Valence State of Iron in the Presence of Ascorbic Acid and Ethylenediaminetetraacetic Acid

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The valence state of iron in solutions of pH 2.6–6.0 in the presence of ascorbic acid (AA) and ethylenediaminetetraacetic acid (EDTA) was investigated by reduction–oxidation titration. The results indicate that 2 mol of Fe(III) was reduced by 1 mol of AA in the pH range. The reduction rate of Fe(III) by AA decreased drastically as the pH value increased. Addition of EDTA prevented Fe(III) from being reduced by AA; that is, when a 1:1 Fe(III)–EDTA complex was formed, Fe(III) could not be reduced by AA, at pH 2.6–6.0. Ferric iron can be reduced by AA only when the pH is below a limit, somewhere between pH 6.0 and 6.8. Above that pH limit, AA is no longer an effective reducing agent for Fe(III). The results clarify some controversies reported in the literature and have important implications in the formulation of iron and AA additives in foods and pharmaceuticals.

Keywords: *Ferric; ferrous; iron; ascorbic acid; chelation; redox titration*

INTRODUCTION

The bioavailability of iron in foods and pharmaceutical preparations has been the subject of much research. Previous work has established that ferrous iron exhibits greater absorption than ferric iron, and ascorbic acid (AA) enhances the uptake of iron in human and animal experiments (Brise and Hallberg, 1962; Forth and Rummel, 1973; Hallberg, 1981; Hungerford and Linder, 1983). The beneficial effect of AA on iron absorption has been attributed to the reductive and chelating power of ascorbate, but the actual mechanism (reduction or chelation or both) in different pH conditions is still poorly understood (Lynch and Cook, 1980; Gorman and Clydesdale, 1983; Dorey et al., 1993). Reduction of ferric iron by AA has been observed in various pH conditions (Nojeim and Clydesdale, 1981). Recently, Dorey et al. (1993) investigated the valence states of iron species in the presence of AA and oxygen and found that AA was not an effective reducing agent for Fe(III) in a pH range of 6.8–7.4. Their finding disagrees with that of Gorman and Clydesdale (1983) and necessitates a reinterpretation of some previous studies which assumed reduction of Fe(III) by AA at physiological pH [e.g., Simpson and Peters (1987)]. The study of Dorey et al. (1993) also implied that there is an upper pH limit for the reduction of Fe(III) by AA. Beyond that pH limit AA is not an effective reducing agent for ferric iron. Earlier studies also have suggested that ferric–ascorbate complex could be stable in a pH range of 2–11 (Conrad and Schade, 1968; Gorman and Clydesdale, 1983), which contradicts some observations that AA reduces ferric iron in acidic solutions (Nojeim and Clydesdale, 1981). The study of Khan and Martel (1968) has suggested that iron could be reduced by AA in Fe(III)–ethylenediaminetetraacetic acid (EDTA) complex form in a pH range of 1.8–3.45.

One of the difficulties in the previous studies of reactions between iron and AA was the inability to accurately determine the actual concentrations of iron species. Quantification of Fe(II) by complexing agents such as bathophenanthroline (Nojeim and Clydesdale, 1981; Lee and Clydesdale, 1979) or ferrozine (Stookey, 1970) may be overestimated in the presence of a reducing agent, such as AA (Dorey et al., 1993; Gorman and Clydesdale, 1983). The reaction of iron species often is not reversible when pH changes from a higher value to a lower value in the region greater than 4 (Lynch and Cook, 1980). This irreversible reaction of iron adds difficulties in experiments that maintain pH by the addition of alkaline or acid solutions.

We initiated this study to clarify the valence state of iron species in the presence of AA and EDTA in a pH range of 2.6–6.0. The study was made possible by using a reduction–oxidation (redox) titration, which quantifies both Fe(II) and AA in the same solution, and by direct titration of Fe(III) solution against AA. The pH of the solutions above 4 was kept constant using a buffer system such that pH fluctuation was minimal during the whole course of an experiment. A correct understanding of the reaction of AA and iron in the pH range studied is critical for accurate interpretations of bioavailability of iron and AA in nutrition studies. It also has important implications in formulations of many food and pharmaceutical products.

