Study of Ascorbic Acid as Iron(III) Reducing Agent for Spectrophotometric Iron Speciation

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ABSTRACT

The study of ascorbic acid as a reducing agent for iron(III) has been investigated in order to obtain an alternative carcinogenic reducing agent, hydroxylamine, used in spectrophotometric standard method based on the formation of a red-orange complex of Fe(II)-*o*-phenanthroline. The study was optimised with regards to ascorbic acid concentration as well as pH solution. The results showed that ascorbic acid showed maximum capacity as reducing agent of iron(III) under concentration of $4.46.10^{-4}$ M and pH solution of 1-4.Under these conditions, ascorbic acid reduced iron(III) proportionally and performed similarly to that of hydroxylamine. The method gave result to linear calibration over the range of 0.2-2 mg/L withhigh accuracy of 97 % and relative standard deviation of less than 2 %. This method was successfully applied to assay iron speciation in water samples.

Keyword: Iron, speciation, ascorbic acid, *o*-phenanthroline, spectrophotometry

INTRODUCTION

Iron is the second most abundant metallic element in the earth's crust and is essential in the metabolism of plants and animals. If presented in excessive amounts; however, it forms oxyhydroxide precipitate that stains laundry and porcelain. As a result, the recommended limit for iron in domestic water supplies is 0.3 mg/L.[1] Determination of Fe(II) and Fe(III) is important in many natural sciences like geology, agriculture, biology or environmental protection, mainly on account of different bioavailability and metabolism of iron on its two oxidation states. Iron(II) is essential for good health, as it helps transport oxygen in the blood and storage of oxygen by means of haemoglobine and myoglobine.[2] Iron deficiency may lead to low immunity and infectious diseases. If the human body takes in too much iron will cause excretory functional disturbance and likely have greater carcinogenic potential. Both Fe (II) and Fe(III) are important in the biosphere, serving as an active centre of a wide range of proteins such as oxidases, reduces and dehydrates.[3] However the plants need a certain amount of iron(II) to survive. Iron helps them create chlorophyll and aids in several other chemical processes plants perform. However, too much iron can have a toxic effect on the plant, weakening and eventually killing it. It should be noted that plants only absorb ferrous iron particles from the soil, and that other forms of iron will not affect plants.[4]

Many methods have been reported for iron determination including atomic absorption spectrometry (AAS),[5] inductively coupled plasma mass spectrometry (ICP-MS),[6] inductively coupled plasma atomic emission spectrometry (ICP-AES),[7] high performance liquid

chromatography (HPLC),[8] and capillary electrophoresis (CE).[9] Most of these methods have good sensitivity but require very expensive instruments and some of them cannot distinguish between the different oxidation states of metals. The spectrophotometry is commonly used for determination of trace of iron is based on the reaction of iron(III) with various chromogenicre agents, such as azo reagents,[10] triphenylmethane reagents,[11] alizarine yellow R,[12] and nitrosonaphthol sodium sulfonates.[13] Numerous reagents have also been reported for the spectrophotometric determination of iron(II); for example, dipyridine, tripyridine and *o*-phenanthroline.[14] However, these methods have some limitations, such as complicated procedures, relatively long analysis time, and relatively unstable.[15] The standard spectrophotometric determination method based on the formation of a red-orange complex from iron(II) and *o*-phenanthroline is practical and sensitive.[16]

In the standard method procedure, reducing reagent, such as hydroxylamine or hydroquinone, is required for converting iron(III) to iron(II) to form red-orange complex with *o*-phenanthroline. Hydroxylamine is thought to be causing of adverse health effects associated with its absorption through the skin. Hydroxylamine is known as toxic and mutagen compounds. Interacting with haemoglobin, hydroxylamine converts it into inactive met haemoglobin.[17] It is an irritant to the respiratory tract, skin, eyes, and other mucous membranes. It is harmful if swallowed and may cause eczemas and festering of skin. Ascorbic acid is a well-known and biologically important chemical reducing agent. Thus, in this research ascorbic acid was chosen as strong reducing agent and environmentally friendly alternatives for hydroxylamine.

