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A Kinetic and ESR Investigation of Iron(II) Oxalate Oxidation by Hydrogen Peroxide and Dioxygen as a Source of Hydroxyl Radicals

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The reaction of Fe^{II} oxalate with hydrogen peroxide and dioxygen was studied for oxalate concentrations up to 20 mM and pH 2–5, under which conditions mono- and bis-oxalate complexes ($Fe^{II}(ox)$ and $Fe^{II}(ox)_2^{2-}$) and uncomplexed Fe^{2+} must be considered. The reaction of Fe^{II} oxalate with hydrogen peroxide ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$) was monitored in continuous flow by ESR with t-butanol as a radical trap. The reaction is much faster than for uncomplexed Fe^{2+} and a rate constant, $k = 1 \times 10^4\ M^{-1}\ s^{-1}$ is deduced for $Fe^{II}(ox)$. The reaction of Fe^{II} oxalate with dioxygen is strongly pH dependent in a manner which indicates that the reactive species is $Fe^{II}(ox)_2^{2-}$, for which an apparent second order rate constant, $k = 3.6\ M^{-1}\ s^{-1}$, is deduced. Taken together, these results provide a mechanism for hydroxyl radical production in aqueous systems containing Fe^{II} complexed by oxalate. Further ESR studies with DMPO as spin trap reveal that reaction of Fe^{II} oxalate with hydrogen peroxide can also lead to formation of the carboxylate radical anion ($CO_2^{\bullet-}$), an assignment confirmed by photolysis of Fe^{III} oxalate in the presence of DMPO.

Keywords: DMPO, Fenton reaction, hydroxyl radical, iron, oxalate

Abbreviations: DMPO, 5,5-dimethyl-1-pyrroline N-oxide; $Fe^{2+}{}_{aq}$, uncomplexed Fe^{2+}; ox, oxalate

INTRODUCTION

The high reactivity of the hydroxyl radical ($^{\bullet}OH$) with all forms of organic matter makes reactions that lead to its formation important in biological and environmental systems, with electron transfer from Fe^{II} to H_2O_2 in the Fenton reaction as the prime example:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \qquad (1)$$

The rate constant for this reaction (k_1) is governed by the Fe^{II} coordination. The only simple generalization is that a ligand replacement step is necessary, leading to very low rates for complexes such as $Fe^{II}(CN)_6^{4-}$ and Fe^{II}-cytochrome c.[1] The measured rate constants range from 75 $M^{-1}\ s^{-1}$ for

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uncomplexed Fe^{2+} (denoted Fe^{2+}_{aq}) to 2×10^5 M^{-1} s^{-1} for the Fe^{II} complex of aminotris(methylenephosphonic acid).[2] The ligands that have been studied include EDTA, DTPA, citrate, ATP and pyrophosphate.[2–5]

One Fe^{II} complexing agent that has received very little attention is oxalate. Oxalate occurs in human and animal urine, while the calcium salt is a common constituent of kidney stones.[6] It is widely distributed in plants and can be an end-product of microbial metabolism. The present research stemmed from a specific interest in a class of wood rotting fungi known as brown rots, for which the dry rot fungus (*Serpula lacrymans*) is an example. These fungi use $^{\bullet}OH$ to weaken the cellulose structure in the early stages of decay,[7] and several mechanisms have been proposed for formation of the critical Fe^{II}/H_2O_2 combination that allows reaction (1) to take place.[8] The secretion of oxalic acid is also a characteristic of these fungi.[7] Thus there is a need for kinetic data on Fe^{II} oxalate reactions as a source of $^{\bullet}OH$.

It should be noted that reaction (1) can take place in the absence of any external source of H_2O_2, since reaction of Fe^{II} with dioxygen (autoxidation) leads to formation of H_2O_2.[9]

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet-} \quad (2)$$

$$Fe^{2+} + O_2^{\bullet-} + 2H^+ \rightarrow Fe^{3+} + H_2O_2 \quad (3)$$

Thus partial oxidation of Fe^{II} will lead to Fe^{II} and H_2O_2 being present together, and hence to $^{\bullet}OH$ production at a rate determined by k_1. The production of $^{\bullet}OH$ during Fe^{II} oxalate autoxidation was demonstrated in earlier experiments by the standard test of salicylate conversion to 2,3- and 2,5-dihydroxybenzoate.[10]

Our choice of concentrations and pH was determined by the interest in fungal degradation of wood described above. For brown rot fungi, the highest reported concentrations of oxalate are 20–25 mM.[11,12] The release of oxalic acid leads to a fall in pH, from 4.0–6.0 in uninfected wood to 2.0–2.5.[13,14] Accordingly, our experiments have used oxalate concentrations of no more than 20 mM and pH's between 2.0 and 5.0. The oxalate concentration has always been in excess of that for iron. The technique of ESR spectroscopy enabled radicals to be identified and was used in continuous flow experiments for studying the kinetics of reaction (1), while the reaction of Fe^{II} with dioxygen was monitored with an oxygen electrode.