MATERIALS AND METHODS

Reagents. All solutions were prepared using reagent grade chemicals. Solutions of 0.14 M Fe(III) and 0.1 M AA were prepared from ferric chloride (FeCl$_3$·6H$_2$O) and L-ascorbic acid, respectively, using oxygen-free deionized water. Solution of 0.1 M Fe(II) was prepared from ferrous sulfate (FeSO$_4$·7H$_2$O) in oxygen-free deionized water the pH of which had been adjusted to 2.6 using a HCl solution. Buffer solutions with pH ranging from 4.0 to 6.0 were prepared from oxygen-free 0.1 M potassium hydrogen phthalate (KHC$_8$H$_4$O$_4$) solution by adding appropriate amounts of HCl or NaOH. Solution of 0.065 M potassium permanganate (KMnO$_4$) was prepared using oxygen-free deionized water. Solution of 0.1 M disodium ethylenediaminetetraacete (Na$_2$C$_{10}$H$_{14}$O$_8$N$_2$·2H$_2$O) was prepared using oxygen-free water.

Titration of AA, Fe(II), Fe(III), and Their Mixtures. Redox titration was performed using an automatic titrator

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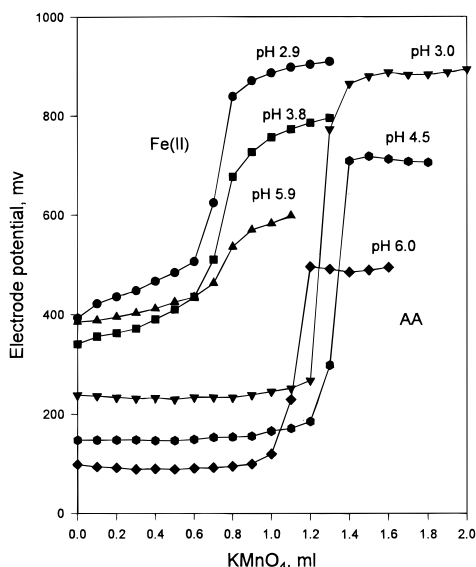
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Figure 1. Titration curves of AA and Fe(II) solutions with 0.065 M KMnO$_4$ solution in pH ranges from 2.9 to 6.0. All end points are sharp, and the results are quantitative.

(Orion Model 960 autochemistry system, Orion Research Inc., Boston, MA) which has control over the titration increment volume and speed. Titration was carried out in a reaction vessel that was constantly purged with nitrogen gas above the liquid level during the titration to minimize oxygen contamination. The reaction vessel was immersed in a water bath maintained at a constant temperature of 25 °C. A combination redox electrode and a combination pH electrode (both with silver chloride reference electrode) were used to monitor the electropotential and pH, respectively, of the solution. The automatic titrator had a built-in software algorithm that calculated end points using a Gran function.

AA and Fe(II) species in solutions of pH ranging from 2.6 to 6.0 were determined by titrating with 0.065 M permanganate solution. The pH of solution was maintained constant by manually adding 0.1 M NaOH or HCl solutions when the pH was less than 4.0 and by the use of phthalate buffer solution when the pH was above 4.0 during a titration. Most of the titration was carried out with an increment titrant volume of 0.1 mL and time interval of 10 s with constant stir by a stirrer.

