absorbance was studied to observe the nature of the curve and is shown in Fig. 2. It is found that the trend of the curve is upward but flattened up to absorbance value 0.3352 and then the trend is sharp upward which indicates that the molar ratio of 0.01:444.87 iron to ammonia is optimal for this determination procedure which corresponds to pH 10.38.

Influence of time

Influence of time on absorbance after the formation of chelate complex was also studied. It is found that the absorbance value recorded maximum just after chelate formation with iron which is termed as below 5 min and the absorbance value is gradually decreases with the increase of time kept after chelate formation. Because in presence of oxygen from air the ferric thioglycolate complex is undergo autoreduction and forms colorless ferrous complex[19].This study was carried out up to 6 h after the formation of chelate and is shown in Fig. 3.

Influence of temperature

Influence of temperature on the formation of chelate complex was studied at temperature 20, 30, 40 and 50 °C and the findings are shown in Fig. 4. From the graph it is found that absorbance value increases up to 30 °C and then downward rapidly. In the temperature range 20–30 °C the increase in absorbance may be due to complete complex formation and above 30 °C absorbance decreases rapidly may be because of ferric thioglycolate complex reduces to ferrous complex relatively faster than ambient condition. From this nature of the curve it can be said easily that calibration curve should be done freshly for the determination of iron by this method.

Construction of calibration curve and Beer's Law

The mathematical expression of the fundamental law of spectrophotometry, which is well known as Beer-Lambert law is

$$A = \varepsilon cl \qquad \qquad \dots (1)$$

Table 6 — Influence of ammonia on the absorbance

Molar ratio of iron to ammonia	Mean absorbance of 5 replica ± MD	SD
0.01: 148.30	0.3286 ± 0.0008	0.0011
0.01: 222.55	0.3304 ± 0.0003	0.0004
0.01: 296.58	0.3313 ± 0.0004	0.0006
0.01: 370.72	0.3332 ± 0.0003	0.0009
0.01 : 444.87	0.3352 ± 0.0007	0.0009

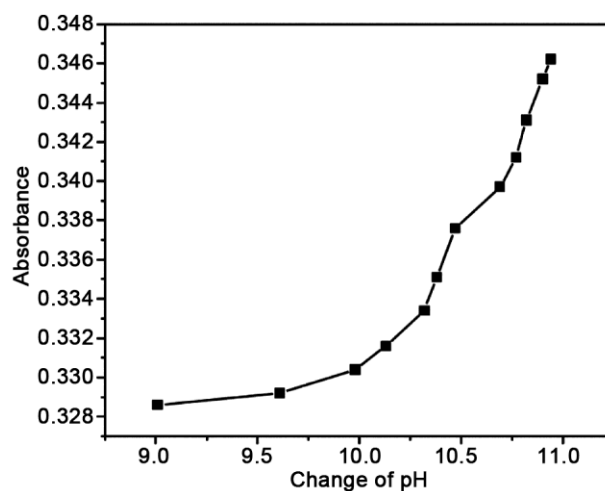*Conditions:Molar ratio of iron:TGA:TA::0.01: 12.37:7.47, T 26 °C, t below 5 min*

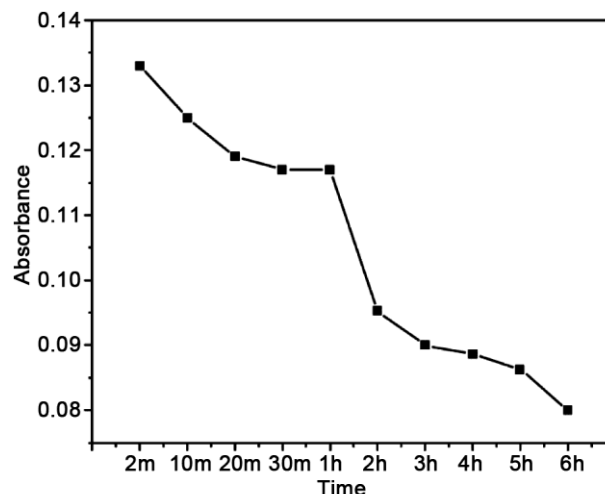Fig. 2 — Plot showing the effect of pH on absorbance.

