RESEARCH ARTICLE

4-Aminoantipyrine as a New Electrophilic Coupling Reagent for Spectrophotometric Determination of Iron(III) in Water, Industrial Effluent and Soil Samples

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ABSTRACT: A Novel, rapid, sensitive and selective spectrophotometric method, using 4-aminoantipyrine (AAP) as an electrophilic coupling reagent and iminodibenzyl (IDB) as a chromogen, is proposed for the determination of trace amounts of iron(III) in water, industrial effluent and soil samples. The method is based on the reduction of iron(III) by AAP, which subsequently couples with IDB in a mild hydrochloric acid medium to form a blue coloured product having λ_{max} at 620 nm. The colour is stable up to 24 h and obeys Beer's law in the concentration range of 0.2 - 4.8 µg ml^{-1}. The optimum reaction conditions and other important analytical parameters were established to enhance the sensitivity of the proposed method. The interference due to various non- target ions was also investigated. The proposed method was applied to the analysis of iron(III) in water, industrial effluent and soil samples. The performance of the proposed method was evaluated in terms of student's *t*-test and variance ratio *F*-test that indicated the significance of the proposed method over reference spectrophotometric method.

KEYWORDS: Spectrophotometry, 4-aminoantipyrine, iminodibenzyl, iron(III), water, industrial effluents and soil.

INTRODUCTION

4-Aminoantipyrine (AAP) is an important derivative of the 5 - pyrazolone class and is used for the detection and determination of number of compounds[1]. However, literature on the use of 4-aminoantipyrine as an electrophilic coupling reagent in analytical chemistry is very scanty. Iminodibenzyl (IDB), a member of dibenzazepine class of tricyclic compounds having a central ring constituted of seven atoms, has been reported as a spectrophotometric reagent for the determination of metals[2]. Survey of the literature revealed that no attempts have been made to develop a non-kinetic or non-catalytic spectrophotometric method for the determination of iron(III) using oxidative electrophilic coupling reaction. The authors report the first-ever use of IDB as a chromogen and AAP as an electrophilic reagent for spectrophotometric determination of iron(III) in waters, industrial effluents and soil samples.

Iron is widespread in the environment and plays an important role as an essential element. Natural waters contain chemical forms of iron depending on geological area and other chemical components. Iron(III) can precipitate readily by the formations of hydrous iron oxide and hydroxides which can absorb other trace metals. Thus, iron ion controls the mobility, bioavailability and toxicity of other trace metals in natural water system. Therefore, methods for the determination of iron are needed to predict the fate and mobility of metals in natural waters[3].

A variety of well established methods for quantitative analysis of iron have been developed; they are inductively coupled plasma mass spectrophotometry (ICP-MS), atomic absorption spectrometry (AAS), electrochemical methods and ion chromatography (IC). Though all of these methods are highly sensitive, main disadvantages are the necessity of expensive and sophisticated instrumentation[4]. There is therefore, a need for simple and rapid methods of analysis that can be readily applied routinely by laboratories to large number of environmental samples. From consideration of speed of analysis, the availability of instrumentation and the relative freedom from routine maintenance and cost, visible spectrophotometry was selected as the ideal measurement technique. Spectrophotometric methods are the most frequently employed for the routine analysis of iron.

Review of literature reveals that several spectrophotometric methods have been reported for

the determination of iron in environmental samples. The visible spectrophotometric methods used for the determination of iron(III) may be broadly classified into four types. The detection chemistry of type I methods are based on complexation reactions; the reagents used in this type include hydroxyurea[5], thiobarbituric acid[6], thiocyanate[7,8], and 2-(5-bromo-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl) amino]aniline[9] to mention a few. Type II methods involve formations of mixed ligands; salicylic acid and some of its derivatives acetyl salicylic acid and salicylamide have been reported for iron(III)- salicylate pupurine complex[10].