The speciation in Fe^{II} oxalate systems is more complicated than for the polydentate ligands of most previous studies (e.g. EDTA) because mono-, bis- and tris-oxalate complexes can all be present. It follows that an understanding of speciation is an essential preliminary to kinetic experiments. The speciation can be computed from stability constants, as long as concentrations and pH are sufficiently defined.

MATERIALS AND METHODS

Materials

Unless otherwise stated, chemicals were from Sigma or Aldrich and were of analytical quality. The Fe^{II} source was $Fe^{II}SO_4 \cdot 7H_2O$, dissolved in deionized water (at 100 mM) shortly before use and maintained at 0°C.[15] Solutions of oxalate at the required pH were prepared from stock solutions of oxalic acid and K_2-oxalate, the pH being monitored with a glass electrode. Hydrogen peroxide solutions were prepared by dilution of 27.5% (100 vol) reagent and assayed on the day of use by $\varepsilon_{240nm} = 43.6$ M^{-1} cm^{-1}.[16] The spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO) was dissolved in water and purified by stirring with activated charcoal in subdued light.[17] After removal of the charcoal by brief centrifugation, the solution was divided into aliquots and stored at −15°C. DMPO was assayed by $\varepsilon_{226nm} = 7.22$ mM^{-1} cm^{-1}.[18] The absence of a significant ESR signal and presence of an absorption peak at 226 nm were confirmed on the day of use.

Speciation Calculations

Chemical speciation calculations were conducted with the MINTEQA2 program[19] which includes correction for ionic strength using the Davies equation.[20]

$$\log \gamma_i = 0.509\, z_i^2 \{(I^{1/2}/(1+I^{1/2})) - 0.24I\}$$

where c_i is the concentration and γ_i the activity coefficient of an ion with charge z_i, and I (= 1/2 $\Sigma(c_i z_i^2)$) is ionic strength. Table I shows the relevant stability constants as published and after correction to I = 0 for insertion in MINTEQA2. The MINTEQA2 database is designed for environmental studies and includes stability constants for species arising from Fe^{2+} and Fe^{3+} hydrolysis ($Fe^{II}(OH)_n^{(2-n)+}$ and $Fe^{III}(OH)_n^{(3-n)+}$). In the present study these species always comprised less than 1% of total iron and hence will not be further discussed. The database also includes sulphate as complexing anion (e.g. $Fe^{II}(SO_4)_{(aq)}$ and $Fe^{II}(SO_4)_2^{2-}$).

ESR Spin-trapping and Continuous Flow Investigations

ESR spectra were recorded on a Bruker ESP-300E spectrometer equipped with an X-band klystron and 100 kHz modulation. Typical conditions were: centre field, 3483 G; sweep width, 100 G; frequency, 9.78 GHz; power, 20 mW. The standard scan had a time constant of 164 ms and was acquired over 335 s, but for fast scans these times were reduced to 41 ms and 84 s respectively. The sample (200 µl) was contained in a long pasteur pipette (outside diameter 4 mm) sealed at the end, which was filled from reagents mixed in a test-tube. The National Institute of Environmental Health Sciences spin-trap database was used for adduct assignment. For continuous-flow ESR experiments a three-way mixer was employed as described by Croft *et al.*[2] The three streams contained Fe^{II} in oxalate buffer, H_2O, and Bu^tOH. The solutions to be mixed were made up in deionized water and deoxygenated before and during use by purging with O_2-free nitrogen. The overall flow rate, typically 40 $cm^3\ s^{-1}$, was maintained with a Watson Marlow 502 peristaltic pump, positioned on the inlet tubing. The delay time between mixing and passage through the ESR cavity was 30 ms.

Monitoring of Dioxygen

Changes in dioxygen concentration were monitored with a Clark-type oxygen electrode. The solubility of dioxygen in air-saturated water at 25°C was taken as 254 µM.[25]

TABLE I Equilibrium constants for speciation computations

Species	Equilibrium constant (K) in standard notation[a]	log K as measured[b]	ionic strength (I) (mol l^{-1})	log K corrected to $I \rightarrow 0$[c]	Reference
Hox^-	K_1		0	4.266	[21]
H_2ox	β_2		0	5.518	[21]
$Fe^{II}(ox)^0$	K_1	3.05	1.0	4.21	[21,22]
$Fe^{II}(ox)_2^{2-}$	β_2	5.01	1.0	6.07	[22]
$Fe^{II}(ox)_3^{4-}$	β_3	5.22	0.5	5.22	[23]
$Fe^{III}(ox)^+$	K_1	7.53	0.5	9.33	[24]
$Fe^{III}(ox)_2^-$	β_2	13.64	0.5	16.0	[24]
$Fe^{III}(ox)_3^{3-}$	β_3	18.49	0.5	20.3	[24]

[a] The table uses the standard notation for equilibrium constants: $K_1 = ML/(M.L)$, $\beta_2 = ML_2/(M.L^2)$ and $\beta_3 = ML_3/(M.L^3)$, where M is the cation and L is the ligand (in this case, $oxalate^{2-}$).
[b] All measurements were made at 25 °C.
[c] Activity coefficients (γ_i) for I = 0.5 or 1.0 mol l^{-1} as appropriate were calculated from the Davies equation (see text).

