

Kinetics of Fe(III) Reduction by Ascorbic Acid in Aqueous Solutions

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The reaction of Fe(III) and ascorbic acid (AA) in food products and digestive tracts affects the efficiency and uptake of these two nutrients. We investigated the kinetics of Fe(III) reduction by AA at pH 5 and 6 in a model system at 25 °C. The results indicate that the reduction of Fe(III) by AA is of zero order with respect to AA. The reaction order with respect to Fe(III) cannot be represented by a simple kinetic model at pH 5 or 6. The major stage of the reduction (about 80%, stoichiometrically), however, could be represented by a general equation of $-d[\text{Fe(III)}]/dt = k[\text{Fe(III)}]^{1.811}$ where k is a rate constant and $[\text{Fe(III)}]$ is the total ferric concentration. The rate constant decreased 1 order of magnitude as pH increased from 5 to 6. Aging of Fe(III) solution slows its reduction rate at pH 6 but not at pH 5.

Keywords: *Fe(III); ascorbic acid; reduction; kinetics; reaction order; aging effect*

INTRODUCTION

Iron and ascorbic acid (AA) are two important nutrients found in many foods and pharmaceutical products. These nutrients are often fortified or enriched in processed food products in pure chemical forms to boost nutritional values or preserve the quality of food. The intricate interaction between iron and AA in food or digestive systems have been of great interest of investigation because it directly impacts on the availability and utilization of these nutrients in human and/or animal bodies (Brise and Hungerford, 1962; Lynch and Cook, 1980; Dorey et al., 1993).

Many reports on the interaction of iron and AA in fortified food system and the mechanism of the beneficial effect of AA on iron absorption present controversial results especially near physiological pH due to the analytical difficulty in the determination of iron species and AA in a mixture of both species (Nojeim and Clydesdale, 1981; Lee and Clydesdale, 1979; Stookey, 1970) and the complexity and poor reversibility of Fe(III) hydrolysis in response to pH fluctuations (Spiro and Saltman, 1969; Lynch and Cook, 1980). Previous studies have shown that Fe(III) could be reduced by AA in aqueous solutions below pH 6.2 (Nojeim and Clydesdale, 1981; Hsieh and Hsieh, 1997). Above pH 6.8, AA is not an effective reducing agent for Fe(III) (Dorey et al., 1993). Indirect evidence has suggested that as the pH increases to 5 and above, the reduction rate of Fe(III) by AA becomes quite slow. We (Hsieh and Hsieh, 1997) have demonstrated that the speed of reduction of Fe(III) by AA was pH dependent; that is, the higher the pH, the slower the rate. In the titration experiments between Fe(III) and AA, when pH was 4 and below, the titration could be performed fairly quickly, namely, within a few minutes, with a sharp end point and consistent results. When the pH was 5, the titration

could only be performed at a slow pace, namely, an hour or so, to get a sharp end point and consistent results (Hsieh and Hsieh, 1997). Therefore, if the kinetic information is not available, one could get incorrect and inconsistent experimental results in the reaction of Fe(III) and AA at pH 5 and above due to nonequilibrium conditions. However, the kinetics of Fe(III) reduction by AA at pH level above 4 remains largely unclear. We initiated this study to investigate the kinetics of Fe(III) reduction by AA at pH 5 and 6 in a model aqueous system at 25 °C and at the concentration of millimolar range. Due to the uncertainty of hydrolysis, polymerization, and complex formation of Fe(III) in the pH range, our main purpose was not to explain the exact reaction mechanisms. Rather, we attempted to identify the kinetic order of Fe(III) and AA in the reaction and the quantitative rate expression that is useful in the related studies. We observed directly the changes of total Fe(III) concentration in the presence of AA by measuring the absorbance at 375 nm and minimized the pH fluctuation during the experiments by using a potassium hydrogen phthalate buffer system.

