Oxidizing Intermediates Generated in the Fenton Reagent: Kinetic Arguments Against the Intermediacy of the Hydroxyl Radical

David A. Wink,¹ Christian B. Wink,¹ Raymond W. Nims¹ and Peter C. Ford²

¹Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, Maryland; ²Department of Chemistry, University of California, Santa Barbara, California

It has long been recognized that the aqueous mixture of hydrogen peroxide and ferrous ion, known as the Fenton reagent, generates powerful oxidants. Furthermore, the chemical intermediates and reaction pathways of the type generated by this reagent have been implicated in oxidative damage in biological systems. Although the subject continues to be debated, the hydroxyl radical (·OH) is generally invoked as the predominant oxidizing intermediate formed by the Fenton reagent. However, recent results from this laboratory have demonstrated that the principal pathway for the Fenton-mediated oxidation of N-nitrosodimethylamine does not involve -OH, but instead must involve the intermediacy of another strongly oxidizing species. This conclusion was based on stopped-flow spectrophotometric observation of a transient, A, believed to be an iron(III) nitrosyl adduct, which was formed at a rate five-fold faster than that predicted for formation of ·OH. Subsequent experiments have shown that methanol and other organic compounds can quench the formation of A. This quenching procedure provides a unique spectrophotometric probe with which to examine the relative reactivities of putative Fenton-type oxidizing intermediates toward organic substrates. Presented here are the results of several such quenching studies, plus an overview of our mechanistic investigations of the Fenton reaction. — Environ Health Perspect 102(Suppl 3):11–15 (1994).

Key words: Fenton reagent, hydroxyl radical, metallo-oxo

Introduction

The reaction between ferrous ion and hydrogen peroxide in acidified aqueous solution was shown by Fenton, in the late 1800s (1), to generate a powerful oxidizing intermediate. The chemistry of the “Fenton reagent” has since been a model upon which metal-mediated oxidation by peroxide in biological media has been based (2). For years, the nature of the specific reactive intermediate(s) involved has been debated. For example, in the 1930s, Haber and Weiss (3) argued that the hydroxyl radical (·OH) is the principal oxidizing species, while Bray and Gorin (4) proposed that a metallo-oxo species, such as solvated ferryl ion, was the predominant oxidant. Later, Merz and Waters (5,6) suggested that the involvement of ·OH was supported by the stoichiometry of the reactions taking place in the presence of Fenton reagent. In 1952, Cahill and Taube concluded, on the basis of experiments utilizing O¹⁸-labeled peroxide, that the oxidant present under acidic conditions was most likely a metallo-oxo species (7). The hydroxyl radical has taken the more predominant role, dating from a 1975 review by Walling (8) which surmised that the lack of effect of differing ionic strength on the rate of reaction of the oxidizing intermediate with Fe²⁺ and methanol at different perchlorate concentrations was evidence that ·OH constituted the predominant oxidizing species generated under acidic conditions (Eq. 1).

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \xrightarrow{\text{Fe}^{3+}} \cdot\text{OH} + \cdot\text{OH}^-
\]

Since the early 1980s, the involvement of oxygen radicals has been proposed in many degenerative conditions, including cancer, senility, autoimmune disorders, etc. (9–12). The cytotoxic and cytostatic actions of cells of the immune system were also thought to result from similar chemical events (13,14). As a result of the increasingly recognized physiological importance of this chemistry, discussion of mechanistic aspects of the Fenton reaction has intensified. Iron complexes with chelating ligands such as EDTA have been examined under physiologically relevant conditions. Electron spin resonance (ESR) spin-trap studies revealed the existence of the 5,5-dimethyl-1-pyrroline N-oxide (DMPO)-·OH adduct, and this has been used as evidence of the generation (and, presumably, the intermediacy) of ·OH in oxidation reactions occurring in the Fenton reagent (15). Competitive kinetic studies have been performed to compare the reactivity of the oxidizing intermediates generated in the Fenton reaction with authentic ·OH generated by radiolysis of water or photolysis of H₂O₂. Rahhal and Richter (16) examined Fe⁴⁺(EDTA) oxidation, and suggested that an oxidant other than ·OH was generated in this system. Koppenol and Rush (17), after studying a number of chelated iron complexes using stopped-flow spectrophotometry, concluded that a metallo-oxo species was generated in neutral solutions, while ·OH was predominant in acidic solutions of nonchelated iron. Sutton et al. (18) arrived at the opposite conclusion, that unchelated iron generated a metallo-oxo species as the primary oxidant, while ·OH was predominant when chelated iron was present. Several review articles and research papers have suggested
oxidation of substrate in the ferrous ion-peroxide reaction under acidic conditions.