Fig. 3 — Plot showing the effect of time on absorbance.

which states that the absorbance (A) depends on the concentration (C) of the solution at specific (1 cm) optical path length (l) [20,21]. As concentration increases, absorption value also increases. Using this law, calibration curve was made over range 0.1–50 mg/L and exposed in this study in three different sets (0.1–0.5, 1–5 & 10–50 mg/L) for convenience of measurements at 535 nm wavelength and are shown on Fig. 5a-d. From these figures, it is clearly seen that, linear calibration curve is obtained for iron concentration range from 0.1 mg/L to 30 mg/L and above that negative deviation from the straight line is observed. The molar absorptivity (€) for the determination of iron by this method is 4.07 × 10⁴ Lmol⁻¹cm⁻¹. According to the relation between sensitivity and molar absorptivity suggested by Sawin[22] this method is moderately sensitive.

Validation

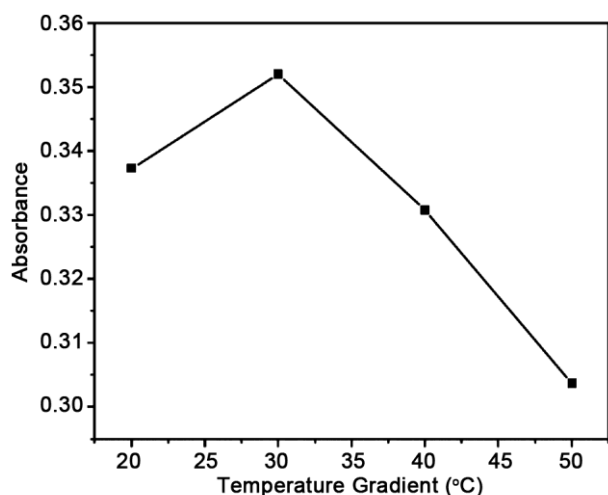The present method for the determination of iron(III) quantitatively in aqueous medium has been validated to make the test results reliable, credible and traceable. The characteristics for method validation such as linearity, accuracy, precision, percent recovery, MD, SD, limit of detection (LOD) and limit of quantification (LOQ) etc. have been done according to the standard procedure[23-25] and the results are shown in the Table 7. This validated method shows good performance on analysis having a correlation co-efficient, $r^2 = 0.9999$.

Application to real sample

This method is successfully applied for the spectrophotometric determination of iron in ceramic materials, clay, sand, glass, stone, soil and any inorganic iron containing compounds after making aqueous solution. Analysis report on few of the stated sample is given in Table 8 and the results of Chevron gas field waste materials and 4 ppm CRM of iron is compared with that of AAS analysis.

Determination of iron in sand, stone aggregate, mill scale

A mixture of sample and anhydrous A. R. sodium carbonate in a weight ratio 1:6 was taken in a platinum crucible and the mixture was covered by a thin layer of sodium carbonate. This mixture was allowed to heat at 800±20 °C to get a tranquil melt and maintained at this condition for more 20 min. The platinum crucible with fused mass was then allowed to cool followed by extraction of fused mass from the platinum crucible with hot water and 1:10 dilute hydrochloric acid. The extracted liquid was then filtered through Whatmann filter paper no. 42 in a certified volumetric flask. After filtration the content was diluted up to the mark. Iron of this solution was determined according to the described method. The result is depicted in the Table 8.

Fig. 4 — Plot showing the effect of temperature on absorbance.

Determination of iron from iron scrap and iron dust

A fixed weight of sample was allowed to react with concentrated hydrochloric acid and heated until the entire sample dissolved. Then this solution was boiled
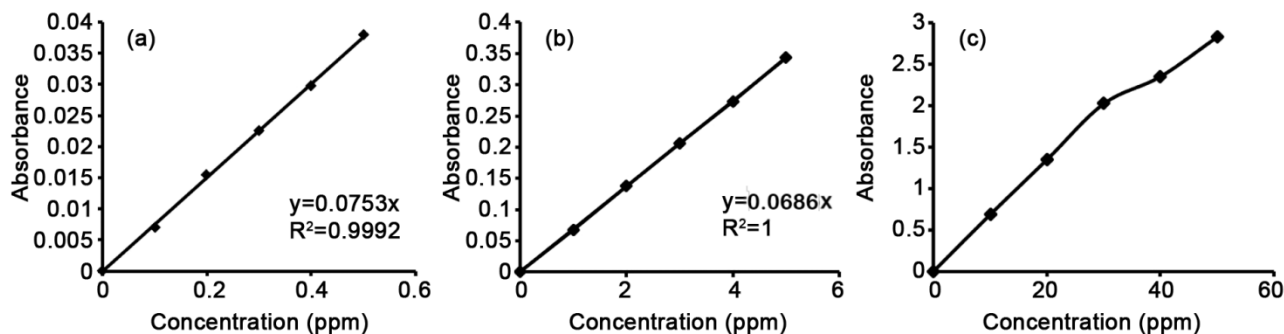