MATERIALS AND METHODS

All solutions were prepared using reagent-grade chemicals. Solutions of 0.14 M Fe(III) and 0.1 M AA solutions were freshly prepared daily from ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and L-ascorbic acid, respectively, using oxygen-free deionized water. Buffer solutions of pH 5 and 6 were prepared from an oxygen-free 0.1 M potassium hydrogen phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$) solution by addition of 0.2 M sodium hydroxide or hydrochloric acid. In the stoichiometrically matched experiments (i.e., AA/Fe equivalent ratio = 1), 60 μL of the 0.14 M Fe(III) solution was first added to 5 mL of the buffer solution in a cuvette and well mixed (the final total concentration of Fe^{3+} was 1.68 mM). Just before the experiment started, 42 μL of 0.1 M AA solution was added to the 1.68 mM Fe(III) buffer solution (the final concentration of AA was 0.84 mM). The disappearance of total Fe(III) was monitored continuously at 375 nm using a spectrophotometer. In the constant AA concentration experiments (i.e., AA/Fe equivalent ratio = 10), 60 μL of 0.14 M Fe(III) solution was added to 5 mL of the buffer solution in a cuvette (the final total Fe^{3+} concentration was 1.68 mM). Just before

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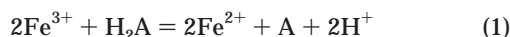
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the experiment, 420 μL of 0.1 M AA solution was added to the 1.68 mM Fe(III) solution (final AA concentration was 8.4 mM). The disappearance of total Fe(III) was monitored continuously at 375 nm using a spectrophotometer.

In the aging experiments, 1.68 mM Fe(III) solution was prepared in the buffer solutions of pH 5 and 6 and aged to a predetermined period of time. Just before the experiment started, an appropriate amount of AA solution was added to the aged Fe(III) solution, and the disappearance of total Fe(III) was monitored continuously at 375 nm using a spectrophotometer. The reduction rate of Fe(III), i.e., $d[\text{Fe}^{3+}]/dt$, was calculated by the difference of total Fe(III) concentration between two consecutive observations divided by the time interval.

Kinetics of Fe(III) reduction by AA was analyzed by the plots of $\log[-(d[\text{Fe}^{3+}]/dt)]$ vs $\log[\text{Fe}^{3+}]$ and $1/[\text{Fe}^{3+}]^{1-m}$ vs time. $[\text{Fe}^{3+}]$ denotes the total concentration of all Fe(III) species in the solution. In a previous study, Hsieh and Hsieh (1997) showed that 1 mol of AA reduces 2 mol of Fe(III) in the buffer solutions of pH 5 and 6, i.e.



where A represents the oxidized product dehydroascorbic acid. The rate of total Fe(III) disappearance at a constant pH can be expressed as

$$-d[\text{Fe}^{3+}]/dt = k[\text{Fe}^{3+}]^m[\text{H}_2\text{A}]^n \quad (2)$$

where $[\text{Fe}^{3+}]$ is the total concentration of all Fe(III) species, k is a rate constant, and m and n are the order of reaction with respect to Fe(III) and AA, respectively. According to eq 1, a stoichiometrically matched, oxygen-free solution of Fe(III) and AA would have a concentration relationship of $[\text{H}_2\text{A}] = 0.5[\text{Fe}^{3+}]$ and eq 2 becomes

$$-d[\text{Fe}^{3+}]/dt = k(0.5)^n[\text{Fe}^{3+}]^{m+n} \quad (3)$$

or in a logarithmic form

$$\log(-d[\text{Fe}^{3+}]/dt) = \log K + (m+n) \log[\text{Fe}^{3+}] \quad (4)$$

where $K = (0.5)^n$ and $k = \text{constant}$. If the reaction follows the expression of eq 4, a plot of $\log[-(d[\text{Fe}^{3+}]/dt)]$ vs $\log[\text{Fe}^{3+}]$ should yield a linear relationship in which the slope represents $m+n$.

In the AA/Fe³⁺ equivalent ratio = 10 experiment, $[\text{H}_2\text{A}]$ was relatively constant throughout the reaction (i.e., between 100 and 90% of the original concentration) and the rate expression in eq 2 could be approximated by a pseudo- m th-order reaction, i.e.

$$-d[\text{Fe}^{3+}]/dt = k[\text{H}_2\text{A}]^n[\text{Fe}^{3+}]^m = k'[\text{Fe}^{3+}]^m \quad (5)$$

or

$$\log(-d[\text{Fe}^{3+}]/dt) = \log k' + m \log[\text{Fe}^{3+}] \quad (6)$$

where $k' = k[\text{H}_2\text{A}]^n = \text{constant}$. A plot of $\log(-d[\text{Fe}^{3+}]/dt)$ vs $\log[\text{Fe}^{3+}]$ would generate a linear relationship with a slope of m . The value of n , i.e., the reaction order with respect to AA, could be deduced from the difference between the slopes of eq 4 and 6.