Investigation of NDMA Denitrosation

Product analyses of the denitrosation of NDMA by the Fenton reagent have shown the following reaction stoichiometry (20,21).

\[
\text{Fe}^{2+} + \text{HD}_{2}O + \text{NDMA} \rightarrow \text{Fe}^{3+} + \text{CH}_{3} \text{NH}_{2} + \text{NO}0 + 2\text{H}_{2}O
\]

Kinetics studies using stopped-flow spectrophotometric techniques have demonstrated that in the reaction with NDMA an intermediate (transient A) is generated with a \(k_{A} = 400 \text{ M}^{-1} \text{sec}^{-1}\), a rate five times faster than that for formation of ferric ion \(k_{f} = 76 \text{ M}^{-1} \text{sec}^{-1}\) (Figure 1). That the appearance of an oxidizing intermediate also occurred at a rate faster than formation of Fe\(^{3+}\) was shown using methylene blue (MB) oxidation as a probe (Figure 2). The disappearance of the 600 nm absorption, characteristic of MB, was first-order in peroxide, such that \(k_{\text{peroxide}} = 556 \text{ M}^{-1} \text{sec}^{-1}\) (Figure 2, inset). This value is similar to that reported (20) for the appearance of transient A (\(k_{A} = 400 \text{ M}^{-1} \text{sec}^{-1}\)). As previously discussed, the formation of -OH from ferrous ion also must involve concomitant formation of ferric ion (Equation 1); thus transient A does not result from the oxidation of NDMA by -OH (20).

To investigate further the possible role of -OH in the formation of A, the reaction of this radical with NDMA was examined using authentic -OH, generated by pulse radiolysis (20). A reaction between -OH and NDMA did occur, but did not lead to formation of A. Moreover, the second-order rate constant for attack of -OH on the nitrosamine was \(3.3 \pm 0.4 \times 10^{8} \text{ M}^{-1} \text{sec}^{-1}\), considerably different than required for the behavior shown in the stopped-flow kinetics study. The NDMA concentration at which the extent of transient formation in the Fenton reaction was half-maximal (i.e., half that observed at infinite [NDMA]) was calculated from the negative X-intercept \((-I_x)\) of Figure 1.4 (inset) to be \(92 \pm 10 \text{ mM}\). At this concentration, half of the active oxidant is being consumed by reaction with NDMA and the remaining half by reaction with other components of the medium, i.e.,

\[
\text{Fe}^{2+} + \text{H}_{2}O_{2} \rightarrow \text{Fe}^{3+} + \text{H}_{2}O + \text{OH}^-
\]

\[
\text{Fe}^{2+} + \text{H}_{2}O_{2} \rightarrow \text{Fe}^{3+} + \text{H}_{2}O + \text{OH}^-
\]

with \(k_{\text{nitrosamine}}\), \(k_{\text{ferrous}}\), \(k_{\text{peroxide}}\), and \(k_{\text{sulfate}}\) being the respective rate constants for reaction of the Fenton oxidant with NDMA, ferrous ion, hydrogen peroxide, and sulfate ion. The reported rate constants for the reactions of -OH with Fe\(^{3+}\), H\(_2\)O\(_2\), and SO\(_4^{2-}\) are \(4.3 \times 10^{7}, 2.7 \times 10^{7}\), and \(6.9 \times 10^{7} \text{ M}^{-1} \text{sec}^{-1}\) (22), respectively, indicating that NDMA could be no greater (under the conditions described in Figure 1) than \(2.7 \times 10^{7} \text{ M}^{-1} \text{sec}^{-1}\) if the primary Fenton oxidant was -OH. This value is at least an order of magnitude smaller than that observed in the pulse radiolysis of NDMA (3.3 \times 10^{8} \text{ M}^{-1} \text{sec}^{-1}\), (20), thus the oxidizing intermediate under the present Fenton reaction conditions is shown by both its spectrophotometric and kinetics behavior to be different from -OH. We have also used kinetics simulation techniques to evaluate a more complex model invoking formation of -OH, which included a feedback loop via Fe\(^{3+}\) reduction of the hydroperoxide radical; however, this simulation was unable to duplicate the experimentally observed kinetics (Appendix 1).