Fig. 5 — Calibration curve for CRM solution of (a) 0.1 to 0.5 ppm, (b) 1 to 5 ppm and (c) 10 to 60 ppm.

Table 7 — Summary on method validation of iron(III)

Sl. No.	Parameter	Done	Procedure	Observation
1	Specificity	Yes	Test for interference that is likely to respond to the test	Interference has no impact on the results
2	Linearity	Yes	Construction of a calibration curve by fitting with simple linear regression between concentration of six or more calibration standards and their responses	Correlation coefficient $r^2 = 0.9999$
3	Range	Yes	The interval between the upper and lower concentration of Fe in the sample	0.1 to 30 ppm
4	Accuracy	Yes	7 Replicate analyses of 2ppm sample to measure the closeness between the average test result and the accepted reference value of the iron sample	Error(%)= 0.33
5	Precision	Yes	7 Replicate analyses of the same sample under different measurement conditions e.g., between-day, between-analyst	S.D. = 0.0547
6	Percent recovery	Yes	Use of spiked sample and analyte by the method under validation both in its original state and spiking of a known mass of the analyte to the portion	99.63%
7	Limit of detection (LOD)	Yes	The lowest concentration of analyte in a sample that has been detected under the stated conditions of the test	S.D. = 0.0034 LOD = 0.0108
8	Limit of quantification (LOQ)	Yes	Analysis of sample with known lowest concentration of analyte which has been quantified with acceptable precision and accuracy under the stated conditions of the test	LOQ = 0.0345
9	Ruggedness	Yes	Reproducibility of test results obtained for same sample under different lab, different analyst different instrument, and different days	Results are precise. SD=0.0066
10	Uncertainty	Yes	7 successive measurements of 2 ppm standard sample	U = $\pm$ 0.0389

Table 8 — Analysis report for iron content in different samples

Types of sample	Mean iron content by UV-visible spectrophotometer $\pm$ MD	Mean iron content by AAS $\pm$ MD	SD
Mill scale	64.21% $\pm$ 0.012	N/D	0.0158
Sand	3.64% $\pm$ 0.0080	N/D	0.0122
Iron Chips	87.37% $\pm$ 0.0050	N/D	0.0071
Iron Scrap	81.48% $\pm$ 0.0280	N/D	0.0380
Stone Aggregate	6.11% $\pm$ 0.0180	N/D	0.0229
Buriganga River Water	0.45% $\pm$ 0.0048	N/D	0.0069
Chevron gas field waste materials	3.0774 ppm $\pm$ 0.0005	3.1208 ppm $\pm$ 0.0005	UV 0.0005/ AAS 0.0006
CRM of iron	3.9950 ppm $\pm$ 0.0003	3.9835 ppm $\pm$ 0.0004	UV 0.0.0005/ AAS 0.0005

Conditions:Molar ratio of iron:TGA:TA:ammonia::0.01: 12.37:7.47:444.87, T 26 °C, t below 5 min.

with concentrated nitric acid for 20 min to oxidize the entire ferrous ion into ferric ion. The resulting solution was cooled, filtered and transferred to a certified volumetric flask and diluted up to mark with water. Iron of this solution was determined according to the stated method. The result is given in the Table 8.

Determination of iron in Buriganga river water

Buriganga River water (collected from Mitford Hospital Ghat, Babubazar, Dhaka) was filtered to remove insoluble matter. Then aliquot of this water (filtrate) was boiled with requisite amount of concentrated nitric acid to convert all the iron into Fe^{3+} ion. Finally the iron concentration of this river water was measured according to the stated method. The result is given in the Table 8.