Reaction of Fe(III) and AA. Aliqouts of the Fe(III) and AA solutions (2 mL) were premixed in 100 mL of deionized, deoxygenated water and titrated with 0.065 M permanganate solution while a constant pH was maintained during the titration. The reactions of Fe(III) and AA in the pH range of 2.6−6.0 were also investigated by directly titrating Fe(III) with AA solution. Reactions of Fe(III) and AA below pH 3 were fast, such that the titration could be carried out with a time interval of 10 s or less between each 0.1 mL titrant addition. As the pH increased, the reaction of Fe(III) and AA became progressively slower, such that slower titration speeds were required. For example, titration of Fe(III) with AA at pH 5 must be carried out with a 240 s time interval between each 0.1 mL titrant addition to have an accurate end point. The effect of EDTA on the reaction of iron and AA was investigated by titrating mixtures of EDTA and Fe(III) solutions with 0.1 M AA. All of the titration experiments were repeated at least three times.

Statistical Analysis. Statistical analysis of linear regression and comparisons of means were carried out using the SAS computer package (SAS Institute Inc., 1990).

RESULTS AND DISCUSSION

Redox titration using permanganate as titrant can determine AA and Fe(II) in separate solutions in the pH range from 2.9 to 6.0 as indicated in Figure 1. Maintaining a constant pH during the titration is
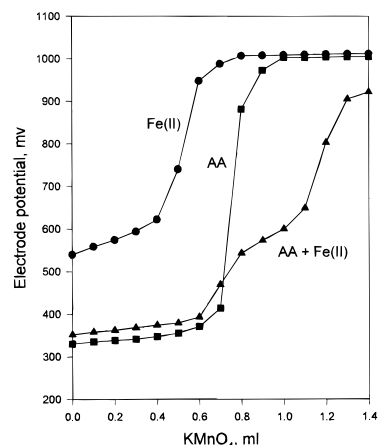
critical to the accuracy of determinations because the electropotential of the solution is pH dependent. Addition of phthalate buffer up to 0.2 M in the solution did not affect the titration, indicating an insignificant buffer solution effect on the titration (data not shown). Phthalate buffer, however, has poor buffering capacity above pH 6.1. It is critically important to minimize the fluctuation of pH in the iron solution during the experiment because the reaction may not be reversible when the pH fluctuates above the value of 4.5. Potassium phthalate buffer solutions did a good job of maintaining a constant pH in the range between 4.0 and 6.0. Below pH 4, phthalate buffer and Fe(III) form a precipitate, which reduces the sensitivity of the titration. For that reason, all titrations below pH 4 were performed without the buffer solution and a constant pH level was maintained by manually adding HCl or NaOH solutions through a syringe titrator (Hach Model 16900-01, Hack Co., Loveland, CO) during the titration. The reaction of iron was much more reversible below pH 4 when the pH fluctuated.

The redox titration with permanganate can determine both AA and Fe(II) species in a mixture at low pH. Figure 2 gives an example of such a titration carried out at pH 2.9. Both AA and Fe(II) have distinguishable and quantitative end points. As the pH of a solution increased, the resolution of the end points for AA and Fe(II) in a mixture decreased and became indistinguishable at pH >4.3. The redox titration method, therefore, is suitable for determining AA and Fe(II) species at pH <4.

Figure 3 shows the titration of a solution containing 350 μmol of Fe(III) and 204 μmol of AA at pH 2.9. The results of the titration indicate that all 350 μmol of iron was reduced to Fe(II) and 29 μmol of AA remained. That is, 350 μmol of Fe(III) was reduced by 175 μmol of AA. The results of the permanganate titration experiments in the pH range of 2.9−4.3 indicate that 1 mol of AA is able to reduce 2 mol of Fe(III).

Reactions of Fe(III) and AA in the pH range between 4.3 and 6.0 can be investigated by directly titrating Fe(III) against AA. This direct titration method can also be applied to pH below 4.3 without the buffer. Examples of titrating Fe(III) with AA at pH 2.6 and 5 are given in Figure 4. Both titration curves in Figure 4 indicate that Fe(III) was quantitatively reduced by AA in a molar ratio of 2:1 at the two pH levels. Titration of Fe(III) with AA at pH 5, however, had to be carried out at a much slower speed than that at pH 2.6;
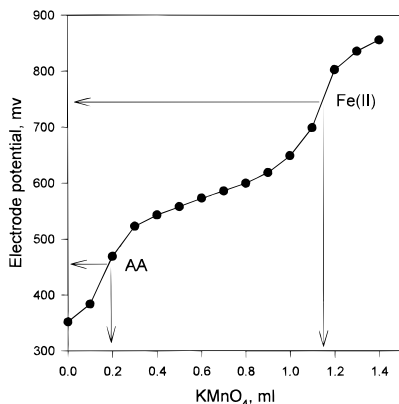