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

RESULTS AND DISCUSSION

Figure 1 gives the absorbance in response to the changes of Fe(III) concentration in the mM range in the buffer solutions at 375 nm. Although a maximum absorbance at 312 nm was found for Fe(III), the

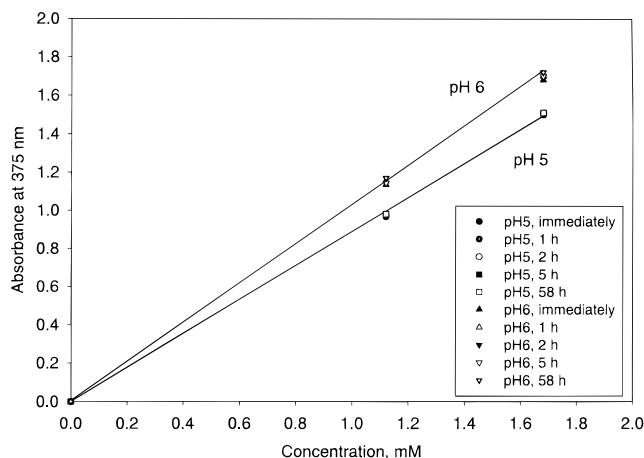


Figure 1. Standard curves for the determination of Fe(III) at 375 nm at pH 5 and 6. The age of Fe(III) solutions up to 58 h did not change the standard curves significantly. Different symbols represent measurements at different times up to 58 h.

wavelength of 375 nm was chosen because it responded linearly to the Fe(III) concentrations between 0 and 1.8 mM, which is the entire range of the experiments. The buffer solution, AA, and Fe(II) have negligible absorbances at 375 nm. The 375 nm absorbance of Fe(III) at pH 6 was significantly higher ($p \leq 0.01$) than that at pH 5. Aging of Fe(III) solution up to 58 h did not change the standard curves significantly ($p \leq 0.01$) at the two pH levels. Although ferric chloride in the absence of chelating agents at pH 5 and 6 may be in the form of polymeric oxo- and hydroxo-bridged complexes (Spiro and Slatman, 1969), the absorbance at 375 nm apparently responded to the total concentration of Fe(III) rather than individual species.

Figure 2 gives plots of $\log(-d[\text{Fe}^{3+}]/dt)$ vs $\log[\text{Fe}^{3+}]$ at pH 5 under the stoichiometrically matched (AA/Fe equivalent ratio = 1) and the constant AA (AA/Fe equivalent ratio = 10) conditions. The plots generated two segments of linear relationship during the course of Fe(III) reduction by AA in both the matched and constant AA cases. The results imply that at least two distinct stages of reactions were involved in each case. The first stage of the reaction (about 85%, stoichiometrically) appeared to be in an order of $m+n = 1.720 \pm 0.35$ (matched AA case) and $m = 1.895 \pm 0.37$ (constant AA case). The values of m and $m+n$ were not significantly different ($p \leq 0.01$), which implies that AA was of apparent zero-order kinetic (i.e., $n = 0$). The second stage of the reaction (about 15%, stoichiometrically) was an erratic, nonrational higher order reaction in which $m+n = 4.106 \pm 0.9$ (matched AA case) and $m = 4.704 \pm 0.8$ (constant AA case). The second stage of the reaction also indicated a zero-order kinetics with respect to AA because the n value was not significantly different from zero ($p \leq 0.01$). The reason for the erratic higher order of the reaction toward the end of the reaction is not clear. In the pH range of 5 or higher, iron chloride solution may form two discrete fractions of oxo and hydroxo polymers, one of low and one of very high molecular weight (Spiro and Saltman, 1969). The two stages of the reduction may have been caused by those two discrete fractions of Fe(III) polymers.