Type III methods use therapeutic agents as ligands for complexation of iron(III). Example includes norfloxacin in ammonium sulphate-sulphuric acid media and subsequent measurement of the yellow 1:2 complex at 377 nm[11]. Type IV methods are based on catalyzed reactions, for example, using *o*-phenylenediamine-hydrogen peroxide[12,13]. Among the reagents belonging to the type I, thiocyanate has in the past been extensively used for spectrophotomteric determination of iron, but it suffers from a number of disadvantages. For example, optical densities of ferric thiocyanate solutions depend upon the conditions used from the reaction (temperature, acidity, excess of reagent); the solutions may suffer from measure of fading and do not completely follow the Beer–Lambert's law[14]. Type II and Type III are more of academic curiosity rather than for live applications in different environmental matrices. Type IV reagents have targeted applications. Besides, all the methods have limitations of interference and relatively long analysis time. 1-10 Phenanthroline is unquestionably a very good reagent for the determination of iron. However, it has a serious limitation that iron should be brought into a solution and reduce to iron(II) state by boiling with acid and hydroxylamine[4] or ascorbic acid[15].

Therefore, it is incumbent on us to develop suitable methods that can be successfully applied directly to samples having complex matrices and containing iron ranging from traces to percentage levels. In this endeavor, a robust method, overcoming most shortcomings associated with the existing thiocyanate method and meeting all the desired characteristics as expected from a standard method, has been developed. The proposed method exploits oxidative coupling reaction between AAP as an electrophilic coupling reagent and IDB as chromogen to yield blue derivative. This novel, sensitive, selective and rapid method was applied for the determination of iron(III) in water, industrial effluent and soil samples. Our research school is the first to report the use of oxidative electrophilic reactions for the spectrophotometric determination of metals[2].

MATERIALS AND METHODS

Reagents and apparatus

Stock solution of iron(III) (1000 µg ml^{-1}) was prepared by dissolving 0.8634 g of ammonium ferric sulphate dodecahydrate in 100 ml of distilled water; 1.0 ml of 2 N sulphuric acid was added. Solutions of the required strength were prepared by diluting this stock solution with distilled water.

Aqueous solution of AAP (0.1% *w/v*) was prepared by dissolving 100 mg of the compound in 100 ml of distilled water. The solution was stored in an amber bottle to protect from sunlight. IDB was received as a gift sample from Max Pharma, India. Fresh solution of IDB (0.1% *w/v*) was prepared in distilled alcohol. Solutions of diverse ions were prepared by dissolving their corresponding salts in distilled water. All other chemicals used were of Analar grade. Specord 50 UV-vis spectrophotometer with 1.0-cm silica quartz matched cell was used for measuring the absorbance.

Procedure

Appropriate volumes of standard solutions of iron(III), 1.0 ml of 1 N (*v/v*) hydrochloric acid, 1.0 ml of 0.1% (*w/v*) AAP and 2.0 ml of 0.1% (*w/v*) IDB were added to a series of 25-ml calibrated flasks. The contents were mixed thoroughly and the solutions were made up to the volume with distilled ethyl alcohol. The absorbance of the resultant blue coloured complex was measured at 620 nm against the corresponding reagent blank prepared under identical conditions but without iron(III) and the calibration graph was constructed. Concentration of iron(III) in test solution was calculated from the regression equation computed from the Beer's law data as a reference. The concentration of iron(III) determined using AAP and IDB and optical characteristics are presented in Table 1.

Determination of Iron(III) in Water Samples

Lake water, tap water, well water and treated

Table 1. Spectral data for the determination of iron(III) by the proposed method.

Parameters	Data
Colour	Blue
λ_{max} (nm)	620
Stability (h)	24
Beer's law (µg ml^{-1})	0.2 - 4.8
Molar absorptivity (L mol^{-1}cm^{-1})	0.872 x 10^4
Sandell's sensitivity (µg cm^{-2})	0.0058
Regression equation[a]:	
Slope 'a'	0.198
Intercept 'b'	0.006
Correlation coefficient 'r'	0.9967

[a]Regression curve: y = ax + b where x is the concentration of iron(III) in µg ml^{-1} and y is the absorbance.

industrial effluent samples were collected in polyethylene containers cleaned with nitric acid, and then filtered through Whatman No. 41 filter paper. Filtered samples were stored at 4 °C until analyzed. Before the analysis, 1.0 ml of H_2O_2 (10 % w/w) was added to 9.0 ml of each sample for the complete oxidation of iron(II) to iron(III). The sample solution was boiled and cooled to eliminate the unreacted H_2O_2.

