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Effects of pH and phosphate on the oxidation of iron in aqueous solution

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Summary

Studies have been initiated to evaluate the catalytic effect of monohydrogen phosphate ions on the oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) ions in an aqueous solution under atmospheric oxygen conditions. The reactions were performed with an initial concentration of 1×10^{-4} M ferrous sulfate in solutions containing varying concentrations of phosphate buffer (0.005–0.0175 M) over the pH range of 6.6–7.1. The final ionic strength of the solutions were adjusted to 0.1 M with sodium chloride and the temperature was kept constant at 25 ± 0.5 °C. The rates of oxidation reactions were measured by following the increase in UV absorbance due to the formation of ferric ion in solution. The reactions appeared to follow pseudo-first-order kinetics and were very prone to catalysis by monohydrogen phosphate at any given pH. $H_2PO_4^-$ seemed to have no effect on the reaction. HPO_4^{2-} was the sole catalytic species with a second-order rate constant of 116.74 $M^{-1} \cdot min^{-1}$. The buffer independent pH–rate profile showed a sigmoidal behavior with the pseudo-first-order rate constant increasing with increasing pH. The sigmoidal nature of the experimental pH–rate profile could possibly suggest a change in the reactivity of the oxidizing species which might follow complex kinetics. The effects of ionic strength and temperature on the reaction rates were also evaluated.

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Introduction

The absorption of iron from the gastrointestinal (G.I.) tract is poor and variable because of the low aqueous solubilities of some of the iron salts especially at the ferric (Fe^{3+}) states. In order to ensure adequate absorption, an iron compound must be solubilized before or during digestion so that iron can enter the non-heme iron pool in the gut. With the world wide prevalence of iron deficiency especially among adult women and children, investigations of factors responsible for poor oral absorption of dietary or supplemental iron seem necessary. It has been observed that in a vegetarian diet, non-heme iron is absorbed very poorly because of the inhibitory action of a variety of components particularly phosphates (Bhagavan and Bloor, 1978). Since the aqueous solubility of ferric ions in the presence of counter ions such as the various phosphates is extremely low, the poor bioavailability of iron in the presence of phosphate appears to be due to the precipitation of ferric phosphate in the G.I. tract. Thus assessment of dietary iron availability should include not only the amount of iron ingested but also an estimate of its availability based on the intake of substances that affect its absorption.

Most previous studies on the oxidation of iron in aqueous solutions have been confined to narrow pH-ranges, high starting iron salt concentration and/or limited to the investigation of one particular mechanism (George, 1956; Goto et al., 1970). Cher and Davidson (1955) have studied the kinetics of the oxygenation of ferrous ion in phosphoric acid solution at low pH range of 1–2. This article discusses the kinetics of oxidation of ferrous ion in neutral to slightly alkaline solutions and provides information about the effect of phosphate (besides pH) affecting the overall stability of ferrous ion.

Materials and Methods

Materials

Ferrous sulfate was obtained from Merck Chemicals. All other chemicals were of reagent grade. Deionized distilled water was used throughout. A pH-range of 6.6–7.1 was maintained by using phosphate buffer in the concentration range of 0.005–0.0175 M. The ionic strength of all the solutions was adjusted to 0.1 M by the addition of sodium chloride.

Apparatus

A Corning Model 125 pH-meter was used for the measurement of hydrogen ion activity. Ultraviolet spectral measurements were performed with a Bausch and Lomb Spectronic 2000 recording spectrophotometer utilizing 1-cm pathlength quartz cuvettes.

The oxidation reactions involving ferrous ions in aqueous solutions were studied spectrophotometrically by following the increase in absorbance at 275 nm which accompanied oxidation. An initial concentration of 1×10^{-4} M ferrous sulfate could be used in these studies because of the sensitivity of UV spectral technique.

Fig. 1 illustrates typical spectral changes accompanying the oxidation of ferrous to ferric ion in neutral and slightly alkaline solutions in presence of phosphate buffer. Plots of $\log(A_\infty - A_t)$ versus time were linear according to the first-order expression:

$$\log(A_\infty - A_t) = \log(A_\infty - A_0) - \frac{k_{obs} \cdot t}{2.303} \tag{1}$$

where A_0, A_t and A_∞ are the absorbances at zero time, time t and infinite time, respectively, and k_{obs} is the pseudo-first-order rate constant derived from the slopes of the plots. In these experiments, the absorbance at time zero was negligible and adjusted to zero using a phosphate buffer blank.