Data Simulation

For non-linear regression analysis, GraFit (version 3.0; Erithacus Software) was employed.

RESULTS AND DISCUSSION

Fe^{II} and Fe^{III} Oxalate Speciation

Figure 1(a) shows the calculated speciation of Fe^{II} as a function of pH, for 20 mM total oxalate and an ionic strength of 60 mM (correct for 20 mM K_2-oxalate). The sharp pH dependence is due to the complexing species being the oxalate dianion, which becomes protonated with pK = 4.0 (at 60 mM ionic strength). The speciation is also dependent on the oxalate concentration and (to a lesser extent) on the ionic strength. It will be noted that although conditions can be selected to make either the mono- or bis-oxalate complex predominant, there will always be significant amounts of other species present. The tris-oxalate complex is only predominant at much higher oxalate concentrations than those of the present study. Figure 1(b) shows a similar plot for Fe^{III}; the much higher binding constants lead to almost complete conversion to the tris-oxalate complex under the conditions of this study, regardless of pH.

Kinetic Measurements for Reaction of Fe^{II} Oxalate with H_2O_2

The production of hydroxyl radicals on mixing of Fe^{II} oxalate with H_2O_2 was confirmed by ESR monitoring of the $^{\bullet}CH_2CMe_2OH$ radical from Bu^tOH. The signal from this radical was used in kinetic measurements by continuous flow, as in previous studies of Fe^{II} and Ti^{III} complexes.[2,26] The rate constant of reaction of $^{\bullet}OH$ with Bu^tOH ($k = 5.8 \times 10^8\ M^{-1}\ s^{-1}$)[2] is much higher than values for oxalate ($k = 5.3 \times 10^6\ M^{-1}\ s^{-1}$ for ox^{2-}; $k = 3.2 \times 10^7\ M^{-1}\ s^{-1}$ for Hox^-)[27] or H_2O_2 ($k = 3 \times 10^7\ M^{-1}\ s^{-1}$)[2], while the reaction of $^{\bullet}OH$ with Fe(II) cannot exceed a diffusion controlled limit of $k = 4 \times 10^9\ M^{-1}\ s^{-1}$.[28] The efficient scavenging of $^{\bullet}OH$ by Bu^tOH could therefore be ensured by working with $[Bu^tOH] > [oxalate]$, $[Bu^tOH] > [H_2O_2]$, and a 30:1 ratio for $[Bu^tOH] : [Fe^{II}]$. The concentration of $^{\bullet}CH_2CMe_2OH$ ($R^{\bullet}$) was monitored at a delay time (t) of 30 ms after mixing. It has been shown that the decay of $^{\bullet}CH_2CMe_2OH$ in such systems is by bimolecular termination, leading to a steady-state concentration derived by analysis of the following reactions:[2]

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

$$^{\bullet}OH + Bu^tOH \rightarrow H_2O + {}^{\bullet}CH_2CMe_2OH$$

$$2\,{}^{\bullet}CH_2CMeOH \rightarrow \text{non-radical products}$$

If $[R^{\bullet}]$ is monitored as the hydrogen peroxide concentration is increased, the concentration initially rises (because radicals are formed more rapidly) but then declines (because the reaction is largely over before entry into the ESR cavity). For $[H_2O_2]_o >> [Fe^{2+}]_o$, the dependence of $[R^{\bullet}]$ on $[H_2O_2]_o$ is given by;[2,29]

$$[R^{\bullet}] = \text{constant}\,([H_2O_2]_0)^{1/2} \exp\{-k_1[H_2O_2]_0 t)/2\} \quad (4)$$

This implies,

$$[H_2O_2]_0 \text{ for } [R^{\bullet}]_{max} = (k_1 t)^{-1} \quad (5)$$

It should be noted that this model has not included the reaction of $R^{\bullet}$ with Fe^{III} or Fe^{II}. The $^{\bullet}CH_2CMe_2OH$ radical has a very high rate of bimolecular removal ($2k_1 = 10^9\ M^{-1}\ s^{-}$)[2], while studies with a wide range of Fe complexes have shown no evidence for significant oxidation by Fe^{III}.[2,30] For reaction of $^{\bullet}CH_2CMe_2OH$ with Fe^{II} complexes of EDTA and aminophosphonic acids, a rate constant of $k \approx 10^6\ M^{-1}\ s^{-1}$ has been estimated.[2] Simulations show that this rate of reaction has a scarcely detectable effect on plots of $[R^{\bullet}]$ versus $[H_2O_2]_o$, while if the rate is 100-fold higher, $[H_2O_2]_o$ for $[R^{\bullet}]_{max}$ will be almost unchanged (and