Figure 2. Titration curves of AA and Fe(II) solutions and their mixture at pH 2.9. The end points of AA and Fe(II) in the mixture are distinctive, and the results are quantitative.

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Figure 3. Titration of a solution containing 204 μmol of AA and 305 μmol of Fe(III) with 0.065 M KMnO$_4$ at pH 2.9 The results showed 29 μmol of residual AA and 350 μmol of Fe(II), which imply that all 350 μmol of Fe(III) was reduced by 175 μmol of AA in a molar ratio of 2:1.
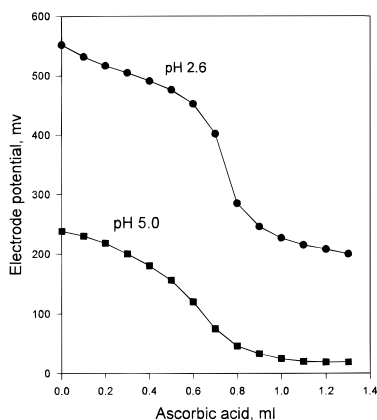

Figure 4. Titration of 140 μmol of Fe(III) by 0.1 M AA solution at pH 2.6 and 5.0, respectively. The titration at pH 5.0 had to be performed at a much slower speed (240 s/0.1 mL of titrant added) than that of pH 2.6 (10 s/0.1 mL of titrant added) to get a sharp and quantitative end point. The results indicate that Fe(III) was quantitatively reduced at both pH levels; however, at pH 5.0 the rate of reduction is much slower.

otherwise, no quantitative end points could be obtained. This fact implies that the rate of Fe(III) reduction slowed substantially at pH 5. The maximum speed of the titration at pH 5 was 240 s/0.1 mL addition of the permanganate solution, which was at least 50 times slower than could be done at pH 2.6. The reduction rate of Fe(III) by AA is, therefore, pH dependent: the higher the pH, the slower the reduction rate.

Both redox titration methods used in this study indicate that Fe(III) can be quantitatively reduced by AA below pH 6.0 and Fe(II) is the dominating iron species at equilibrium. The results disagree with those of Conrad and Shade (1968) and Gorman and Clydesdale (1983), who suggested the formation of ferric ascorbate complex over a wide range of pH values. This study showed that the reduction rate of Fe(III) by AA could be fairly slow at pH above 5. Ferric iron could possibly form ascorbate complex before an equilibrium is reached, which is slow at a higher pH due to the kinetic effect. At equilibrium, all Fe(III) should be reduced to Fe(II) if AA is in excess and the pH is less than 6. Rates of AA oxidation under aerobic conditions have been found to be accelerated as pH increased (Khan and Martell, 1967; Hsieh and Harris, 1987, 1993). Rates of AA oxidation under anaerobic conditions, however, were found not to follow the trend (Huelin, 1953; Finholt et al., 1966). The reactions of Fe(III) and
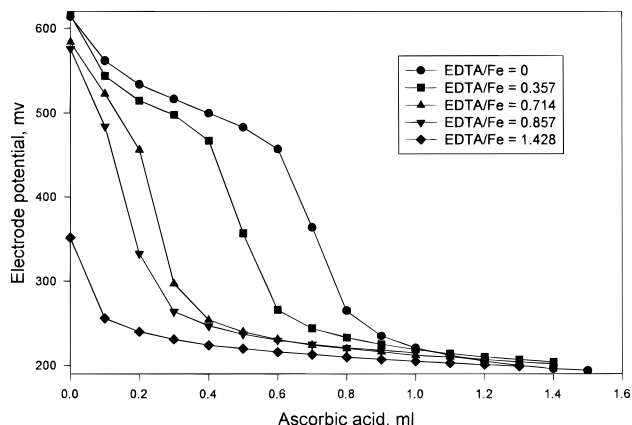