The plots of $\log(-d[\text{Fe}^{3+}]/dt)$ vs $\log[\text{Fe}^{3+}]$ of the 20-h-old Fe(III) solutions at pH 6 are presented in Figure 3. Two distinctive stages of reaction in each of the matched AA case and constant AA case were also

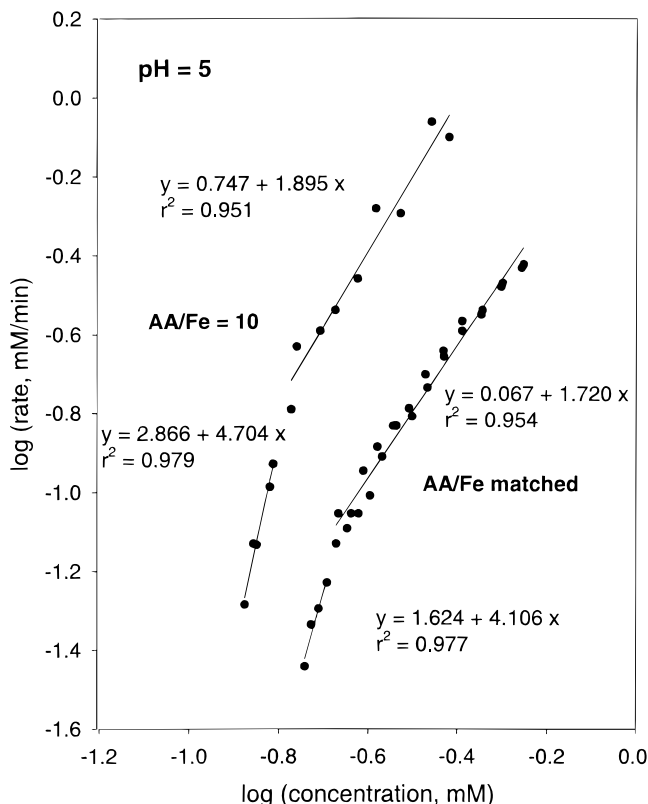


Figure 2. Plots of $\log(d[\text{Fe}^{3+}]/dt)$ vs $\log [\text{Fe}^{3+}]$ at pH 5. The slopes indicate the reaction order with respect to Fe^{3+} (AA/Fe = 10) and that with respect to Fe^{3+} and AA combined (AA/Fe matched). All AA/Fe ratios are in equivalent units.

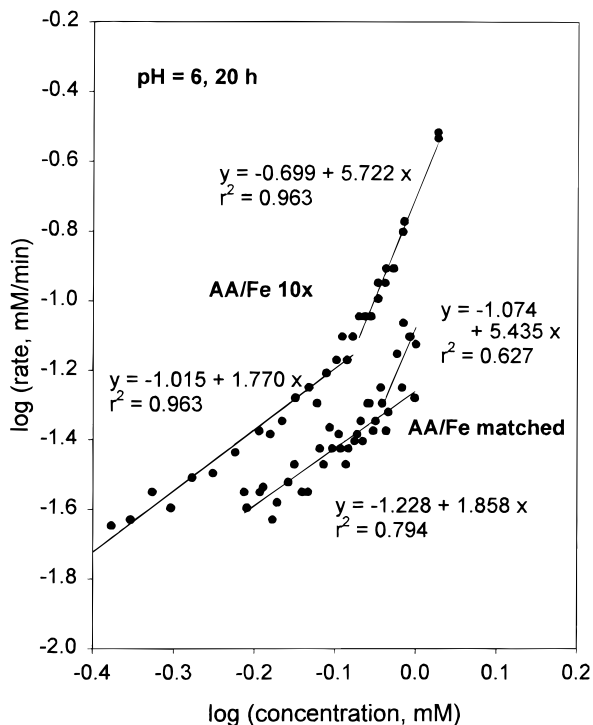


Figure 3. Plots of $\log(d[\text{Fe}^{3+}]/dt)$ vs $\log [\text{Fe}^{3+}]$ at pH 6 for a 20-h old Fe(III) solution. The slopes indicate the reaction order with respect to Fe^{3+} (AA/Fe = 10) and that with respect to Fe^{3+} and AA combined (AA/Fe matched). All AA/Fe ratios are in equivalent units.

observed. Contrary to the situation at pH 5, the first stage of the reaction (about 20%, stoichiometrically) was an erratic higher order reaction ($m + n = 5.435 \pm 1.1$