The present paper describes the effectiveness of ascorbic acid as an alternative reducing agent, which is environmentally friendly, to reduce iron(III) to iron(II) for spectrophotometric determination based on formation of red-orange complex of iron(II)-*o*-phenanthroline which absorb wavelengths at 509.5 nm.

EXPERIMENT

Reagent and apparatus

All solutions were prepared with pro-analysis grade chemicals in de-ionized water. Stock solution of iron (III) was prepared by dissolving 0.072 g of $FeCl_3$ in 250 mL 0.2 M sulphuric acid solution. Stock solution of iron (II) 100 ppm was prepared by dissolving 0.07 g of $FeNH_4SO_4.6H_20$ in 100 mL 0.2 M sulphuric acid solution. Working solutions of iron (III) and iron(II) were prepared by the suitable dilution of these stock solutions as required. Preparation of 0.1 % *o*-phenanthroline solution was conducted by dissolving a 100 mg of *o*-phenanthroline monohydrate in 100 mL water containing two drops of hydrochloric acid. Hydroxylamine solution (10%) was prepared by dissolving a 10 g of hydroxylamine hydrochloride in 100 mL distilled water. Ascorbic acid solution was prepared by dissolving a 786 mg of ascorbic acid in 100 mL water. Sodium acetate of 1M was prepared by dissolving 1.64 g of $NaC_2H_3O_2$ in 20 mL of distilled water.

A Shimadzu spectrophotometer (model UV-1601) was used for the absorbance measurements. The pH measurements were done using LH (667 413) pH meter.

Procedure for determination of iron(II)

The following components were mixed in a 100 mL volumetric flask: a solution containing suitable amounts of iron(II) ions, 1mL of 1M HCl, 1 mL of 1M $NaC_2H_3O_2$ and 20 mL of 0.1 % *o*-phenanthroline. Then, the flask was diluted to the mark with distilled water and mixed. Awaiting time of 5 -10 min was selected as sufficient for the generation of the

Fe(II)-*o*-phenanthroline complex. The absorbance of this solution was measured at 509.5 nm versus blank solution containing all reagents except of iron(II).

Proposed procedure for determination of total iron

Aliquots of standard solutions containing suitable amounts of iron ions were placed into 100 mL volumetric flask. Then, volumes of 1 mL of ascorbic acid were added. After allowing the mixture to stand for 5 min, the following reagents were added: 1mL of 1 M HCl, 1mL of 1 M $NaC_2H_3O_2$ and 20 mL of 0.1 % *o*-phenanthroline. Then, the flask was diluted to the mark with distilled water and mixed. Waiting time of 5 min was selected as sufficient for the generation of the Fe(II)-*o*-phenanthroline complex. The absorbance of this solution was measured at 509.5 nm versus blank solution containing all reagents except of iron(II).

Determination of iron(III)

Iron(III) was determined by subtracting the absorbance of total iron with the absorbance of iron(II). Then, the obtained absorbance was plotted to standard curve of iron(III).

RESULTS AND DISCUSSION

The method described in this work is based on the reaction between iron(II) and *o*-phenanthroline to form a red-orange complex which absorb wavelengths at 509.5 nm. Total iron was determined by adding ascorbic acid in order to reduce iron(III) to iron(II), and the total iron obtained was complexed with *o*-phenanthroline to form the analytically measured Fe(II)-*o*-phenanthroline complex. The chemical reaction variables such as reactant concentrations and reaction pH were optimized in order to achieve the highest sensitivity for the determination of iron.

$$2Fe^{3+} + C_6H_8O_6 \rightarrow 2Fe^{2+} + C_6H_6O_6 + 2H^+ \quad E^o = 0.68V \qquad (1)$$