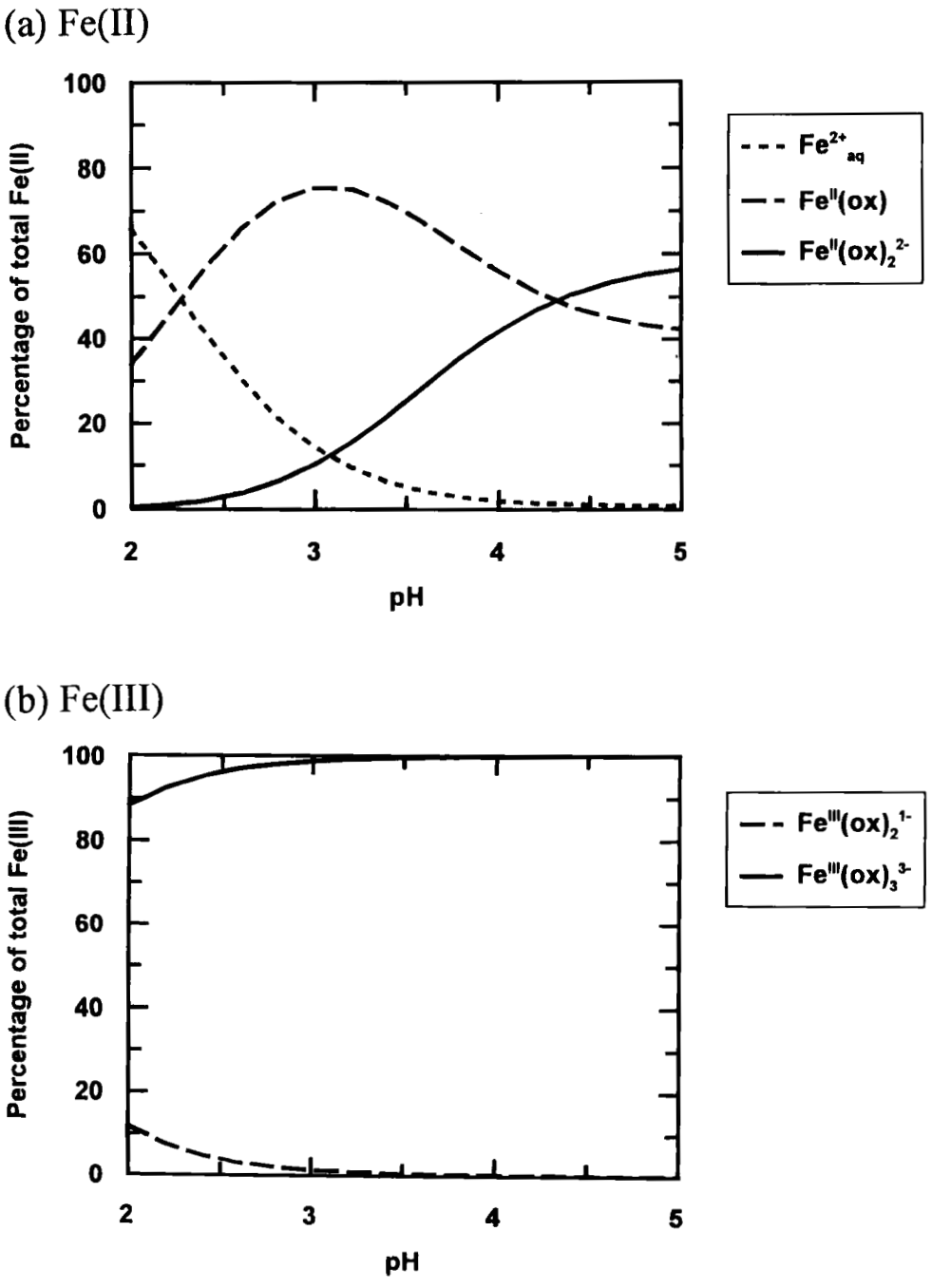

FIGURE 1 Fe^{II} oxalate and Fe^{III} oxalate speciation as a function of pH. The speciation was calculated for 20 mM total oxalate, I = 60 mM, 25°C using MINTEQA2. Total Fe^{II} or Fe^{III} was set at 10^{-6} M, giving results that are valid for any iron concentration provided uncomplexed oxalate is present at a much higher concentration than that of iron. (**a**) Speciation plot for Fe^{II}. The trisoxalate complex ($Fe^{II}(ox)_3^{4-}$) was always less than 1 % of the total and is therefore not shown. (**b**) Speciation plot for Fe^{III}. The following species were always less than 1 % of the total and are therefore not shown: Fe^{3+}_{aq}, $Fe^{III}(ox)^{+}$.

hence equation (5) is still valid) but $[R^{\bullet}]_{max}$ will be strongly decreased.[2] In practice, the ESR signals observed with Fe^{II}-oxalate were similar in size to those with Fe^{II}-EDTA (unpublished results), implying that the simple kinetic analysis described above is appropriate.