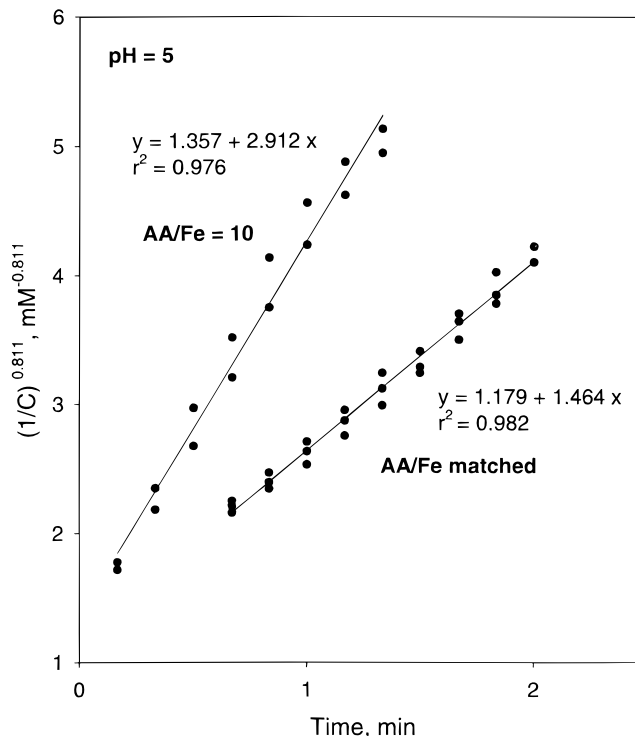


Figure 4. Plots of $(1/C)^{0.811}$ vs time at pH 5 for the AA/Fe matched and AA/Fe = 10 cases. All AA/Fe ratios are in equivalent units.

for the matched AA case and $m = 5.722 \pm 0.8$ for the constant AA case) followed by a more consistent $m + n = 1.858 \pm 0.28$ (matched AA) and $m = 1.770 \pm 0.31$ (constant AA) second stage (80%, stoichiometrically). Both stages of the reaction indicated that the values of m and $m + n$ were not significantly different ($p \leq 0.01$), i.e., a zero-order kinetics with respect to AA at pH 6. Statistical analysis indicated that the order of reaction with respect to Fe(III) was not significantly different between pH 5 and pH 6 at the major stages of the reaction. We estimated the average order of the major stages of the reaction with respect to Fe(III) at pH 5 and 6 to be 1.811 ± 0.309 .

The rate constants of the reduction of Fe(III) by AA were estimated by the integration of eq 3 under a stoichiometrically matched AA and Fe condition, i.e.

$$[\text{Fe}^{3+}]^{-(m+n-1)} = [\text{Fe}^{3+}]_0^{-(m+n-1)} - k^*t \quad (7)$$

where $[\text{Fe}^{3+}]_0$ was the initial concentration of all Fe(III) species. Since $n = 0$ and $m = 1.811$, eq 7 reduces to

$$[\text{Fe}^{3+}]^{-0.811} = [\text{Fe}^{3+}]_0^{-0.811} - k^*t \quad (8)$$

That is, the plots of $1/[\text{Fe}^{3+}]^{0.811}$ vs time would generate a linear relationship in which the slope represents the value of k because $k' = (0.5)^0 k = k$.

Figure 4 is a plot of $1/[\text{Fe}^{3+}]^{0.811}$ vs time at pH 5. The rate constants were found to be $1.464 \pm 0.44 \text{ mM}^{-0.811} \text{ min}^{-1}$ for the AA/Fe matched case and $2.912 \pm 0.52 \text{ mM}^{-0.811} \text{ min}^{-1}$ for the AA/Fe = 10 case at $25 \pm 2^\circ \text{C}$. Although the reduction of Fe(III) is of zero order with respect to AA, AA did have a positive effect on the reduction rate. When the AA/Fe equivalent ratio was 10, the reduction rate of Fe(III) doubled to that of the Fe/AA matched case. The positive effect of AA may be explained by two factors: (1) Addition of 10 times AA