The reactions were initiated by the addition of 0.8 ml of 1×10^{-3} M ferrous sulfate solution to 7.2 ml of phosphate buffer. Immediately following the mixing, an aliquot was transferred to the cuvette inside the cell compartment. The starting concentration of ferrous ion in the final solution was 1×10^{-4} M. At appropriate intervals, the absorbances were read at 275 nm. The point where no more significant change in absorbance was noted, was taken as the infinity reading. Kinetic studies involving variations in temperature were performed using a Beckman DU-7 spectrophotometer equipped with a thermostatically-controlled cell compartment.

An independent confirmative test was performed according to the method described by Nebergell et al. (1976) to identify the formation of ferric ions in the aqueous reaction mixture. When a small quantity (5 ml of 1×10^{-3} M) of ferrous

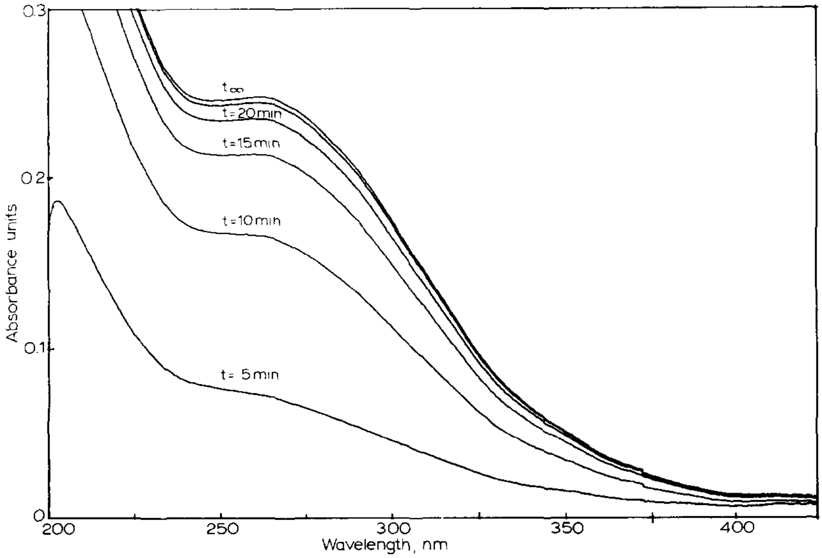

Fig. 1. Spectral changes for oxidation of ferrous ion (1×10^{-4} M) in 0.01 M phosphate buffer pH 6.7 at 25°C ($\mu = 0.1$). The curves are labelled as to minutes after the start of the reaction; t_∞ is approximately 25 min.

sulfate solution following acidification with 1 N HCl was added to an excess of ammonium thiocyanate, a faint pink hue to the solution appeared. This indicated the presence of trace quantity of ferric ion in ferrous sulfate sample. But when the same test was repeated following the addition of dibasic sodium phosphate to the ferrous sulfate solution, a deep red color appeared which confirmed the presence of high concentration of ferric ions in the solution. This test suggests that it is indeed the formation of ferric phosphate that is being observed spectrophotometrically.

Results and Discussion

The oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}) in aqueous solution was studied over the pH range of 6.6–7.1 at 25°C and at an ionic strength of 0.1. The reaction appeared to be significantly dependent on pH as well as phosphate concentration. Below pH 6.6, the reaction was extremely slow and ferrous ion seemed to be the stable oxidation state for iron. The reaction rate became increasingly faster above pH 6.6 and beyond a pH of 7.1, the oxidation rate was too fast to be measured with a conventional spectrophotometer.

Fig. 2 exhibits typical first-order plots for the oxidation of ferrous ion in an aqueous solution of pH 6.9 containing varying concentrations of phosphate buffer. The excellent linearity of the plots and direct dependency of the rate on the phosphate buffer concentration clearly suggest a pseudo-first-order behavior of the oxidation process.

