The Fenton Reaction in Aerated Aqueous Solutions Revisited

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The oxidation of alcohols in aerated acidic aqueous solutions by the Fenton reagent is a chain reaction. The length of the chain depends linearly on the number of CH_nOR ($n = 1$ or 2) groups in the alcohol. The reaction is accelerated by increasing the concentration of $Fe(H_2O)_6^{2+}$, but this cation is also active in at least one of the termination steps of the chain reaction. Addition of ethanol to a solution containing sucrose shortens the chain length. Saturation with dioxygen, instead of air, increases the chain length. An increase in alcohol concentration increases the chain length up to a limiting value. A complicated mechanism, which is in agreement with all these observations, is proposed. However, efforts to simulate the mechanism succeed only in simulating the $Fe(H_2O)_6^{3+}$ yield, but indicate that the observed process is considerably faster than the predicted one. In the latter the rate-determining step is the Fenton reaction, the rate constant of which is well known.

Introduction

In 1894 Fenton reported that alcohols are oxidized in the presence of H_2O_2 and $Fe(H_2O)_6^{2+}$.[1] After more than 110 years of further research the active intermediate in, and detailed mechanism of, this kind of reactions are still ambiguous and repeatedly discussed in many publications and reviews.[2–9] In 1934 Haber and Weiss[10] suggested that the key step in this process is:

$$H_2O_2 + Fe(H_2O)_6^{2+} \rightarrow {}^{\cdot}OH + OH^- + Fe(H_2O)_6^{3+} \quad (1)$$

This reaction and its analogous reactions

$$H_2O_2 + M^nL_m \rightarrow {}^{\cdot}OH + OH^- + M^{n+1}L_m \quad (2)$$

the "Fenton-like" reactions, play an important role in a variety of catalytic and biological processes. In biology this reaction is believed to be the main source of the ROS (Reactive Oxygen Species) in the cell[11–13] that cause a variety of diseases, for example cancer, arteriosclerosis, essential hypertension, Alzheimer's disease, amyloidosis, osteoarthritis, etc.[14,15]

However, detailed kinetic studies indicate that hydroxyl radicals are not formed in all these reactions.[3,4,16–23] The detailed mechanism seems to involve one of the following routes:[19]

$$H_2O_2 + M^nL_m \leftrightharpoons L_mM^{n}\cdot H_2O_2 \text{ or } L_{m-1}M^{n}\cdot H_2O_2 + L \quad (3)$$

followed by:

$$L_{m-1}M^n \cdot H_2O_2 \xrightarrow{+L} M^{n+1}L_m + {}^{\cdot}OH + OH^- \quad (4a)$$

$$L_{m-1}M^n \cdot H_2O_2 \rightarrow L_{m-1}M^{n+2}{=}O + H_2O \quad (4b)$$

$$L_{m-1}M^n \cdot H_2O_2 \xrightarrow{+RH} M^{n+1}L_m + R^{\cdot} + H_2O + OH^- \quad (4c)$$

$${}^{\cdot}OH + RH \rightarrow R^{\cdot} + H_2O \quad (5)$$

$$L_{m-1}M^{n+2}{=}O + {>}RH \xrightarrow{+L} M^{n+1}L_m + R^{\cdot} + OH^- \quad (6)$$

Thus, in the presence of an organic substrate, RH, ultimately the same radicals $R^{\cdot}$ are formed. The results point out that the choice of the route to $R^{\cdot}$ depends on the nature of M, L, RH and its concentration, and on the medium, for example the pH.[3,4,19,24,25] Recent results indicate that for $M_nL_m = Fe(H_2O)_6^{2+}$ reaction (4b) is the dominant pathway.[16–18,26]

We decided recently to use the Fox assay[27] to determine the concentrations of H_2O_2 and hydroperoxides in biological samples. In this assay, the yield of Fe^{III} is measured by the absorption of its complex with xylenol orange. The addition of alcohols to the aerated reaction mixture increases the Fe^{III} yield. Thus, for example, in solutions containing 0.1 M sorbitol the yield is $[Fe(H_2O)_6^{3+}]/[H_2O_2] = 14$. This result clearly points out that the $[Fe(H_2O)_6^{3+}]$ is formed in a chain reaction. Two mechanisms have been suggested in the literature for this process. The first, suggested by Wolff,[27] is as follows:

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$$H_2O_2 + Fe(H_2O)_6^{2+} \rightarrow HO^{\cdot} + OH^- + Fe(H_2O)_6^{3+} \quad (7)$$

$$HO^{\cdot} + R_2CHOH \rightarrow H_2O + R_2C^{\cdot}OH \quad (8)$$

$$R_2C^{\cdot}OH + O_2 \rightarrow R_2C(OO^{\cdot})OH \quad (9)$$

$$R_2C(OO^{\cdot})OH \rightarrow R_2C{=}O + HO^{\cdot}_2 \quad (10)$$

$$HO^{\cdot}_2 + Fe(H_2O)_6^{2+} \rightarrow H_2O_2 + Fe(H_2O)_6^{3+} \quad (11)$$

However, it is difficult to suggest which reaction terminates this chain process as the rates of reactions (8), (9), (10), and (11) are known and are high. Thus, second-order reactions between the radicals are not expected to compete with these reactions.

The second mechanism, proposed by Gebicki,[28] has reaction (12) instead of (11) in the reaction mechanism [reactions (7)–(10) are analogous to Wolff]:

$$2\ HO^{\cdot}_2 \rightarrow H_2O_2 + O_2 \quad (12)$$

This mechanism is clearly wrong as it predicts a yield of $[Fe(H_2O)_6^{3+}]/[H_2O_2]$ of 2.

We decided, therefore, to investigate the mechanism of this reaction in detail. The results indeed point out that the mechanism is considerably more complicated, although some of its aspects remain unresolved.

Results and Discussion

Initially, the effect of $Fe(H_2O)_6^{2+}$ concentration on the kinetics of the FOX assay with the substrate sucrose was studied. The results (Figure 1) are quite surprising. As expected, the rate of the process increases with $Fe(H_2O)_6^{2+}$ concentration. However the yield of $Fe(H_2O)_6^{3+}$ clearly decreases with the increase of $Fe(H_2O)_6^{2+}$ concentration, in other words the results point out that $Fe(H_2O)_6^{2+}$ is involved in at least one of the chain-termination steps. This termination step cannot be due to the competition between reactions (13) and (8a).

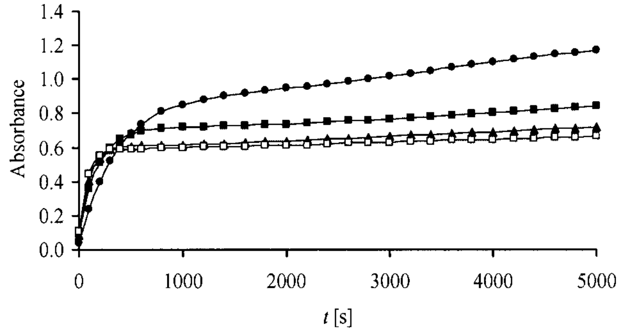

Figure 1. Kinetic measurements for different concentrations of iron(II) ammonium sulfate. The test solutions contained 10 mM sucrose, 2 μM H_2O_2, and the FOX reagent at a concentration of 250 μM (●), 400 μM (■), 600 μM (▲), and 800 μM (□) of iron(II) ion in air-saturated solutions.

$$^{\cdot}OH + Fe(H_2O)_6^{2+} \xrightarrow{H^+} H_2O + Fe(H_2O)_6^{3+} \quad (13)$$

with $k_{13} = 3.0 \times 10^8\ M^{-1}\,s^{-1}$,[29]

$$R_2CHOH + HO^{\cdot} \rightarrow R_2C^{\cdot}OH + H_2O \quad (8a)$$

with $k_{8a} = 2.3 \times 10^9\ M^{-1}\,s^{-1}$,[30] as even at the highest $[Fe(H_2O)_6^{2+}]$, $k_{13}[Fe(H_2O)_6^{2+}]$ is only 1.5% of k_{8a}[sucrose].