Determination of Iron(III) in a Soil Sample

Soil sample (0.5 g) was slurred with 2.0 ml water in a 250-ml long stem beaker. Distilled HNO_3 (40 ml) was added and the mixture was heated at 70 °C for 2 h. The mixture was cooled and 5.0 ml of 30% H_2O_2 was added drop wise. Heating was continued for 1 h with intermittent stirring by gentle swirling of the beaker. The cooled content was filtered and washed and diluted to 250 ml with distilled water in a standard flask.

RESULTS AND DISCUSSION

Absorption Spectrum of Coloured Derivative

The absorption spectrum of the blue coloured product was recorded with a Specord 50-UV-vis spectrophotometer by scanning the wavelength in the region of 400-740 nm. Wavelength of maximum absorption was found at 620 nm. The reagent blank showed negligible absorption at this wavelength (Fig. 1).
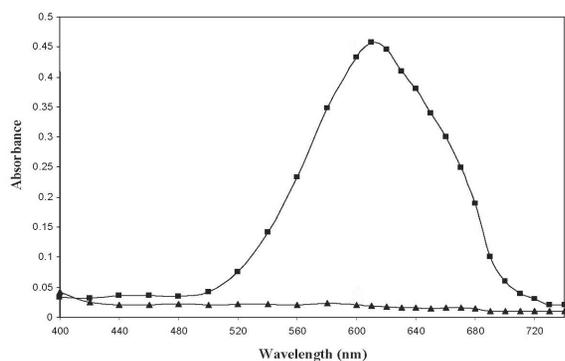

Fig 1. Absorption spectra of the reaction product of Fe^{3+} + AAP + IDB (-■-) and reagent blank (-▲-).

Effect of Time

Under the optimized conditions, although the colour developed almost instantaneous, 1 min was allowed to obtain the maximum and constant absorbance. The blue colour derivative was stable up to 24 h. The absorbance varied by ± 2 % in a day.

Effect of Temperature and Solvent

The colour development was independent of temperature in the range of 20-35 °C. The intensity of

the blue colour when diluted with water decreased rapidly, but ethyl alcohol stabilized the colour and was stable up to 24 h (± 2 % variation).

Acids such as sulphuric acid, nitric acid and perchloric acid; bases such as sodium hydroxide, ammonia; and solvents such as acetone, acetic acid and acetonitrile, were not effective in stabilizing the blue colour. As against this, methyl alcohol and ethyl alcohol showed profound influence on the stability of colour. Ethyl alcohol is preferred for routine analysis as it is nontoxic and cost-effective.

Order of Addition

The sequence of addition of reactants is also important as it influenced, to a great extent, the intensity and stability of the coloured product. The intensity and the stability of the colour was less when we followed the sequence of addition either (i) IDB-acid-substrate-AAP or (ii) substrate-acid- IDB-AAP. But, the sequences (iii) substrate-acid-AAP-IDB or (iv) AAP-acid-substrate-IDB, gave more intense and stable blue colour. This was expected as the reactions in the sequences of addition (i) and (ii) produced radical cation while, electrophilic substitution reaction was involved in the sequences (iii) and in (iv).

Reaction mechanism

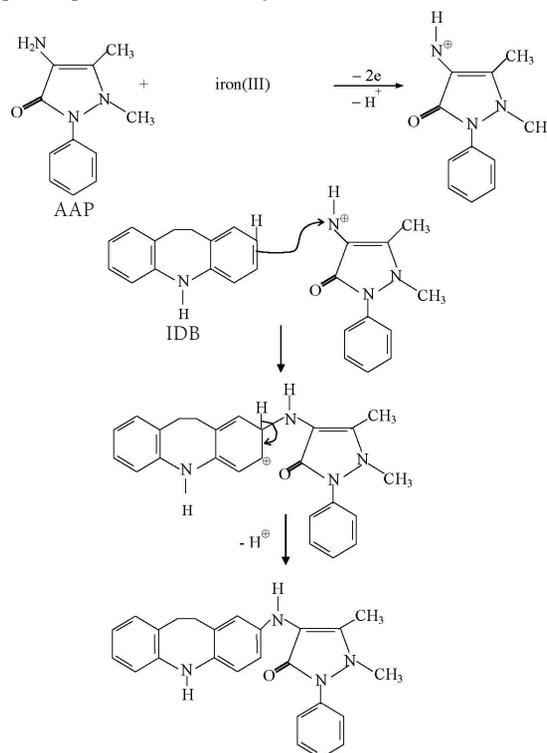The chemical reaction in the present spectrophotometric study of iron(III), involves the

Fig 2. Proposed mechanism of the reaction between AAP and IDB.

reduction of iron(III) by AAP and subsequent coupling of the oxidized AAP with IDB in mild acidic medium to form a blue product having λ_{max} at 620 nm. The factors affecting the colour development, reproducibility, sensitivity and adherence to Beer's law were investigated by optimizing the analytical variables. A general reaction mechanism is proposed in Fig. 2.