Buffer concentration effect on oxidation rates

At a constant pH and in the presence of excess phosphate buffer, the rate constants k_{obs} for the oxidation reaction were greatly affected by general acid–base catalysis by the buffer components. Typical plots for the catalytic effect of phosphate buffer at various pHs are shown in Fig. 3, giving reasonably linear relation-

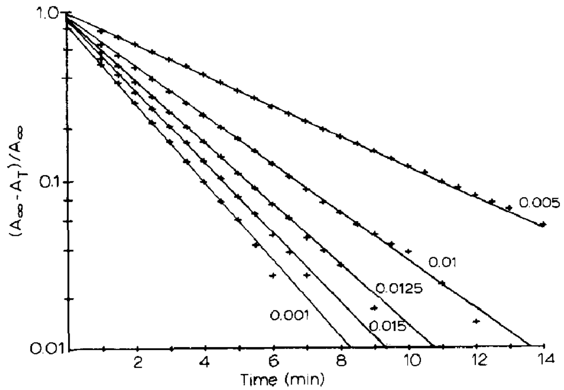

Fig. 2. Typical pseudo-first-order plots for the oxidation of ferrous ion in varying concentrations of phosphate buffer solutions at pH 6.9, 25°C ($\mu = 0.1$).

ships at constant pH in all cases. Extrapolation of such plots to zero buffer concentration provides, as intercepts, the pseudo-first-order rate constants k_{H^+} corresponding to the non-buffer catalyzed oxidation process. The rate constant, k_{obs}, may be defined in the phosphate buffer system as:

$$k_{obs} = k_{H^+} + k_{H_2PO_4^-}[H_2PO_4^-] + k_{HPO_4^{2-}}[HPO_4^{2-}] \tag{2}$$

where $k_{H_2PO_4^-}$ and $k_{HPO_4^{2-}}$ are second-order catalytic rate constants associated with the dihydrogen and monohydrogen phosphate species, respectively. Eqn. 2 can be rewritten in terms of total buffer concentration $[B]_T$

$$k_{obs} = k_{H^+} + \left[k_{H_2PO_4^-} + \left(k_{HPO_4^{2-}} - k_{H_2PO_4^-}\right)f_{HPO_4^{2-}}\right][B]_T \tag{3}$$

where $f_{HPO_4^{2-}}$ is the fraction of monohydrogen phosphate ion. Slopes of the plots shown in Fig. 3 and described by Eqn. 3 were calculated for each pH and plotted against the fraction of monohydrogen phosphate anion at that pH. Such a plot, as shown in Fig. 4 provided a second-order catalytic rate constant of 116.74 $M^{-1} \cdot min^{-1}$ for the HPO_4^{2-} buffer component. No significant catalytic effect could be assigned to the $H_2PO_4^-$ component.

Effect of ionic strength

The oxidation rates of 1×10^{-4} M ferrous sulfate solutions containing 0.015 M phosphate buffer at pH 6.7 under atmospheric oxygen pressure and 25°C were measured with the addition of various amounts of sodium chloride so that ionic strength ranged from 0.1 to 0.4. The rate constants were calculated according to Eqn. 1.

Fig. 5 depicts the plot of log k_{obs} versus $\sqrt{\mu}$. The plot is reasonably linear with a positive slope of 0.64. Within limited range of μ, plot of log k_{obs} versus $\sqrt{\mu}$ should

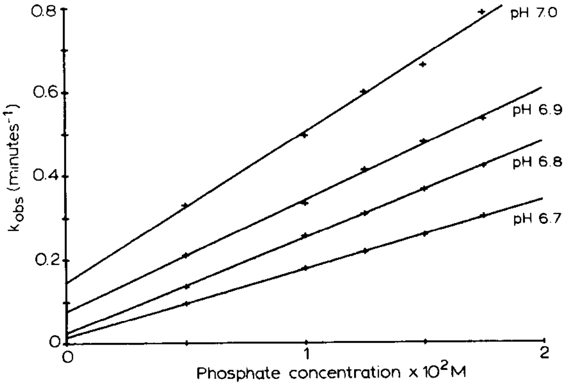

Fig. 3. The effect of phosphate buffer concentrations on the observed rate constants for the oxidation of iron at various pH values (25°C, $\mu = 0.1$).