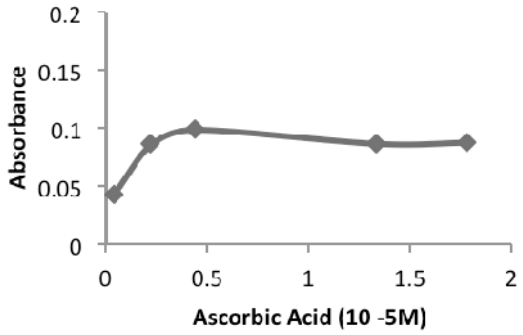

Figure 1. Effect of Ascorbic Acid on iron(III) reduction (Condition: Fe^{3+}. 0.5 ppm with 0.1% Phen, pH 4, and ascorbic acid 0.04 to 1.78×10^{-5}M).

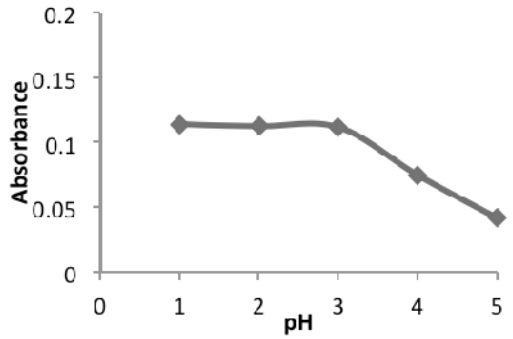

Figure 2. Effect of pH on iron(III) (Condition: Fe^{3+}: 0.5 ppm; ascorbic acid 4.46×10^{-6} M; Phen 0.1%, pH: 1-5).

The Influence of Concentration of Ascorbic Acid

Ascorbic acid was first studied as the reducing agent of iron(III) to iron(II) quantitatively. According to stoichiometry, 1 mole of ascorbic acid is required to reduce 2 mole of iron (III) as illustrated in equation 1. The effect of ascorbic acid concentration to reduce iron (III) to iron (II), which is observed as the absorbance intensity of iron(II)-phenanthroline, was investigated from $4.46.10^{-6}$-$1.78.10^{-4}$ M. This concentration range represents the amount of ascorbic acid from below up to above stoichiometric amount required for reducing solution containing $4.46.10^{-6}$ M of iron (III).

Higher concentration of ascorbic acid, the higher iron(III) is reduced to iron(II) and forms red complex of Fe(II)-*o*-phenanthroline. Figure 1 illustrates the absorbance of Fe(II)-*o*-phenanthroline formed increases by increasing ascorbic acid concentration with maximum absorbance intensity obtained at 0.446×10^{-4} M. No changing in absorbance was observed when the amount of ascorbic acid was increased from 0.446 - $1.78.10^{-4}$ M. This indicated that the reaction has reached equilibrium under the addition of 0.446×10^{-4} M of ascorbic acid solution, and the reaction went to completion and all iron(III) was expected to be reduced to iron(II), as shown by constant absorbance. Therefore, 0.446×10^{-4}M of ascorbic acid was used for further experiment.

Effect of pH

Iron (III) is soluble at low pH, and hence the pH of iron (III) sample plays a vital role in solubility of Fe^{3+} as ion; besides, capacity of most reducing agents is influenced by pH solution. Therefore, the effect of pH on the effectiveness of ascorbic acid as reducing agent for iron(III) to iron(II) was examined over the range pH 1-5 under the optimum ascorbic acid amount (0.446×10^{-5} M). Iron (III) solution was highly acidic (pH=1), adjusting the pH was carried out by adding different amount of sodium acetate before adding *o*-phenanthroline. This effect is illustrated in Figure 2, which shows that constant absorbance was observed when pH solution was increased from 1 to 3. This is because all iron(III) is in a soluble form of Fe^{2+}at acidic pH.[16]