In the second set of experiments the effect of the nature of the organic solute on the yield of $Fe(H_2O)_6^{3+}$ was studied. The yield of Fe^{III} increases with the number of CH_nOX groups. The results are summarized in Table 1 and Figure 2. These results again clearly demonstrate that a competition between reactions (13) and (8) cannot determine the chain length. As recent results[16–18] point out that the Fenton reaction in acidic solutions proceeds via:

$$Fe(H_2O)_6^{2+} + H_2O_2 \rightarrow (H_2O)_5Fe^{IV}{=}O^{2+} + H_2O \quad (4b')$$

it seemed reasonable to suggest that the chain length is determined by the competition between reactions (14) and (15):

$$(H_2O)_5Fe^{IV}{=}O^{2+} + Fe(H_2O)_6^{2+} \xrightarrow{2\,H^+} 2\ Fe(H_2O)_6^{3+} \quad (14)$$

$$(H_2O)_5Fe^{IV}{=}O^{2+} + R^1R^2HCOH \rightarrow 2\ Fe(H_2O)_6^{3+} + {}^{\cdot}CR^1R^2(OH) \quad (15)$$

The specific rates of these reactions are unknown, although it is reasonable to suggest that reaction (14) proceeds via an inner-sphere mechanism and, as the specific rate of water exchange of $Fe(H_2O)_6^{2+}$, reaction (16), is $3.2 \times 10^6\ M^{-1}\,s^{-1}$,[31]

$$Fe(H_2O)_6^{2+} + H_2O^* \rightarrow Fe(H_2O)_5(H_2O^*)^{2+} + H_2O \quad (16)$$

it is estimated that $k_{14} \leq 3 \times 10^6\ M^{-1}\,s^{-1}$.

It is not unreasonable to suggest that k_{15} increases linearly with the number of CH_nOX groups. Therefore, the simplest mechanism that explains the results is that outlined in reactions (4b′)–(14) below. All the reactions in this group, with the exception of reactions (15) and (14), are known reactions. Furthermore, reaction (14) has been proposed in the literature[16] and reactions analogous to reaction (15) have also been proposed.[16,36]

$$Fe(H_2O)_6^{2+} + H_2O_2 \rightleftharpoons [(H_2O)_5Fe^{II}{\cdot}H_2O_2] \rightarrow (H_2O)_5Fe^{IV}{=}O^{2+} + H_2O \quad (4b')$$

with $k = 56\ M^{-1}s^{-1}$ [16]

$$(H_2O)_5Fe^{IV}{=}O^{2+} + R^1R^2CHOH \rightarrow Fe(H_2O)_5(OH)^{2+} + R^1R^2C^{\cdot}OH\ (\equiv R^{\cdot}) \quad (15)$$

$$R^{\cdot} + O_2 \rightarrow RO_2^{\cdot} \quad (9')$$

with $k \geq 1 \times 10^9\ M^{-1}s^{-1}$ [32]

$$RO_2^{\cdot} \rightarrow R^1R^2C{=}O + HO^{\cdot}_2 \quad (10')$$

with $400\ s^{-1} < k < 2600\ s^{-1}$ [33,34]

$$RO_2^{\cdot}\backslash HO^{\cdot}_2 + Fe(H_2O)_6^{2+} \rightleftharpoons (H_2O)_5Fe^{III}{-}OOR^{2+} \quad (17)$$

with $k > 2 \times 10^6\ M^{-1}\,s^{-1}$, $K \approx 200\ M^{-1}$ [35]

$$(H_2O)_5Fe^{III}{-}OOR^{2+} \xrightarrow{H^+} Fe(H_2O)_6^{3+} + HOOR \quad (18)$$

with $k \approx 1500\ M^{-1}\,s^{-1}$ [35]

Table 1. The influence of substrate structure on the chain length in the FOX assay. The test solutions contained 2–7 μM H_2O_2, the FOX assay, and 100 mM organic substrate in air-saturated solutions.

Substrate	CH_nOX groups	k(·OH+ alcohol) [$M^{-1}\,s^{-1}$][54]	[Fe^{III}]/[H_2O_2][a]	[Fe^{III}]/[H_2O_2][a] without XO
None	0		1.9	1.9
Methanol	1	1.0×10^9	3.6	4.4
Ethanol	1	1.9×109	4.8	6.6
Ethylene glycol	2	2.4×10^9	5.7	6.4
Glycerol	3	2.0×10^9	8.3	9.0
Pentaerythritol	4[b]	3.3×10^9	9.6	
Sorbitol	6		13.7	14.5
Glucose	6	1.5×10^9	14.5	18.7
Sucrose	11	2.3×10^9	24.4	
Diethyl ether	1	3.6×10^9	4.6	
Dioxane	2[b]	2.8×10^9	4.9	

[a] Average of several determinations, error limit ±10%. [b] The CH_2OX groups are not adjacent.