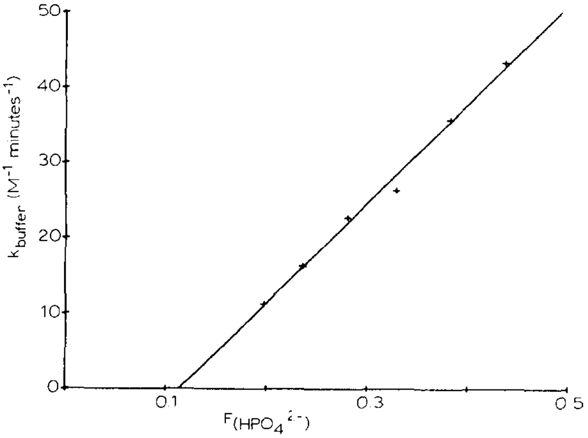

Fig. 4. Second-order phosphate catalytic rate constant as a function of the fraction of monohydrogen phosphate ion in total buffer.

yield theoretical slopes equal to $2AZ_AZ_B$, where A is a solvent parameter (0.509 for water at 25°C) for the solvent at a given temperature and Z_A and Z_B are the charges on reaction species A and B, respectively (Butler, 1964). The positive slope can be used as evidence for the reaction between two like-charged species. A slope of less than one suggests that a parallel pathway for the oxidation in addition to ion–ion reaction is also taking place which is unaffected by salt concentration, i.e. at least one of the reactants is uncharged. The ionic strength effect can also be due to the secondary salt effect. Increase in ionic strength has been found to decrease the pK_{a_2} of phosphoric acid and as a result the fraction of HPO_4^{2-} at a constant pH will be higher and the reaction rate will be faster.

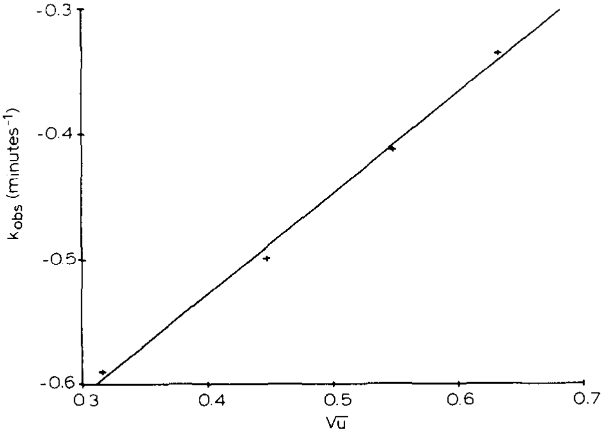

Fig. 5. Plot of log k_{obs} versus $\sqrt{\mu}$ for the oxidation of iron at 25°C, pH 6.7 in 0.025 M phosphate buffer.

Effect of temperature

The effect of temperature on the oxidation of Fe^{2+} ion was determined by measuring the oxidation rate at 20, 25, 30 and 35°C in 0.01 M phosphate buffer pH 6.7 and $\mu = 0.1$. The logarithm of the observed rate constant was plotted against the reciprocal of the absolute temperature according to the following Arrhenius equation:

$$\log k_{obs} = \log A - \frac{E_a}{2.303\,RT} \tag{4}$$

where log A, the intercept of the plot, is the logarithm of the collision frequency and E_a is known as the activation energy required for the reaction. Fig. 6 illustrates such an Arrhenius plot. The activation energy calculated from the slope was 14.2 kcal/mol. Because of the uncertainty in formulating a detailed reaction mechanism, the calculated activation energy can not be assigned to any particular reaction.

pH–rate profile

The pH-dependence of the overall oxidation rate of iron at 25°C and $\mu = 0.1$ is shown in Fig. 7. The rate constants (k_{H^+}) used in the construction of the graph were obtained from the intercepts of the plots of k_{obs} versus total phosphate buffer concentration at various pH values, i.e. at zero buffer concentration (Fig. 3).

The sigmoidal nature of the pH–rate profile along with more than an order of magnitude increase in the rate over the pH range of 6.6–7.1 suggests a change in the molecular structure or environment of the reacting species and the species that is formed at higher pHs appears to have a greater oxidation potential. Qualitatively, this observation suggests that at lower pHs (pH < 6.6) the ferrous ion is the preferred oxidation state. Therefore, the G.I. absorption of iron is likely to take place in the stomach or in the early part of the small intestine where the environment is more favorable for Fe^{2+} ion stabilization. Solid dosage forms containing the