Figure 2 shows results from two experiments in which $[R^{\bullet}]$ was monitored as a function of $[H_2O_2]_o$. In each case an estimate for k_1 was obtained by fitting the data to equation (4), using (5) for an initial estimate of k_1. The interpretation of such experiments requires consideration of the rate of equilibration of Fe^{2+}_{aq} and its oxalate complexes with oxalate, since concentration changes in oxalate (at mixing) and Fe^{II} complexes (by reaction) are involved. As a generalization, the rate of equilibration of complex ions is closely related to the rate constant for substitution of inner sphere water on the aqueous ion.[31,32] This rate constant is fast for Fe^{2+}_{aq}; $k = 3 \times 10^6\ s^{-1}$, as compared with $k = 3 \times 10^3\ s^{-1}$ for Fe^{3+}_{aq}.[32] Accordingly, the equilibration of Fe^{II} with oxalate is likely to be fast in relation to the delay of 30 ms between mixing and observation. The following analysis is based on this premise.

The Fe^{II} speciation for the experiment in Figure 2(a) is shown in Table II. The only species that comprise more than 2% of total Fe^{II} are Fe^{2+}_{aq} and the mono-oxalate, $Fe^{II}(ox)$. The rate constant for Fe^{2+}_{aq} ($k_1 = 75\ M^{-1}\ s^{-1}$)[2] is far lower than the observed rate ($k_1 = 6.6 \times 10^3\ M^{-1}\ s^{-1}$). This implies that radical production is almost exclusively attributable to $Fe^{II}(ox)$, which accounts for 62.5% of the total Fe^{II}. Assuming that equilibration is rapid (see above), the results imply a rate constant close to $k_1 = 1 \times 10^4\ M^{-1}\ s^{-1}$ for $Fe^{II}(ox)$. It is notable that this is higher than the rates for DTPA, citrate and EDTA (1.4×10^3, 5×10^3 and $7 \times 10^3\ M^{-1}\ s^{-1}$ respectively,[3,4] although somewhat higher rates have been observed for pyrophosphate[5] ($1 \times 10^5\ M^{-1}\ s^{-1}$) and ethylenediaminetetrakis(methylenephosphonic acid) ($2 \times 10^5\ M^{-1}\ s^{-1}$)[2].

The higher pH of Fig 2(b) leads to a slightly faster reaction than that in Figure 2(a) (see Table II). In this experiment the proportion of $Fe^{II}(ox)$ is the same as in Figure 2(a), but almost all remaining Fe^{II} is present as $Fe^{II}(ox)_2^{2-}$ (see Table II). The relative rates of reaction in Figure 2(a) and 2(b) (and other data, not shown) are consistent with similar k_1 values for $Fe^{II}(ox)$ and $Fe^{II}(ox)_2^{2-}$.

Kinetic Measurements for Fe^{II} Oxalate in Reaction with Dioxygen

The uptake of dioxygen was used for continuous monitoring of Fe^{II} oxidation in air-equilibrated oxalate at a preset pH. The addition of catalase after 10–30 min led to no detectable burst of dioxygen evolution, implying that there was no significant accumulation of H_2O_2 (data not shown). The simplest reaction scheme is for all four 1-electron stages of O_2 reduction to be coupled to Fe^{II} oxidation, implying a Fe^{II}: O_2 stoichiometry of 4.0:

$$O_2 + 4Fe^{2+} + 4H^+ \rightarrow 2H_2O + 4Fe^{3+}$$

Burkitt and Gilbert[33] have deduced Fe^{II} : O_2 stoichiometries between 3 and 4 from similar studies with a wide range of chelators, with values below 4.0 being attributed to reaction of $^{\bullet}OH$ with the chelator instead of with Fe^{II}. It follows from these stoichiometries that O_2 uptake does not give an exact rate for Fe^{II} oxidation, but is nevertheless suitable for an initial study. The rate of oxidation is strongly dependent on pH, as shown in Figure 3. The maximal O_2 uptake rate in Figure 3 is $3.8 \times 10^{-7}\ M\ s^{-1}$, obtained with $[Fe^{II}]$ and $[O_2]$ at 8×10^{-4} and $2.5 \times 10^{-4}\ M$ respectively. This corresponds to an apparent bimolecular rate constant, $k = 1.9\ M^{-1}\ s^{-1}$, for reaction of Fe^{II} with O_2.