Comparative study of the present method with AAS

4 ppm CRM solution of iron and waste material from Chevron gas field were taken for comparison. Waste material from Chevron gas field or well-water waste materials was treated as follows.

The waste materials known as well-water waste materials obtained from well-head of gas field during

separation and purification of gas supplied by Chevron, Bangladesh. After collecting the waste material, the solid part was separated by filtration and then dried at 100 ℃. A certain amount of this solid waste material was dissolved in 1.5 M hydrochloric acid and then boiled for 20 min with 5.0 ml concentrated nitric acid for the conversion of all iron into ferric ion. Finally, the concentration of iron was measured by this validated TGA method and AAS. The results are shown in Table 8.

Conclusions

In this paper a simple, sensitive, selective and inexpensive method was developed with iron, TGA, TA and ammonia to form chelate complex at molar ratio of iron: TGA:TA:ammonia :: 0.01:12.37:7.47:444.87. This procedure is spontaneous with time and temperature dependable method. This developed method may be used for the determination of iron in any inorganic iron containing compound or alloys or matrices. A comparison of the present method with AAS was done and found to be very close having deviation 0.0434 and 0.0115, respectively for chevron gas field waste material and CRM solution of iron. The sensitivity of the present method in terms of molar absorptivity and precision in terms of standard deviation are found to be reliable for the determination of iron. The wide applicability, simplicity and less inference make the developed method an excellent choice among available method.

Supplementary Data

Supplementary data associated with this article are available in the electronic form at http://www.niscair. res.in/jinfo/ijca/IJCA_59A(06)790-796_SpplData.pdf.

Acknowledgement

Reference

1   Zygmunt M, Spectrophotometric Determination of Elements, 1st Edition (1976) 306.
2   Ovenston T C & Parker C A, *Anal Chim Acta*, 3 (1949) 277.
3   Vydra F & Kopanica M, *Chemist-Analyst*, 88 (1963) 52.
4   Harvey A E Jr, Smart J A & Amis E S, *Anal Chem*, 27 (1955) 26.
5   Hibbitis J O, Davis W F & Menke M R, *Talanta*, 8 (1961) 163.
6   Yamamura S S & Sikes J H, *Anal.Chem*, 38 (1966) 793.
7   Struszynski M, Marczenko Z & Nowtica T, *Przemyst Chem*, 32 (1953) 293.
8   Smith G. F, McCurdy W H Jr & Diehl H, *Analyst*, 77 (1952) 418.
9   Crawley R H & Aspinal M L, *Anal Chim Acta*, 13 (1955) 376.
10   Penner E M & Inman W R, *Talanta*, 9 (1962) 1027.
11   Agreen A, *Acta Chem Scand*, 8 (1954) 266.
12   Ogawa K & Tobe N, *Bull Chem Soc Japan*, 39 (1966) 223.
13   Bassett J, Denney R C, Jeffery G H & Mendham J, Vogel's Textbook of Quantitative Inorganic Analysis including Elementary Instrumental Analysis, 4th ed.(1978) 743.
14   Swank H. W & Mellon M G, Ind. *Eng Chem Anal Edn*, 10 (1938) 7.
15   Mostafa M. G, *In Methods of Statistic*, (1984) 81.
16   Hughes H K, *Anal Chem*, 24 (1952) 1349; 33 (1961) 1968.
17   Sandra D, Sandrine D &G Berthon, *J Inorg Biochem,* 81 (2000) 301.
18   Zygmunt M & Maria B, Separation, Preconcentration and Spectrophotometry in Inorganic Analysis, Analytical Spectroscopy Library, (2000) 159.
19   Robert K C & George M R, *Biochem J*, 6 (1929) 1242.
20   Testolin G, Erba D, Ciappellano S & Bermano G, *Food Addit Contam*, 13 (1996) 21.
21   Blank A B, *Zh Analit Khim*, 17 (1962) 1040.
22   Sawin S B, *CRC Crit. Rev Anal Chem*, 8 (1979) 55.
23   Nawaporn A & Hansa C, Guidance for Method Validation in Chemical Analysis, Bureau of Cosmetics and Hazardous Substances, Thailand.
24   Alankar S & Vipin B G, *Drug Dev Therap*, 2 (2011) 1.
25   Navya C N, Pravallika D & Navya S D, *IOSR J Pharma*, 5 (2015) 7.