In this context, the simple model for the oxidation of NDMA indicated in Equations 3 to 7 was proposed to account for the appearance of transient A via the reaction of NDMA with an oxidizing intermediate X.

\[
\text{Fe}^{2+} + \text{H}_{2}O_{2} \rightarrow \text{Fe}^{3+} + \text{H}_{2}O + \text{OH}^-
\]

\[
\text{X} + \text{NDMA} \rightarrow \text{A}
\]

\[
\text{Fe}^{2+} + \text{H}_{2}O_{2} \rightarrow \text{Fe}^{3+} + \text{H}_{2}O + \text{OH}^-
\]

\[
\text{X} + \text{NDMA} \rightarrow \text{A}
\]
For NDMA concentrations ≥ 25 mM, this model could be numerically fit to the observed temporal absorbance changes obtained from stopped-flow spectrophotometry (Figure 3). In the absence of NDMA, the spectral changes show a single exponential increase in [Fe^{3+}]. The kinetics of the reaction are first-order in [Fe^{3+}] and in [H₂O₂], with k₅ = 76 M⁻¹ sec⁻¹. According to the model, k₄ = k₅/k₃ if it is assumed that k₄, k₅, and k₆ are all the same under these conditions. In the presence of NDMA, k₅ must be such that the formation of transient A is governed by k₄/k₅ = 0.087 M⁻¹, as dictated by the −Iₓ of the plot in Fig. 1A (inset) where k₅ = k₄(−Iₓ)⁻¹. Since k₄ is rate-limiting for the formation of A, and the transient was observed at 5 mM NDMA and 20 mM H₂O₂, then k₄ must be >> 2500 M⁻¹ sec⁻¹. In this context, the kinetics simulation shown in Figure 3 used the estimated rate constants k₄ = 560 sec⁻¹, k₅ = 5000 sec⁻¹, k₆ = 10⁻³ M⁻¹ sec⁻¹, k₇ = 870 sec⁻¹, and k₈ = 95 M⁻¹ sec⁻¹, to generate the observed plots (20).

This simplified model was successful for the qualitative simulation of the temporal absorbance behavior as illustrated by Figure 3, but leaves unanswered questions such as the nature of the putative intermediates X, Y, and transient A. Notably, the absorption spectrum of the transient displays maxima at 590 and 450 nm, features which are observed also in the spectrum of the iron(II) nitrosyl complex (H₂O₂)₂Fe(NO)²⁺ (Figure 1B). Thus, it appears likely that transient A is a closely related species. As we have shown previously, the formation of A displays a substantial deuterium isotope effect when the substrate is NDMA-d₆, thus C–H bond cleavage would appear to be directly involved in the rate-limiting process.

To ascertain the relationship between formation of transient A and of the denitrification products, a competitive kinetics study was performed in the presence of MeOH. When [NDMA] was held constant at 100 mM, the addition of varying amounts of MeOH resulted in quenching of the degradation of the nitrosamine to nitrate. A plot of 1/[NO₃⁻] versus [MeOH] was linear (Figure 4A), with −Iₓ(MeOH) = 20 mM (a value representing the [MeOH] necessary to quench half the denitrification products). Under identical conditions, the formation of A, as monitored by stopped-flow spectrophotometry, was also observed to decrease with increasing [MeOH]. A plot of 1/Abs versus [MeOH] was linear, with −Iₓ(MeOH) = 20 mM (Figure 4B), suggesting that for this concentration of NDMA (100 mM) most of the nitrate was formed via transient A. This would be consistent with the interpretation of X by NDMA being very efficient under these conditions unless a reactant such as methanol or another quencher (see below) is present.

In contrast, when [NDMA]<10 mM, plots of 1/[nitrate] versus 1/[NDMA] displayed marked deviation from linearity, indicating that more nitrate was formed than could be accounted for if transient A were the only precursor to this product. This suggested that another reactive intermediate capable of oxidizing NDMA, for example Y, might play an important role in the reaction stoichiometry of NDMA degradation under these conditions. Methanol might also be expected to quench the oxidation of NDMA by Y, especially if Y were the hydroxyl radical. Examination of the methanol quenching of nitrate formation for [NDMA] = 1 mM gave a linear 1/[nitrate] versus [MeOH] plot over the [MeOH] range 0.2 to 10 mM. Under these conditions, the [MeOH] necessary to quench half the nitrate formation (i.e., −Iₓ(MeOH)) was shown to be 17 mM. However, since the bulk of nitrate formation would have occurred via reaction of NDMA with Y, this implies that the reaction of MeOH with Y is at least a factor of 17 less extensive than the reaction of NDMA with Y. However, the rate constant for the reaction of MeOH with ·OH has been determined to be 9.7×10⁹ M⁻¹ sec⁻¹, a factor of 3 larger than that for the reaction of NDMA with ·OH (see above). Thus, one can conclude that Y is not the hydroxyl radical.