During one-electron transfer between metal complexes and dioxygen, the metal complexation is likely to remain unchanged, e.g.

$$Fe^{II}(ox)_n^{(2-2n)+} + O_2 \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} Fe^{III}(ox)_n^{(3-2n)+} + O_2^{\bullet-} \quad (6)$$

For each value of n (from 0 to 3) an equilibrium constant (K_6) for (6) can be derived, as shown in

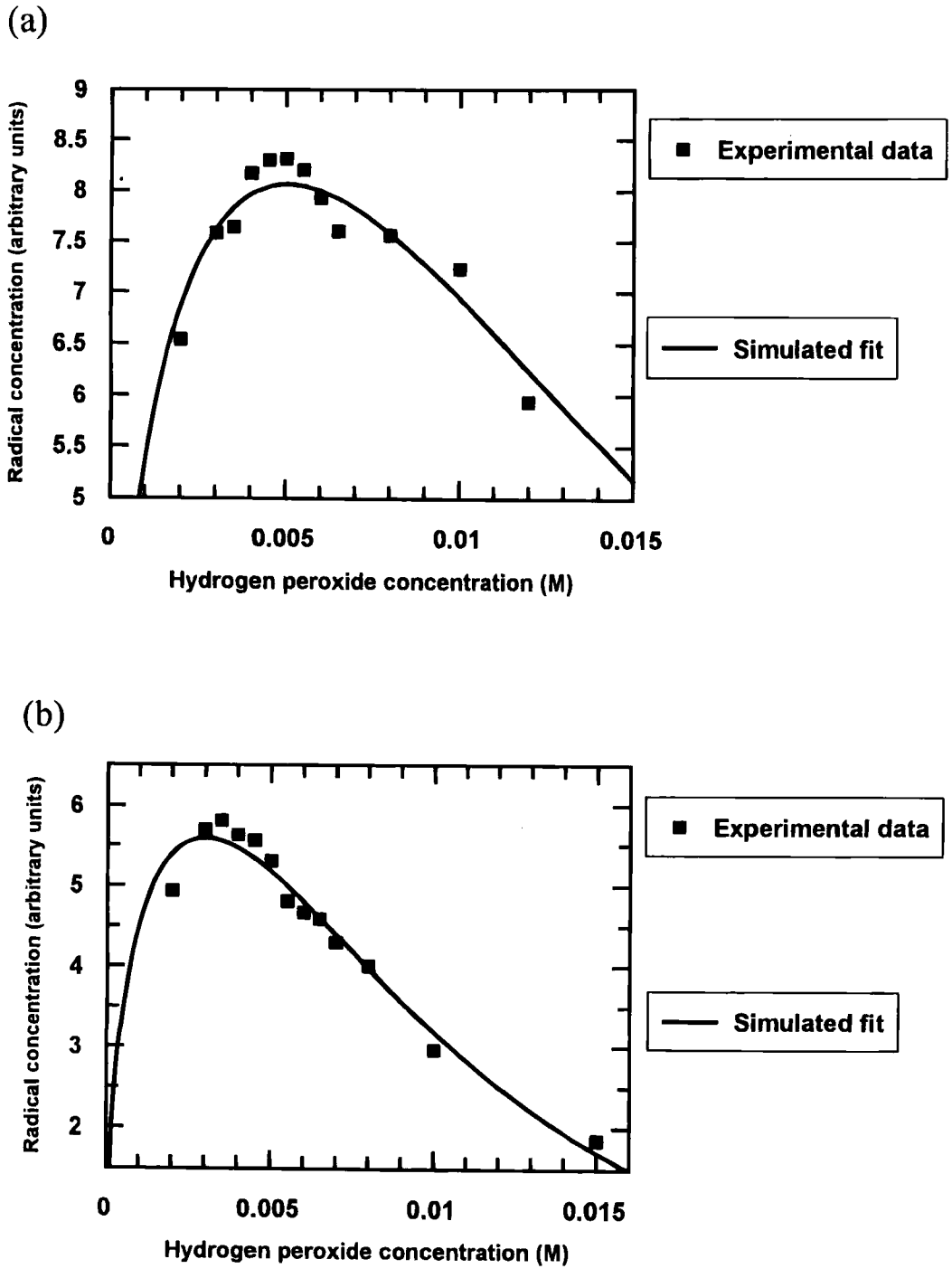

FIGURE 2 Kinetic study by continuous flow ESR for reaction of Fe^{II} oxalate with H_2O_2. Fe^{II} oxalate and H_2O_2 were mixed in a continuous flow system in the presence of t-butanol. The ESR signal at 30 ms after mixing was monitored as $[H_2O_2]$ was varied. All concentrations are given for immediately after mixing. The Fe^{II} concentration was 1 mM. (**a**) 10 mM K_2-oxalate and 10 mM oxalic acid, mixed to give pH 2.70 (measured before addition of Fe^{II}). (**b**) 10 mM K_2-oxalate plus 1 mM oxalic acid. In each case the curve is a simulated fit to equation (4). For the deduced k_1 values, see Table II.