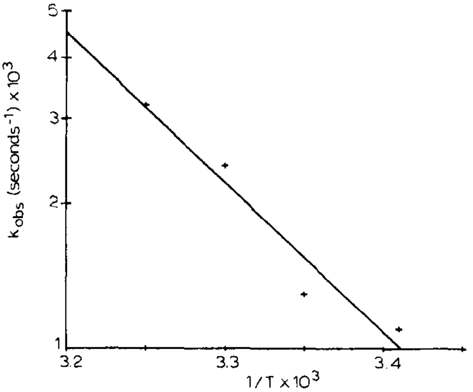

Fig. 6. Arrhenius plot for the oxidation of Fe^{2+} at $\mu = 0.1$ and pH = 6.6 in 0.01 M phosphate buffer.

ferrous salt should undergo rapid disintegration and dissolution so that the active component can remain in solution as Fe^{2+} salt and not being oxidized to the poorly soluble Fe^{3+} salts. Phosphate has a significant effect on the oxidation of Fe^{2+} ions. Therefore, presence of phosphate in the diet appears to have a dual inhibitory effect on iron absorption. It catalyzes the oxidation step and subsequently precipitates the oxidized iron as inorganic phosphate salt.

The mechanism of oxidation of iron from the ferrous to the ferric state in aqueous solution has been the subject of much speculation. Observations from the present study can be summarized as the following. (1) Increase in alkalinity causes an increase in the buffer-independent oxidation rate. The profile shows a sigmoidal behavior. (2) The reaction has a pseudo-first-order dependency on the phosphate concentration. Monohydrogen phosphate dianion appears to be the sole catalytic species acting as a general base. (3) The reaction requires a moderate energy of activation (14.2 kcal/mol) and exhibits a positive salt effect.

The concentration of dissolved oxygen has no apparent effect on the reaction rate. Reactions run under a nitrogen atmosphere and with solutions purged with nitrogen were found to have almost identical rates to those performed under atmospheric oxygen conditions, when other variables such as ferrous ion concentration, phosphate concentration, pH, ionic strength and temperature of the reaction were held constant. Therefore, assumption of singlet oxygen involvement in the oxidation process seems logical because it is regenerated in the course of the reaction.

A mechanism consistent with these observations and other experimental findings is being proposed in the following paragraph

$$Fe^{2+} + OH^- \rightleftharpoons FeOH^+ \tag{5}$$

$$O_2^- + H^+ \rightleftharpoons HO_2 \tag{6}$$

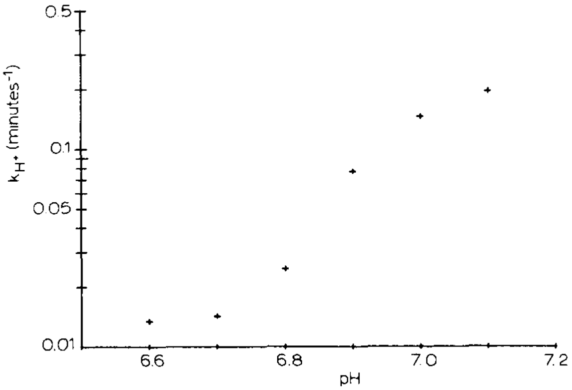

Fig. 7. Log k_{H^+}-pH profile for the iron oxidation at 25°C and $\mu = 0.1$. The points are experimental values.

Involvement of both $FeOH^+$ and HO_2 in the oxidation process has been proposed and verified by Goto et al. (1970) and Cher and Davidson (1955), respectively. The next step may be a slow proton transfer from HO_2 to $FeOH^+$ according to the following equation.

$$FeOH^+ + HO_2 \rightarrow FeOH_2^{2+} + O_2^- \qquad (7)$$

The proton transfer step as shown in Eqn. 7 may explain the general buffer catalysis by HPO_4^{2-} ion as well as the low activation energy required for the reaction. The final step is the rapid breakdown of $FeOH_2^{2+}$ to Fe^{3+} and water.

The ionic strength effect on the reaction rate may be a secondary effect. The overall picture of pH dependency on the oxidation process appears to be complex as it involves both pH-dependent formation of $FeOH^+$ and HO_2. One explanation that can be put forward is the ionization of $FeOH_2^{2+}$ species according to Eqn. 8

$$FeOH_2^{2+} \underset{K_{a_1}}{\rightleftharpoons} Fe^{2+}(OH)^- + H^+ \qquad (8)$$

$$pK_{a_1} = 6.7\ (I = 0.7)$$

The ionized species may be the more easily oxidizable species. Further studies are needed to unequivocally establish the mechanism of iron oxidation in aqueous solution.

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