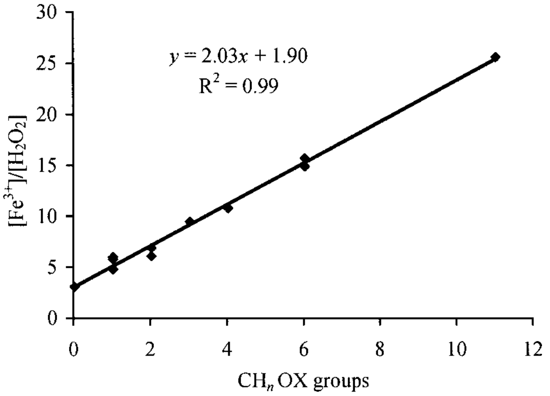

Figure 2. The influence of substrate structure [number of CH_nOX (n = 1 or 2) groups] on the chain length in the FOX assay. The test solutions contained 2–7 μM H_2O_2, 100 mM organic substrate, and the FOX reagent in air-saturated solutions.

$$(H_2O)_5Fe^{III}\text{–}OOR^{2+} + Fe(H_2O)_6^{2+} \rightarrow (H_2O)_5Fe^{IV}{=}O^{2+} + Fe(H_2O)_6^{3+} + ROH \quad (19)$$

with $k \approx 5\times10^5\ M^{-1}\,s^{-1}$ [35]

$$(H_2O)_5Fe^{IV}{=}O^{2+} + Fe(H_2O)_6^{2+} \xrightarrow{2\,H^+} 2\ Fe(H_2O)_6^{3+} \quad (14)$$

with $k < 3\times10^6\ M^{-1}\,s^{-1}$.

In order to check the validity of this mechanism, the effect of the concentration of the organic substrate on the yield of $Fe(H_2O)_6^{3+}$ was measured; the results for ethanol and sucrose are presented in Figure 3. The results point out that the yield of $Fe(H_2O)_6^{3+}$ initially increases with [R^1R^2HCOH], as expected, but surprisingly reaches a limiting value at high substrate concentrations. These results indicate that at low [R^1R^2HCOH] the competition between reactions (14) and (15) influences the chain length, but this competition has no effect at high concentrations. Indeed, a careful analysis of Figure 1 shows that the effect of [$Fe(H_2O)_6^{2+}$] on the yield of $Fe(H_2O)_6^{3+}$ is large at low [$Fe(H_2O)_6^{2+}$] and reaches a limiting value at higher concentrations. These results suggest that another termination step contributes to the mechanism. This conclusion is supported by the effect of adding C_2H_5OH to reaction mixtures containing sucrose (Figure 4). The results clearly demonstrate that the addition of ethanol shortens the chain length rather than extending it as predicted in reactions (4b′)–(14). This effect might be due to the fact that, in principle, two types of radicals R· are formed: a) a terminal α,ω radical ·CH(CHR′OH)OH (≡ ·R^1), b) a β radical ·C(CHR′OH)(CHR′′OH)OH (≡ ·R^2).

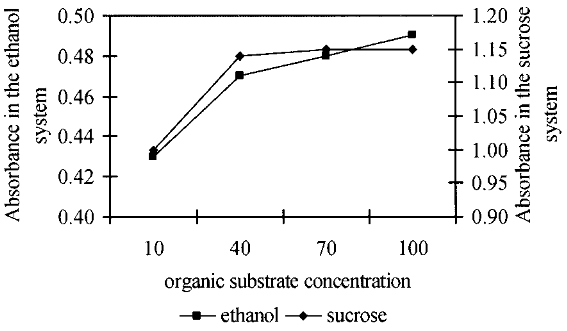

Figure 3. The influence of increasing concentrations of ethanol and sucrose on the absorbance of Fe^{III}XO formed. The test solutions contained 5 μM H_2O_2 with 10–100 mM ethanol and 2 μM H_2O_2 with 10–100 mM sucrose and the FOX reagent in air-saturated solutions.