TABLE II Fe^{II} speciation in kinetic studies for reaction of Fe^{II} oxalate with H_2O_2

Experimental data	Fig 2(a)	Fig 2(b)
k_1 as measured (M^{-1} s^{-1})[a]	$(6.6 \pm 0.3) \times 10^3$	$(1.11 \pm 0.04) \times 10^4$
pH before addition of Fe^{II}	2.70	4.71
pH after Fe^{II} equilibration	2.63	4.64
Fe^{II} speciation:[b]		
Fe^{2+}_{aq}	0.342	0.019
$Fe^{II}(ox)$	0.625	0.627
$Fe^{II}(ox)_2^{2-}$	0.013	0.352
$Fe^{II}(ox)_3^{4-}$	(1.3×10^{-6})	0.001
$Fe^{II}SO_{4(aq)}$	0.020	0.001

[a] The k_1 values were computed from experimental data in Figure 2 by non-linear regression (see text).
[b] The Fe^{II} speciation was computed for concentrations after mixing (see Fig. 2), the values given being the fraction in each state.

Table III. The back reaction, which is more thermodynamically favourable, cannot exceed the diffusion controlled limit of $K_{-6} = 4 \times 10^9$ M^{-1} s^{-1}.[28] This enables a limit to be set for the forward reaction, since $K_6 = k_6/k_{-6}$. The conclusion is that Fe^{2+}_{aq} and $Fe^{II}(ox)$ cannot account for the observed rate of O_2 uptake. The observed rate is, however, plausible for $Fe^{II}(ox)_2^{2-}$. Indeed, the O_2 uptake rate shows a strong correlation with the $Fe^{II}(ox)_2^{2-}$ concentration, when both are plotted as a function of pH (Fig. 3). The bimolecular rate constant deduced above ($k = 1.9$ M^{-1} s^{-1}) is for

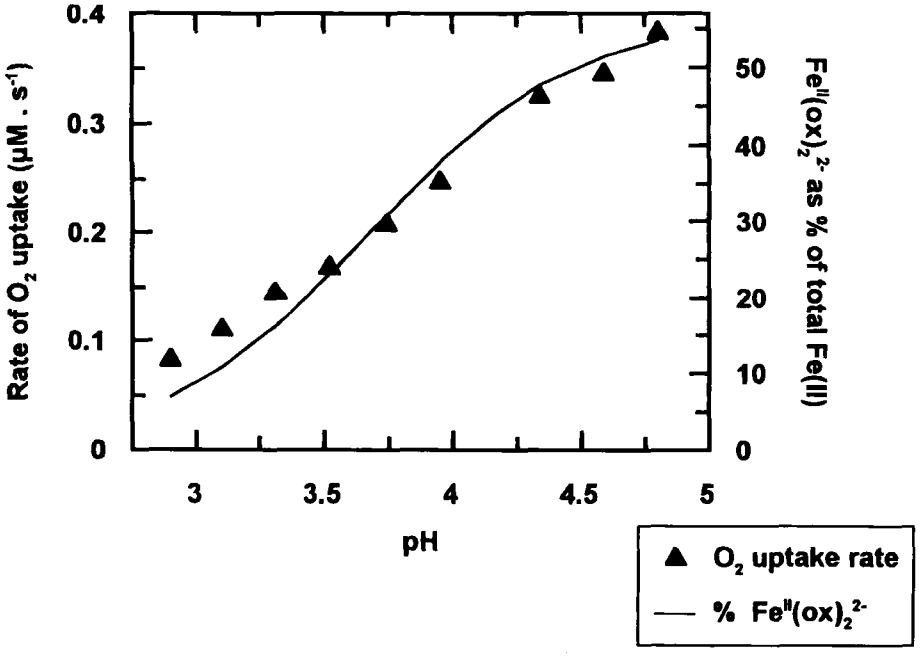

FIGURE 3 Kinetics of Fe(II)-oxalate oxidation by dioxygen as a function of pH. A set of solutions with pH's between 3.0 and 4.8 was prepared from 20 mM K_2-oxalate and 20 mM oxalic acid. For each solution, the rate of O_2 uptake was measured after addition of 0.8 mM Fe^{II}. The pH values were corrected for equilibration with Fe^{II} before plotting (the change being less than 0.1 pH unit). The Figure also shows the pH dependence of the $Fe^{II}(ox)_2^{2-}$ concentration, plotted as a percentage of total Fe^{II}.