The above observations, with NDMA as substrate, clearly show the presence of two oxidizing intermediates under the Fenton reagent conditions, neither of which shows the competition reactivity characterized previously for the hydroxyl radical in aqueous solution. The specific natures of X and Y are as yet undetermined. However, the fact that X is formed (apparently reversibly) in a 1/1 stoichiometry from Fe²⁺ and H₂O₂ leads one to speculate that this is simply a ferrous hydrogen peroxide complex, i.e., Fe(H₂O₂)₂(H₂O). Furthermore, since the formation of Y from X is apparently unimolecular, a ferryl species, e.g., FeO²⁺, should certainly be considered for the former. The fact that the FeO²⁺ cation has recently been shown to be relatively unreactive toward alcohols (16) is at least consistent with the apparent reactivity of Y as shown by the competition studies.

Figure 3. Temporal absorbance changes, monitored at λ = 450 nm, in solutions initially containing 5 mM Fe³⁺, 20 mM H₂O₂, pH 2.5, plus 0 or 100 mM NDMA. Solid lines without symbols represent experimental data, while data points derived from computer simulation based upon Equations 3 to 7 are represented by (C) when NDMA was 0 mM and by (D) when NDMA was 100 mM.

X + H₂O₂ → Y  
A + H₂O₂ → NO₃⁻ + CH₃NH₂
+ CH₂O + Fe²⁺  
Y + Fe²⁺ → 2Fe³⁺

1/[
NO₃⁻]
(mM)-¹  

1/Abs₆₃₅

[MeOH] (mM)

[MeOH]

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Table 1. The concentrations of substrate required to cause 50% quenching of transient A.

| Substrate  | [NDMA] mM | $-I_4$(substr) mM | Ratio $^b$ | $\cdot\text{OH}^\bullet\text{OH}$ (×10^8 M$^{-1}$ sec$^{-1}$)$^c$ | Ratio $^d$ |
|------------|-----------|------------------|------------|---------------------------------|------------|
| NDMA       | -         | 20 ± 2           | 1          | 3                               | 1          |
| Methanol   | 100       | 20 ± 2           | 5          | 10                              | 3          |
| t-Butanol  | 200       | 28 ± 2           | 7          | 6                               | 2          |
| Benzyl alcohol | 200   | 0.8 ± 0.3        | 250        | 84                              | 24         |
| DMSO       | 200       | 2.8 ± 0.5        | 72         | 100                             | 29         |
| DMPO       | 200       | 20 ± 2           | 10         | 42                              | 12         |

$^a$The concentration of substrate required to quench half the maximal amount of transient A, derived as the negative X-intercept of the plot of 1/abs versus [substrate]. $^b$Ratio of $k_f/k_p$, as derived from Scheme 1. $^c$Known rate constants for the reactions of $\cdot\text{OH}$ with the various substrates. $^d$Ratio of the rate constant for the reaction of $\cdot\text{OH}$ with NDMA to the known rate constants for the reactions of $\cdot\text{OH}$ with the other substrates. For example, using the equation derived in Scheme 1, for which the rate constant for the reaction between $\cdot\text{OH}$ and benzyl alcohol is 8.9 × 10$^8$ M$^{-1}$ sec$^{-1}$, [NDMA] = 200 mM and $k_p$ = 3.3 × 10$^8$ M$^{-1}$ sec$^{-1}$, the substrate concentration required to cause 50% quenching of transient A, if hydroxyl is involved, may be predicted by using $k_f$/[NDMA] = [benzyl alcohol], or (3.3 × 10$^8$ M$^{-1}$ sec$^{-1}$)(200 mM)/(8.9 × 10$^8$ M$^{-1}$ sec$^{-1}$) = 24 mM.