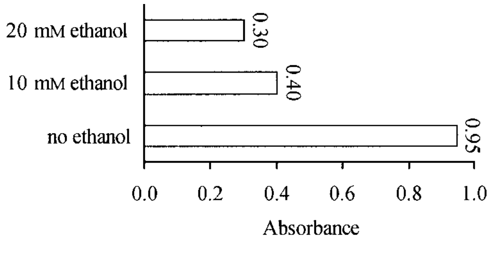

Figure 4. The absorbance of Fe^{III}XO formed in sucrose-containing solutions in the presence of different concentrations of ethanol. The test solutions contained 10 mM sucrose, 2 μM H_2O_2, the FOX reagent, and 0–20 mM of ethanol in air-saturated solutions.

Both these radicals react with dioxygen to yield the $R^iO_2^{\cdot}$ radicals. However, these eliminate $HO_2^{\cdot}$ at considerably different rates

$$^{\cdot}O_2CH(CHR'OH)OH \rightarrow HO_2^{\cdot} + CH(O)(CHR'OH) \quad (10'a)$$

with $k = 400\ s^{-1}$ [33,34]

$$^{\cdot}O_2C(CHR'OH)(CHR''OH)OH \rightarrow HO_2^{\cdot} + C(O)(CHR'OH)(CHR''OH) \quad (10'b)$$

with $k = 2600\ \mathrm{s^{-1}}$ [33,34]

For sugars, there is an additional, faster elimination step for the group next to the ether linkage ($k > 7000\ \mathrm{s^{-1}}$).[33,34]

All the $HO_2^{\cdot}$ radicals contribute to the chain process via reactions (17), (18), and (19). However the $RO_2^{\cdot}$ radicals, which are longer lived for the $^{\cdot}O_2CH(CHR'OH)OH$ radicals (α,ω-attack), yield some $HO_2CH(CHR'OH)OH$ via reaction (18). The latter product is usually unstable and might decompose by reaction (20)[37]

$$HO_2CH(CHR'OH)OH \rightarrow C(O)(CHR'OH)OH + H_2O \text{ or } HC(O)(CHR'OH) + H_2O_2 \qquad (20)$$

The decomposition into the acid is acid catalyzed, whereas the aldehyde analog preferably forms in more basic solutions and at higher temperatures.[37] Thus, part of reaction (20), which competes with reaction (4b′), is clearly a termination step. This mechanism also explains the role of $Fe(H_2O)_6^{2+}$ in shortening the chain length at high $[HCR^1R^2OH]$. Under these conditions the competition between reaction (10) and reaction (17) is clearly a function of $[Fe(H_2O)_6^{2+}]$ and, as reaction (17) is partially followed by reactions (18) and (20), reaction (17) contributes to a termination step. This contribution of $Fe(H_2O)_6^{2+}$ to the termination step is limited by the fact that reaction (19) competes with reaction (18) and reaction (4b′) with reaction (20).

This reaction scheme also explains the effect of the number of CH_nOX groups on the $Fe(H_2O)_6^{3+}$ yield: clearly, with more CH_nOX groups the ratio $[R^{2\cdot}]/[R^{1\cdot}]$ increases. This decreases the contribution of reaction (20), which is partially a termination step. In order to check this hypothesis the effect of pentaerythritol was checked. The result, Table 1, clearly demonstrates that the chain length corresponds to four CH_nOH groups, even though they are not adjacent. However, it should be noted that the rate of $HO_2^{\cdot}$ elimination from $^{\cdot}O_2CH(OH)C(CH_2OH)_3$ is not known and might be considerably higher than that of $R'O_2^{\cdot}$ radicals. It should be noted that there is a large variety in the rates of $HO_2^{\cdot}$ elimination reactions;[33,34,38,39] the source of these differences is not yet fully understood.[33,34,38,39]