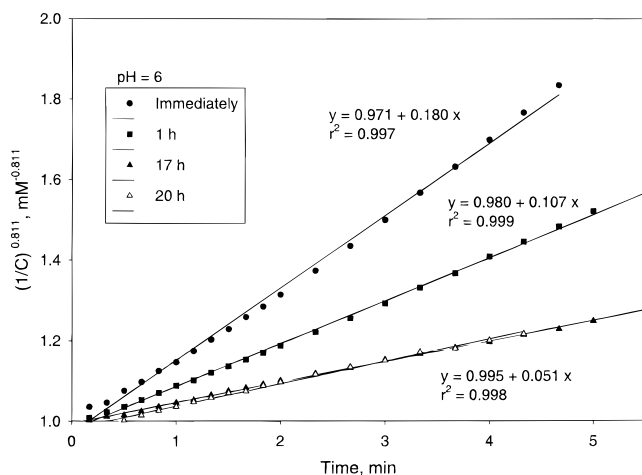


Figure 5. Plots of $(1/\text{Fe}^{3+})^{0.811}$ vs time at pH 6. The slopes represent rate constants. As the age of the Fe(III) solution increased to 17 h, the rate constants decreased.

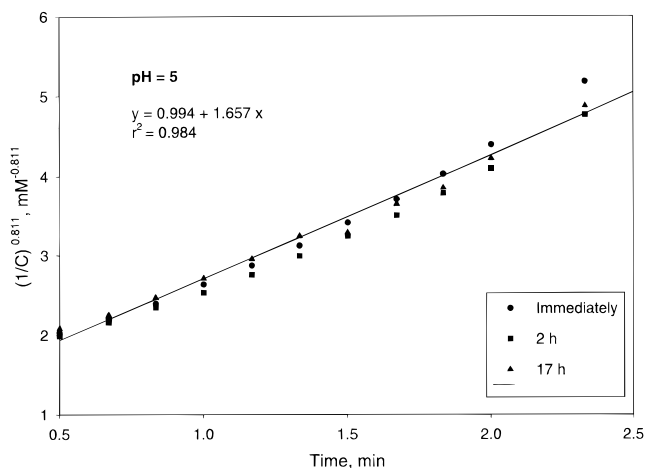


Figure 6. Plots of $(1/\text{Fe}^{3+})^{0.811}$ vs time at pH 5. The slopes represent rate constants. No significant aging effect of the Fe(III) solution on the reduction rate was observed.

decreased the pH in the buffer solution by approximately 0.2 unit. If the reduction rate increases 10 times every one pH unit decrease in the solution (see the discussion in the following section), a 0.2 unit decrease in pH would explain the 60% increase in the reduction rate. (2) AA might compete with the hydroxyl group for Fe(III) in the formation of complexes and decrease the hindrance of the reduction (increased the reduction rate).

There was an aging effect of Fe(III) solution on the reaction at pH 6. The reaction rate constant of Fe(III) at pH 6 was $0.180 \pm 0.09 \text{ mM}^{-0.811} \text{ min}^{-1}$ with freshly prepared Fe(III) solution, $0.107 \pm 0.07 \text{ mM}^{-0.811} \text{ min}^{-1}$ with 1-h-old Fe(III) solution, and $0.051 \pm 0.03 \text{ mM}^{-0.811} \text{ min}^{-1}$ with 17-h-old Fe(III) solution (Figure 5). There was no difference in rate constants between the 17- and 20-h-old Fe(III) solutions at pH 6. The aging effect of Fe(III) solution on the reduction rate was probably due to the increased hydrolysis of Fe(III) with time at pH 6, which hindered the reaction of Fe(III) and AA. No significant ($p \leq 0.01$) aging effect, up to 20 h, on the reaction at pH 5 was observed (Figure 6).

The reduction rate constant of Fe(III) at pH 5 is generally 1 order of magnitude higher than that at pH 6 [fresh prepared Fe(III) solutions], which implies that the reaction is pH dependent and of first order. In a study of iron oxidation by oxygen in seawater, Roekens