Reactivity of X with Oxygen Radical Scavengers

Formation of the absorbing transient, A, solely from intermediate X provides a unique opportunity to examine the competitive kinetics of the reaction of X with additional substrates. Figure 5 illustrates this for a quenching experiment carried out with initial concentrations: 200 mM NDMA, 20 mM H$_2$O$_2$, and 0.5 mM Fe$^{2+}$. When various amounts of t-butanol were added, the amount of transient A observed decreased, with simultaneous generation of a new product displaying an absorption maximum at 500 nm (Figure 5). The absorbance due to this new product was dependent on the concentration of t-butanol. While the exact nature of that species remains uncharacterized, it most likely results from the formation of t-butanol radical and the subsequent reaction of this radical with the nitroso function of NDMA as previously reported (23), followed by decay to a stable product. In analogy to the quenching by methanol, the $-I_4$(ROH) value (the concentration of t-butanol at which 50% of A was quenched) was determined at 450 nm for a constant reaction time of 0.4 sec (to minimize interference with the subsequent absorbance changes).

Determination of the $-I_4$(substrate) values for different substrates allows the direct comparison of the rate constants for the reactions of NDMA and t-butanol and other substrates with X (Scheme 1). Table 1 summarizes the $-I_4$(substrate) values for benzyl alcohol, DMPO, and dimethylsulfoxide (DMSO) and compares the relative reactivities to those previously reported for $\cdot\text{OH}$. The relative reactivity of X toward benzyl alcohol is ten times greater than that predicted for $\cdot\text{OH}$. Similarly, the selectivity for DMSO is five times different than the predicted selectivity of $\cdot\text{OH}$ for DMSO. Little difference is apparent in the case of the substrates t-butanol, methanol, and DMPO.

Many of the previous investigations of Fenton chemistry have based the conclusion as to the presence of $\cdot\text{OH}$ or a metallo-oxo species solely on the competitive kinetics studies involving one or two quenching agents. Based solely on relative reactivities toward the substrates described above, it is quite possible that $\cdot\text{OH}$ could have been mistaken for X. Another interesting aspect is the high affinity of DMPO for X. Under conditions normally used in spin-trapping experiments (19), nearly 90% of the DMPO would react with the intermediate, suggesting that DMPO-$\cdot\text{OH}$ adduct could be formed from this intermediate.

Conclusions

The denitrosation of NDMA, studied with a combination of stopped-flow spectrophotometry and product analysis, has provided a unique opportunity to elucidate the reactivity of novel oxidizing intermediates generated in the Fenton reaction. It now appears that $\cdot\text{OH}$ plays little or no role in the oxidation of substrates under acidic conditions. There are instead two reactive intermediates, X and Y; and we speculate that X is [Fe(Fe$_2$O$_2$)$]^{2+}$ and Y is [FeO]$^{2-}$. For certain substrates, intermediate X displays a reactivity pattern sufficiently similar to that of $\cdot\text{OH}$ that this species may have been mistakenly identified in previous studies as $\cdot\text{OH}$. However, the use of the reaction with NDMA allows relative reactivity comparisons with a broad range of substrates and such comparisons are clearly incompatible with the behavior predicted for intermediacy of $\cdot\text{OH}$.

Appendix

The arguments presented, based on the reaction rate constants for $\cdot\text{OH}$, may not
provide a comprehensive analysis of this potentially complicated reaction. For instance, the formation of superoxide might result in a back reaction with ferric ion to form ferrous ion as shown by Bielski et al. (24). It could be argued that some back reaction could be involved to give the rapid formation of transient A. To provide a comprehensive determination of the involvement of •OH in the formation of transient A, the increase in absorbance at 625 nm characteristic of the transient was simulated by numerical integration of the elementary reaction pathways described in Scheme 2, using a 4th-order Runge-Kutta method (25). As indicated in these equations, the reaction of Fe(III) with peroxide yields •OH and ferric ion. The •OH then could react with NDMA to form the alkyl radical, which then may undergo denitrosation to form the Schiff base (CH₃=NCH₃) and NO (21). NO then could react with Fe(II) to produce transient A (postulated to be a Fe(II)− NO complex, Figure 1B). Transient A then could further react with peroxide to produce NOₓ. Hydroxyl radical could also react with ferrous ion to generate ferric ion, or react with peroxide to yield the perhydroxyl radical (HO₂). HO₂ then could react with ferric ion to form ferrous ion, or react with ferrous ion to yield ferric ion and peroxide. The rate constants for these steps have been reported previously in the literature. A simulation based on this model, where \( k = 76 \text{ M}^{-1}\text{sec}^{-1} \), did predict some accumulation of absorbance attributed to transient A (not shown). However, a plot of (Abs)−1 versus [NDMA]−1 (simulation) yielded (−1)−1 of 5.9 mM, nearly 16 times lower than that experimentally observed (92 mM). The amount of the ultimate nitrogenuous product of NDMA denitrosation (nitrate) formed in this reaction was [NDMA] dependent. The predicted negative (X-intercept)−1 from the model involving •OH as oxidant was 3.4 mM, while the actual observed value was 20 mM. The simulation results confirm that the relative selectivity of •OH is similar to that indicated by the simpler model described above.