When the solutions were saturated with O_2, instead of air, the yield of $Fe(H_2O)_6^{3+}$ increased (Table 2). In these experiments, only the yield was measured as they were performed in sealed syringes. The effect is small, though significant, for one CH_nOX group, and increases somewhat for a larger number of CH_nOX groups. The effect of differing oxygen concentrations on a system containing ethanol was noted previously.[40] As reaction (9′) is very fast, and as $k[^{\cdot}R+Fe(H_2O)_6^{2+}]$ is about three orders of magnitude slower[41] and $[O_2]$ is at least two orders of magnitude larger than that of any of the intermediates, the dioxygen effect cannot be attributed to an acceleration of reaction (9′). Thus, one has to conclude that the dioxygen effect in the ethanol system is due to the reaction of dioxygen with one of the reactive intermediates. No such reaction is known and a plausible one is:

Table 2. The effect of oxygen on the yields in the FOX assay for different substrates (comparison of air- and oxygen-saturated solutions). The test solutions contained 2 μM H_2O_2, the FOX assay, and 100 mM organic substrate (ethanol, glycerol, sorbitol, and sucrose).

Increase on oxygen saturation	$[Fe^{III}]/[H_2O_2]$ (oxygen)[a]	$[Fe^{III}]/[H_2O_2]$ (air)[a]	CH_nOX groups	Substrate
None	–	1.9	1.9	0
Ethanol	+26%	6.2	4.9	1
+56%	12.2	7.8	3	glycerol
+50%	24.2	16.1	6	sorbitol
+48%	42.4	28.6	11	sucrose

[a] Single determination, error limit ±15%.

$$(H_2O)_5Fe^{III}\text{–}OOR^{2+} + O_2 \xrightarrow[H^+]{} (H_2O)_5Fe^{IV}\text{–}OOR^{3+} + HO_2^{\cdot} \qquad (21)$$

followed by:

$$(H_2O)_5Fe^{IV}\text{–}OOR^{3+} \xrightarrow{H_2O} Fe(H_2O)_6^{3+} + RO_2^{\cdot} \qquad (22)$$

or by:

$$(H_2O)_5Fe^{IV}\text{–}OOR^{3+} \xrightarrow{H^+} (H_2O)_5Fe^{VI}{=}O^{4+} + ROH \qquad (23)$$

Reaction (21) might compete with reaction (18) and thus elongate the chain length. Alternatively, the reaction occurring could be:

$$Fe^{IV} + O_2 \rightarrow Fe^{V} + O_2^{\cdot-} \qquad (24)$$

although the relative redox potentials do not support this possibility. Oxidation of $PFe^{IV}{=}O$ by H_2O_2 to $PFe^{III}\text{–}O_2^{\cdot-}$ has been suggested in the literature.[42–44]

The larger dioxygen effect for systems with more than or equal to two CH_nOX groups is attributed to the competition between reactions (9) and reactions (25) (β-elimination of water from 1,2-diol-type radicals):[38]

$$^{\cdot}CH(CHR'OH)OH \rightarrow O{=}CH(C^{\cdot}HR') + H_2O \qquad (25a)$$

$$^{\cdot}C(CHR'OH)(CHR''OH)OH \rightarrow O{=}C(C^{\cdot}HR')(CHR''OH) + H_2O \qquad (25b)$$

Reactions (25) are acid catalyzed and, at pH 1.8, the experimental pH, the rate for the glycol radical, for example, is $2.5\times10^3\ \mathrm{s^{-1}}$.[45] Reaction (25) competes slightly with reactions (9), the rate of which in air saturated solutions is around $7\times10^5\ \mathrm{s^{-1}}$.[32] The radicals formed in reactions (25) form peroxyl radicals, which do not eliminate $HO_2^{\cdot}$ radicals. Clearly, reactions (25) do not occur for radicals derived from alcohols with one CH_nOX group or nonadjacent CH_nOX groups.

In kinetic simulations the general trend of the reactions ($[Fe^{II}]$, [substrate] effect) could be modeled reasonably well by including only the group of reactions (4b′)–(14) outlined above. Reactions for α,ω and β radicals were differentiated and the option included that part of the formed ROOH leads to a termination reaction instead of being returned to the cycle. These reactions do not include any explanations for the [dioxygen] effect and therefore this effect could not be modeled. The reactions (21–25) could not be incorporated in the simulations as the calculations proved to be too complicated. The simulations could be fitted to the $Fe(H_2O)_6^{3+}$ yields but the kinetics were always considerably slower than the observed ones. This stems from the fact that reaction (4b) is the rate-determining step and the rate constant of this reaction is well known.