Optimization of Analytical Variables

In order to achieve high sensitivity, selectivity and optimized analytical system for the determination of iron(III), various experimental parameters including reagents concentration, reaction temperature, reaction time and stability were investigated. The operating conditions were optimized by a univariate approach. For a fixed concentration of iron(III) and AAP the colour intensity remains almost constant with 1.0-3.0 ml of 0.1% (*w/v*) IDB solution. Therefore, 2.0 ml of 0.1% (*w/v*) IDB is optimum for routine analysis. It was found

Table 2. Optimized experimental conditions for the determination of iron(III).

Parameters of study	Range of experimental conditions[a]
Hydrochloric acid (1 N)	0.5 – 2.5 ml (1.0 ml)
AAP (0.025%)	0.5 – 2.5 ml (1.0 ml)
IDB (0.1%)	1.0 – 3.0 ml (2.0 ml)
Temperature	20 - 80 °C (20-35 °C)

[a]Figures in parentheses are the recommended volume and reaction conditions.

that 1.0 ml of 0.025% (*w/v*) AAP solution was optimum to get reproducible result. Range and optimum conditions of the parameters studied are presented in Table 2.

Linearity, Accuracy and Precision

The linearity of the spectrophotometric method for the determination of iron(III) was evaluated under optimum conditions. The regression equation obtained under optimum conditions was: $Y = 0.198\,X + 0.006$; $r = 0.9967$ and $n = 5$, where Y is the absorbance and X is the concentration of iron(III) in mg ml^{-1} 'r' is the correlation coefficient. The calibration curve was linear over the range of 0.2 – 4.8 mg ml^{-1}. All the other quantitative parameters are presented in Table 1.

The accuracy and precision of the proposed method was evaluated by performing recovery tests by standard addition method. These tests were performed by adding known amounts of standard solutions at two different concentration levels to a fixed amount of real samples and the mixtures were analyzed by the proposed procedures. Each test was repeated five times and the results are presented in Table 3.

Method validation

To validate the proposed spectrophotometric method, Student's *t*-test was performed on the results of three real samples (Table 3). Comparison was made between the proposed spectrophotometric method

Table 3. Determination of iron in natural water and soil samples.

Sample	Iron(III) added (mg/L)	Proposed method				Reference method[a]	
		Fe recovered (mg/L ± RSD[b])	Recovery (%)	*t*-value[c]	*F*-value[d]	Fe recovered (mg/L ± RSD[b])	Recovery (%)
Lake water	0.50	0.51 ± 0.62	102.0	1.07	1.98	0.51 ± 0.39	102.0
	1.20	1.22 ± 0.59	101.6	1.62	2.30	1.22 ± 0.51	101.6
	1.50	1.52 ± 0.25	101.3	1.09	1.98	1.52 ± 0.31	101.3
	4.00	4.08 ± 0.53	102.0	1.54	2.03	4.06 ± 0.29	101.5
Tap water	0.50	0.50 ± 0.32	100.0	1.21	2.51	0.50 ± 0.65	100.0
	1.20	1.23 ± 0.50	102.5	2.12	3.20	1.23 ± 0.62	102.5
	1.50	1.52 ± 1.20	101.3	1.56	2.94	1.53 ± 0.91	102.0
	4.00	4.10 ± 0.98	102.5	1.21	3.01	4.12 ± 0.63	103.0
Well water	0.50	0.51 ± 0.24	102.0	0.99	2.03	0.50 ± 0.28	100.0
	1.20	1.24 ± 0.54	98.3	1.82	4.16	1.19 ± 0.37	99.1
	1.50	1.52 ± 0.75	101.3	1.24	2.80	1.48 ± 0.57	98.6
	4.00	4.08 ± 0.65	102.0	1.52	2.14	4.10 ± 0.54	102.5
Treated industrial effluent	0.50	0.51 ± 0.84	102.0	1.01	2.57	0.50 ± 0.24	100.0
	1.20	1.18 ± 0.40	98.3	2.48	3.42	1.22 ± 0.56	101.6
	1.50	1.47 ± 0.31	98.0	1.46	2.56	1.53 ± 0.72	102.0
	4.00	4.12 ± 0.37	103.0	2.31	3.54	4.12 ± 0.57	103.0
Soil	0.50	0.51 ± 0.29	102.0	1.21	2.54	0.51 ± 0.39	102.0
	1.20	1.22 ± 0.68	101.6	1.45	3.92	1.19 ± 0.38	99.1
	1.50	1.51 ± 0.52	100.6	1.60	3.43	1.50 ± 0.31	100.0
	4.00	4.10 ± 0.64	102.5	1.95	4.02	4.12 ± 0.61	103.0