### REFERENCES

1. Fenton HJH. Oxidation of tartaric acid in the presence of iron. J Chem Soc 65:899−910 (1894).
2. Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-induced aging and cancer: a hypothesis. Proc Natl Acad Sci USA 78:6858−6862 (1981).
3. Haber F, Weiss JF. The catalytic decomposition of hydrogen peroxide by iron salts. Proc Roy Soc London Ser A 147:332−351 (1934).
4. Bray WC, Gorin MH. Ferryl ion, a compound of tetravalent iron. J Am Chem Soc 54:2124−2125 (1932).
5. Mist JH, Waters WA. The mechanism of oxidation of alcohols with Fenton’s reagent. Faraday Soc Discuss 2:179−187 (1947).
6. Mist JH, Waters WA. The oxidation of aromatic compounds by means of the free hydroxyl radical. J Chem Soc S15:2427−2433 (1949).
7. Cahill AE, Taube H. The use of heavy oxygen in the study of reactions of hydrogen peroxide. J Am Chem Soc 74:2312−2318 (1952).
8. Walling C. Fenton’s reagent revisited. Acc Chem Res 8:125−131 (1975).
9. Halliwell B, Gutteridge JMC. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch Biochem Biophys 246:501−514 (1986).
10. Minotti G, Aust SD. The requirement for iron(III) in the initiation of lipid peroxidation by iron(II) and hydrogen peroxide. J Bio Chem 262:1098−1104 (1987).
11. Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 219:1−14 (1984).
12. Halliwell B, Gutteridge JMC. Biologically relevant metal ion-dependent hydroxyl radical generation. An update. FEBS Lett 307:08−112 (1992).
13. Imla JA, Chin SM, Linn S. Toxic DNA damage by hydrogen peroxide through the Fenton reaction in vivo and in vitro. Science 240:640−642 (1988).
14. Jonas SJ, Riley PA, Willson RL. Hydrogen peroxide cytotoxicity.

Biochem J 264:651−655 (1989).
15. Lai CS, Piette LH. Further evidence for OH radical production in Fenton’s reagent. Tetrahedron Lett 9:775−778 (1979).
16. Rahhal S, Richter HW. Reduction of hydrogen peroxide by the ferrous iron chelate of diethylenetriamine − N,N,N′,N″,N‴-pentaacetate. J Am Chem Soc 110:3126−3133 (1988).
17. Rush JD, Kopenol WH. Reactions of Fe²⁺/no and FeIV/edda with hydrogen peroxide. J Am Chem Soc 110:4957−4963 (1988).
18. Sutton HC, Vile GF, Winterbourn CC. Radical driven Fenton reactions—evidence from paraphet radical studies for production of tetravalent iron in the presence and absence of ethylenediaminetetraacetic acid. Arch Biochem Biophys 256:462−471 (1987).
19. Yamazaki I, Piette LH. ESR spin-trapping study on the oxidizing species formed in the reaction of the ferrous iron with hydrogen peroxide. J Am Chem Soc 113:7588−7593 (1991).
20. Wink DA, Nims RW, Desrosiers MF, Ford PC, Keefe LK. A kinetic investigation of intermediates formed during the Fenton reagent mediated degradation of N-nitrosodimethylamine: evidence for an oxidative pathway not involving hydroxyl radical. Chem Res Toxicol 4:510−512 (1991).
21. Heur Y-H, Streeter AJ, Nims RW, Keefe LK. The Fenton degradation as a nonenzymatic model for microsomal denitrosation of N-nitrosodimethylamine. Chem Res Toxicol 2:247−253 (1989).
22. Buxton GV, Greenstock CL, Phillip W, Ross AB. Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals (•OH/OH⁻) in aqueous solution. J Phys Chem Ref Data 17:513−886 (1988).
23. Wink DA, Desrosiers MF. Unusual spin-trap chemistry for the reaction of hydroxyl radical with carcinogen N-nitrosodimethylamine. Radiat Phys Chem 38:467−472 (1991).
24. Bielski BHJ, Cabelli DE, Arudi RL, Ross AB. Reactivity of HO₂/O₂ radicals in aqueous solution. J Phys Chem Ref Data 14:1041−1100 (1985).
25. Braun M. Differential Equations and their Applications. New York: Springer-Verlag; 159−163 (1975).