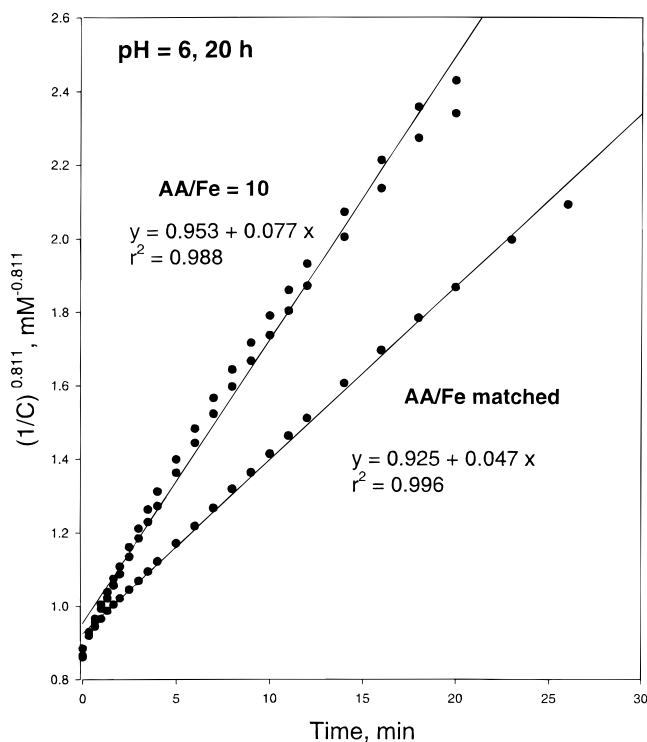


Figure 7. Plots of $(1/\text{Fe}^{3+})^{0.811}$ vs time at pH 6 for a 20-h-old Fe(III) solution for the AA/Fe matched and AA/Fe = 10 cases. All AA/Fe ratios are in equivalent units.

and Grieken (1983) reported that the oxidation rate increased as the pH increased from 5.9 to 8.4. The observations of this study indicate that the reduction rate of Fe(III) by AA decreased as the pH increased from 5 to 6. The results of this study also confirm the observation of Hsieh and Hsieh (1997) that Fe(III) reduction slows as the pH increases. The positive effect of AA on Fe(III) reduction was also observed at pH 6. Addition of 10 times AA with respect to Fe(III) increased the reduction rate by 70% when compared with the matched AA case (Figure 7). The increased rate of Fe(III) reduction was mainly due to the slightly lower pH (0.22 unit) of the excessive AA in the system.

This study is the first to demonstrate that AA is of zero-order kinetics in the reduction of Fe(III). This study also demonstrated that the rate of Fe(III) reduction by AA is highly influenced by pH. The kinetic information of this study is essential for anyone who studies the reaction between Fe(III) and AA at pH 5 and above. For example, when conducting a titration of Fe(III) and AA at pH 5 (at mM level), one should allow at least 4 min equilibration time for each increment addition of the titrant to the system. A titration of this pace would easily last 1–2 h at pH 5. At pH 6, at least 1 h equilibration time should be allowed for each increment addition of titrant to the system. At that pace, a titration would easily last 10–20 h at pH 6. One also needs to consider the aging effect of the Fe(III) solution when pH is close to 6. Results from this study have important implications in the formulation of chemical form of iron and AA in food, and the design of experiment for studying absorptivity of iron in the presence of AA.

CONCLUSIONS

Several conclusions can be drawn from this study. First, the rate of Fe(III) reduction by AA is not propor-

tional to the concentration of AA (i.e., of the zero-order kinetics). Ascorbic acid, however, has a positive effect on the rate of Fe(III) reduction mainly due to the indirect effect of pH and chelation. Reduction of Fe(III) by ascorbic acid at pH 5 and 6 can be represented by a two-stage simple kinetic model in a mM concentration range. The major stage of the reduction (80%) could be represented by the equation of $-d[\text{Fe}^{3+}]/dt = k[\text{Fe}^{3+}]^{1.811}$, where k is a rate constant. That is, the reduction is 1.811th order with respect to Fe(III). The reduction rate of Fe(III) by AA is influenced by pH. As pH increased from 5 to 6, the rate of Fe(III) reduction decreased by 1 order of magnitude. Aging of Fe(III) solution at pH 5 did not change the reduction rate. Aging of Fe(III) solution at pH 6, however, slows down the reduction rate, probably due to the progressive hydrolysis and polymerization of Fe(III) with time. Understanding the kinetics would help us conduct more meaningful experiments that lead to better understanding of the interaction between Fe(III) and AA in food, pharmaceuticals, and intestinal lumen.

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