Finally, it should be noted that when ROOH peroxides other than H_2O_2 are used, the yield of $Fe(H_2O)_6^{3+}$ is considerably lower.[27,46] This suggests that in these reactions the yield of $(H_2O)_5Fe^{IV}{=}O^{2+}$ is considerably lower than in the H_2O_2 system. This suggests that either $R^3O^\cdot$ radicals are formed in these reactions, which might be true also for reaction (19), or that reaction (4c) takes place in these systems to produce another mixture of $R^\cdot$ radicals than reaction (15). $R^3O^\cdot$ radicals might decompose via:[47]

$$CHR'R''O^\cdot \xrightarrow{\text{fast}} {}^\cdot CR'R''OH \tag{26}$$

This is, for example, expected to happen for fatty acid hydroperoxides, which are formed biologically, or for the radical formed from $(CH_3)_3COOH$ via:

$$C(CH_3)_3O^\cdot \rightarrow C(CH_3)_2O + {}^\cdot CH_3 \tag{27}$$

with $k = 2.0\times10^3\ s^{-1}$,[48] etc.

Concluding Remarks

This study clearly demonstrates that the mechanism of the Fenton reaction and Fenton-like reactions, in aerated aqueous solutions containing organic substrates, is considerably more complex than assumed so far. It seems reasonable to propose that reactions analogous to reaction (17) occur also in nonaqueous media. Therefore, also in these media the mechanisms of the Fenton process are probably considerably more complicated than proposed.[6,49–52]

The results point out that the yield of Fe^{III} depends on the nature of the peroxide and the nature of all the organic compounds present in the solution. Thus, the use of the FOX assay for the determination of the total peroxides in biological samples is difficult if at all feasible.

Finally, it is tempting to speculate that analogous reactions in biological systems might lead to considerable deleterious biological processes by damaging sugars bound to the membranes of cells[13] and/or to the sugar moieties in RNA and DNA.

Experimental Section

Materials: All the chemicals purchased from Sigma or Aldrich were of A.R. grade and therefore used without further purification. The water used was deionized and was further purified with a Millipore Milli-Q setup with a final resistivity of >10 MΩ

Preparation of the FOX Reagent: The FOX reagent containing iron(II) ammonium sulfate, H_2SO_4, xylenol orange, and an organic substrate was mixed with hydrogen peroxide to give final concentrations of 2–9 μM hydrogen peroxide, 250–800 μM iron(II) ion, 25 mM H_2SO_4, 100 μM xylenol orange (XO), and 10–100 mM organic substrate. The solutions were incubated for 40 min at room temperature. The absorbance was read at 560 nm. All spectra were run against blanks containing the FOX reagent. In some experiments, the XO was added only after 40 min for the analytical determination of the Fe^{III} yield.

UV/Vis measurements were carried out with a Hewlett–Packard Diode Array spectrophotometer model 8452A, which enables measurements in the range of 190–820 nm at a resolution of ±2 nm.

Determination of the H_2O_2 Concentration: The concentration of H_2O_2 was determined using the iodide method.[53] A stock solution containing KI, NaOH, and ammonium heptamolybdate was mixed with a second stock solution containing potassium hydrogenphthalate to give final concentrations of 0.08 M KI, 3.2×10^{-5} M ammonium heptamolybdate, and 0.02 M potassium hydrogen phthalate (final pH 4.0). The final solution was then mixed with H_2O_2 at concentrations ranging between 1×10^{-6} and 1×10^{-5} M. Addition of H_2O_2 leads to the production of I_3^- via the following reactions:

$$2\ I^- + H_2O_2 + 2\ H^+ \rightarrow I_2 + 2\ H_2O \tag{28}$$

$$I_2 + I^- \leftrightharpoons I_3^- \tag{29}$$

The absorbance of I_3^- was measured at 352 nm (ε = 26500 $M^{-1}\,cm^{-1}$).

Rate Simulations: Kinetic simulations were performed in order to evaluate the effects of H_2O_2, Fe^{II}, and organic substrate concentrations and the input rates and pattern of the mechanism. The simulations explored the formation kinetics of Fe^{III} and were compared to the experimental results. The kinetic calculations were performed with the Pro-Kineticist II program by Applied Photophysics.

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