$$Fe^{3+}(aq) + 3H_2O(l) \rightarrow Fe(OH)_3(s) + 3H^+(aq) \qquad (2)$$

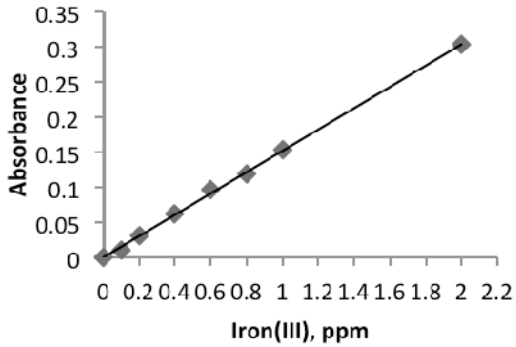

Figure 3. Calibration curve of standard iron(III) solution (Condition: Series solution of 0.1-2 ppm Fe^{3+}; $4.46.10^{-6}$ M ascorbic acid, 0.1% Phen; and pH 1 solution).

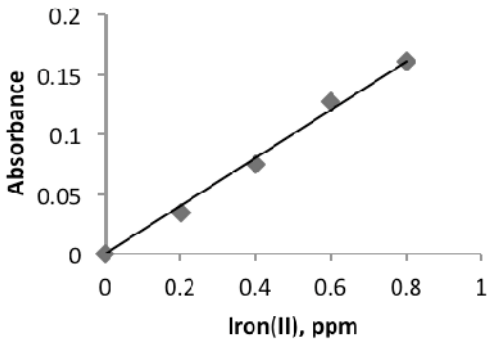

Figure 4. Calibration curve of standard Iron (II) solution (Condition: Series solution of 0.1-2 ppm Fe^{3+}; $4.46.10^{-6}$ M ascorbic acid, 0.1% Phen; pH 1 solution).

Decreasing in absorbance was observed when the pH was increased more than 3; this is probably due to the effect of iron(III) hydrolysis which forms hydrolysis products of Iron(III) up to hydroxide precipitation as shown in equation 2. It can be concluded that under the optimum pH solution (pH < 3) iron is in iron(III) ion form, ensured the complete reduction of iron(III) to iron(II), which form orange-red complex with *o*-phenanthroline. Solution pH of 1 was chosen as optimum and used for further experiment.

Calibration curve

Under the optimum conditions outlined above (i.e., 4.46×10^{-6}M of ascorbic acid and pH of the iron(III) solution of 1), the calibration curve was observed to be linear ($R^2 = 0.999$) for 0.2-2 ppm of iron (III) as shown in Figure 3. The calibration curve produced using proposed procedure was slightly longer in comparison to that obtained from standard method using hydroxylamine (0.1-1 ppm). The limit of detection for iron(III) was 0.12 mg/L and for iron(II) was found to be 0.048 mg/L. The relative standard deviation (RSD) of the procedure was typically below 1 % (n = 3), indicating good reproducibility of the proposed method.

Reduction Efficiency of Ascorbic Acid

In order to find out the reduction efficiency of ascorbic acid, a calibration curve of iron(II) was prepared by directly mixing the acidic iron(II) solution with *o*-phenanthroline to form orange-red complex of Fe(II)-*o*-phenanthroline as shown in Figure 4. The slope of the calibration curve from iron(III) was then compared to that of obtained from iron(II) using ascorbic acid reducing agent. Based on Figure 4, calibration curve of iron(II), y = 0.200x, gave result to a slope of 0.200, which is significantly higher than the slope obtained from iron(III) calibration curve, y = 0.153x, i.e. 0.152. This fact indicates that not all of iron(III) was reduced to iron(II) under optimum concentration of ascorbic acid resulting the efficiency of 76 %.