Figure 5. Titration of 140 μmol of Fe(III) with 0.1 M AA solution at pH 2.9 in the presence of various amounts of EDTA. The more EDTA added, the less titratable iron (Fe[II])); that is, fewer Fe(III) were reduced.
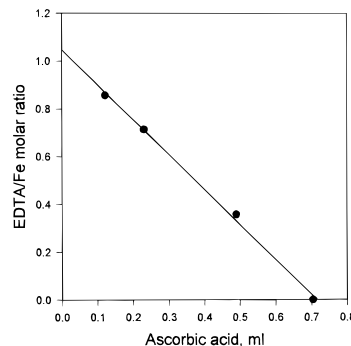

Figure 6. Determination of the EDTA/Fe ratio at which Fe-(III) is completely protected from reduction by AA. The regression line intercepts the *Y*-axis at 1.04, indicating a 1:1 EDTA−Fe complex.

AA studied in this paper were mainly anaerobic, and the rates were probably controlled by the step of Fe-(III) reduction rather than AA oxidation.

Salts of EDTA are one of the most popular chelating agents used in foods (Lindsay, 1985). Titration of Fe-(III) by AA in the presence of various amounts of EDTA was studied (Figure 5). The more EDTA added, the less titratable (ferric) iron, until no iron was titratable at all. A regression analysis confirmed that when a 1:1 EDTA/Fe(III) complex was formed, Fe(III) was not reduced by AA (Figure 6). Thermodynamic consideration also predicts that Fe(III)−EDTA cannot be reduced by AA since the standard reduction potential of the Fe(III)−EDTA complex (0.1 V) is much lower than that of AA (0.39 V). Our results disagree with those of Kahn and Martell (1968), who reported that Fe(III)−EDTA was reduced by AA in a pH range of 1.8−3.45. Kahn and Martell (1968), however, did not directly measure the valence state of iron species in their experiments. They instead measured the amount of dehydroascorbic acid (oxidized species of AA) to infer the reduction of Fe(III). Anaerobic degradation of AA without coupling of metal reduction has been reported (Huelin, 1953; Finholt et al., 1966). Direct determination of iron species in this study indicated that Fe(III)−EDTA was not reduced by AA in the pH range 2.6−6.0.

This result has implications for the formulation of food and pharmaceutical products. First, if maintaining iron in solution is a main concern, adding AA can keep iron in the more soluble ferrous status when the pH is 6 or less. The AA, however, will be oxidized in solution. Second, if maintenance of both iron and AA in the

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products is of concern, adding a ferric chelator such as EDTA could keep iron in solution (in a ferric complex form) and protect AA from being oxidized at the same time.

CONCLUSIONS

Titration studies of reactions between AA and Fe(III) indicate that 2 mol of Fe(III) was reduced by 1 mol of AA in the pH range between 2.6 and 6.0. The reduction rate of Fe(III) by AA was pH dependent; that is, the higher the pH, the slower the rate. At pH >5, the reduction of Fe(III) by AA could be slow enough to generate different results in laboratory studies, if kinetic information is not considered. Kinetics of Fe(III) reduction by AA is important, and further study is required. The titration method, however, is not suitable for quantitative study of the reduction kinetics. Addition of EDTA can prevent Fe(III) from being reduced by AA. When 1:1 Fe(III)−EDTA complex was formed, Fe(III) was not reducible by AA. Ferric iron can be reduced by AA only when the pH is below an upper pH limit and without the presence of a strong ferric chelator. According to the results of this study and that reported in literature, this upper pH limit is somewhere between 6.0 and 6.8.

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