[a] - Reference 4.
[b] - Average of five-determinations, RSD - relative standard deviation.
[c] - Tabulated *t*-value at 95% confidence level is 1.96.
[d] - Tabulated *F*-value at 95% confidence level is 5.27.

and the reference phenanthroline method[4] to find out whether the two methods give the same results at the 97% confidence level. The *t*-test with multiple samples was applied to examine whether the two methods for iron(III) determination differ significantly at the 97% confidence level. Recoveries (Table 3) of iron(III) added to the water samples were 98.6 and 103.0 %. For soil samples, recoveries (Table 3) of iron(III) were 99.1 and 103.0 %. These results indicate that there is no serious interfering species in the water and soil samples.

Effect of Diverse Ions

The interference from foreign ions commonly present in water and soil was studied by adding known amounts of foreign species to a solution of known concentration of iron(III). The tolerance limit for various ions is defined as the concentration of added ion causing less than ± 3% relative error for the iron(III) determination. The developed method is based on the oxidation of AAP with iron(III). Therefore, strong oxidizing or reducing agents are expected to interfere by oxidation of AAP. Vanadium(V), cerium(IV) chromium(VI) and manganese(VII) at a same level of iron(III) caused low recovery. Masking agents like EDTA, fluoride, tartrate and citrate did not interfere in the determination of iron(III). The use of a mixture of tartaric acid (50 mg) and citric acid (5 mg) has been found to have effective masking action on a large number of foreign metal ions. During the interference studies, if a precipitate was formed, it was removed by centrifugation. The possible interference and the maximum tolerable concentration are given in Table 4.

Table 4. Maximum tolerance limit of diverse species on the determination of iron(III).

Foreign ions	Tolerance limit ($\mu g\ ml^{-1}$)
EDTA, F^-, Br^-, Bi^{3+}, Ba^{2+}, Ti^{4+}, citrate, tartarate, oxalate	500
Na^+, Mg^{2+}, Al^{3+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, K^+, Hg^{2+}, Ni^{2+}, CH_3COO^-	200
NO_3^-, Cl^-, Br^-, $SO_4^{2-\dagger}$, NH_4^+, Co^{2+}, Cu^{2+}, PO_4^{3-}	50
Chloramine-T, Chloramine-B, N-bromosuccinimide, N-chlorosuccinimide, N-iodosuccinimide	0.5

CONCLUSIONS

A first-ever use of 4-amino-antipyrine (AAP) as an electrophilic coupling reagent and iminodibenzyl (IDB) belonging to the class of dibenzazepines as chromogen is proposed for the spectrophotometric determination of iron(III). Although a variety of methods are available for the determination of iron(III), the proposed method, besides being simple, inexpensive, sensitive, selective, novel and precise also has the advantage of determination without the need for extraction or heating. The method does not involve complicated reaction conditions and can be compared in sensitivity and precision with other standard methods. Applications of the method in the determination of iron(III) in a variety of real samples has demonstrated its practical utility. The use of the AAP as coupling reagent and IDB, a tricyclic compound having a central ring constituted of seven atoms as chromogenic reagent for the determination of iron(III) will open up new areas of research. A value-addition of this method can be achieved, if the procedure is combined with on-line or at-line system and this is currently under investigation.

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