The reduction efficiency of ascorbic acid was also investigated by comparing the slope of iron(III) calibration curve using reducing agent of ascorbic acid to that of using hydroxylamine, which is used as reducing agent in spectrophotometric standard method. The calibration curve of iron(III) using reducing agent of hydroxylamine resulting linear regression equation of y = 0.157x - 0.0091 with the slope of 0.157, which is quite similar to the slope obtained from ascorbic acid (0.152). Based on this data, the efficiency of ascorbic acid compared to hydroxylamine is 97 %.

The similarity of efficiency ascorbic acid with hydroxylamine in reducing iron(III) to iron(II) as well as the highly coefficient correlation of iron(III) calibration curve (R^2= 0.999), it can be concluded that ascorbic acid can be considered as a safe alternative reducing agent to hydroxylamine for reducing agent of iron(III) to iron(II) which can be applied for determination of iron speciation spectrophotometricaly. However, as only 76 % of iron(III) is reduced to iron(II), for analysing iron(III), a correction factor of 0.76 should be taken into account to bring to the original value. Alternatively, no correction factor is required if the absorbance obtained from iron(III) is plotted to calibration curve of iron(III).

Iron Speciation Determination

To establish the accuracy and validity of the described method, real and synthetic samples containing mixture of 0.2 mg/L free iron (II) and 0.2 mg/L free iron(III) ions were

analysed by proposed method under the obtained optimum conditions and compared the results to those obtained from standard spectrophotometric method.[16]

The analysis results of synthetic samples are presented in Table 1, showing that the proposed procedure (using ascorbic acid reducing agent) gave comparable results to those obtained by the standard method. All of the measurements iron(III) and iron(II)) gave results to high recoveries (close to 100 %) for both methods (proposed and standard methods) with relative standard deviation of typically less than 2 %.

Table 1. Analysis of synthetic samples

Iron samples		Method of analysis	
Iron	Concentration (mg/L)	Proposed method (mg/L)	Standard method (mg/L)
Iron(II)	0.20	0.21±1.57%	0.21±1.57%
Iron(III)	0.20	0.20±0.87 %	0.21±1.65%

*The results are the average of three determinations ±RSD.
* Iron (III) = Total iron – Iron (II)

Table 2. Analysis of river water sample

Iron samples	Method of analysis	
	Proposed method(mg/L)	Standard method (mg/L)
Iron(II)	0.29±0 %	0.29±0 %
Iron(III)	0.18±0.72 %	0.21±0.72 %

*The results are the average of three determinations± RSD
*Iron (III) = Total iron – Iron (II)

The real sample obtained from Brantas river water were analysed for iron(II) and iron(III) by standard and proposed procedure outlined above. The samples were treated with acid to ensure that the iron in soluble form and to prevent adsorption or deposition. The addition of acid to the sample may eliminate the need for adding acid prior to analysis. The analysis data presented in Table 2 shows that the measurements of iron(II) and iron(III) obtained from proposed method are not significantly different to those obtained from standard method. The calculated student's t-test did not exceed the tabulated value at 5% confidence level, which it is clear that there is good agreement between the results obtained by the proposed method and standard method. However, the measurement of iron (III) in real sample using proposed method was lower than that obtained from standard method. Presumably, the real samples contained oxidising agents. So, the available ascorbic acid was not sufficient to reduce iron (III) to iron (II). Therefore, an excess of ascorbic acid concentration is suggested to eliminate expected oxidation agent in real samples.

CONCLUSION

As a simple, inexpensive, and environmentally friendly reducing agent, ascorbic acid showed maximum capacity as reducing agent of iron(III) under concentration of $4.46.10^{-4}$ M and pH solution of 1-3. Under these conditions, it reduced iron(III) proportionally and performed similarly to that of hydroxylamine. When it was applied for spectrophotometric determination of iron speciation in synthetic and real samples, the method gave result to satisfactory results with linear calibration over the range of 0.2-2 mg/L with high accuracy of 97 % and relative standard deviation of less than 2 %.

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