TABLE III Reactivity of Fe^{II} oxalate complexes in 1-electron transfer to dioxygen

Fe^{III}/Fe^{II} couple	$(\log K_{Fe(III)} - \log K_{Fe(II)})^a$	$E^\circ(Fe^{III}/Fe^{II})$, mV[b]	K_6[c]	Limit for k_6 ($M^{-1}\ s^{-1}$)[d]
Fe^{3+}_{aq}/Fe^{2+}		+771	1.8×10^{-16}	$<7 \times 10^{-7}$
$Fe^{III}(ox)^+/Fe^{II}(ox)$	5.12	+430	2.4×10^{-11}	$<1 \times 10^{-1}$
$Fe^{III}(ox)_2^-/Fe^{II}(ox)_2^{2-}$	9.93	+181	1.5×10^{-6}	$<6 \times 10^{3}$
$Fe^{III}(ox)_3^{3-}/Fe^{II}(ox)_3^{4-}$	15.08	−121	2.2×10^{-1}	$<9 \times 10^{2}$

[a] From values for I → 0 in Table I

[b] Redox potentials for $Fe^{III}(ox)_n/Fe^{II}(ox)_n$ couples at 298 K were calculated from, $E^\circ(Fe^{III}/Fe^{II}) = E^\circ(Fe^{3+}_{aq}/Fe^{2+}_{aq}) - (2.303RT/F)(\log K_{Fe(III)} - \log K_{Fe(II)})$, assuming $E^\circ(Fe^{3+}_{aq}/Fe^{2+}_{aq}) = +771$ mV.[34]

[c] For each couple the equilibrium constant for reaction (6) was calculated from, $\log K_6 = (F/2.303RT)\{E^\circ(O_{2(aq)}/O_2^{\bullet-}) - E^\circ(Fe^{III}/Fe^{II})\}$, assuming $E^\circ(O_{2(aq)}/O_2^{\bullet-}) = -160$ mV.[35,36]

[d] See text.

experimental conditions where $Fe^{II}(ox)_2^{2-}$ comprises 53% of total Fe^{II} (see Fig. 3). A rate constant, $k_6 = 3.6\ M^{-1}\ s^{-1}$, can thus be deduced for $Fe^{II}(ox)_2^{2-}$. This is similar to the rate for Fe^{II} DTPA ($k_6 = 3.5\ M^{-1}\ s^{-1}$) but far slower than that for Fe^{II} EDTA ($k_6 = 63\ M^{-1}\ s^{-1}$).[9] The faster rate for EDTA than for $Fe^{II}(ox)_2^{2-}$ is partly explained by its lower Fe^{III}/Fe^{II} redox potential: +114 mV[9] as compared with +181 mV for $E^\circ(Fe^{III}(ox)_2^-/Fe^{II}(ox)_2^{2-})$ (see Table III). However, the redox potential for Fe-DTPA (+90 mV)[9] is below that for Fe-EDTA, indicating that other factors are also important.

ESR Studies with DMPO as Spin Trap

The ESR spectra shown in Figure 4(a) and (b) were obtained after reaction of Fe^{II} oxalate with H_2O_2 in the presence of 10 mM DMPO, at pH 2.5 and pH 4.6 respectively. The spectrum at pH 2.5 is exactly as expected for DMPO-OH, being a 1:2:2:1 quartet with $a^N = a_\beta^H = 14.9$ G.[37] Although DMPO-OH can be formed by decomposition of DMPO-O_2^-,[38] its presence in this experiment is consistent with other evidence for $^\bullet OH$ production, as described above. The nature of the second adduct in Figure 4(b) (splitting constants $a^N = 15.6$ G, $a_\beta^H = 18.6$ G) was revealed as that for $CO_2^{\bullet-}$ when an identical signal was obtained from a solution containing oxalate, Fe^{III} (note, not Fe^{II}) and DMPO after exposure to diffuse sunlight for a few minutes; see Figure 4(c). The absorption of light by Fe^{III} oxalate leads to $CO_2^{\bullet-}$ formation by a charge transfer reaction, which has been widely studied and indeed recommended as an actinometer for measuring total exposure to light:[39]

$$Fe^{III}(C_2O_4)_3^{3-} + \text{light} \rightarrow Fe^{II}(C_2O_4)_2^{2-} + CO_2 + CO_2^{\bullet-}$$

In a further study with conditions as in Fig 4(c) except for varied $[Fe^{III}]$, the signal declined sharply above 0.3 mM Fe^{III}, implying a quenching reaction. The DMPO-OH signal was not observed in these experiments with Fe^{III}.

From comparison of Figure 4(b) and 4(c), it is evident that reaction of Fe^{II} oxalate with H_2O_2 at pH 4.6 in the presence of DMPO leads to a mixture of two adducts, DMPO-OH and DMPO-CO_2^-. The production of the $CO_2^{\bullet-}$ radical from oxalate implies a one-electron oxidation:

$$^-O_2C.CO_2^- \rightarrow CO_2 + CO_2^{\bullet-} + e^-$$

The formation of DMPO-CO_2^- from oxalate plus DMPO in aqueous solution has been observed with several strong oxidants, including the sulphate radical anion,[40] the veratryl alcohol radical cation[41] and Mn^{III}.[42] Popp *et al.*[41] noted that DMPO-CO_2^- was only observed above pH 4, in agreement with the present study (cf. Fig. 4(a) and 4(b)). They attributed its absence at lower pH to a faster decay for the protonated state, DMPO-CO_2H.

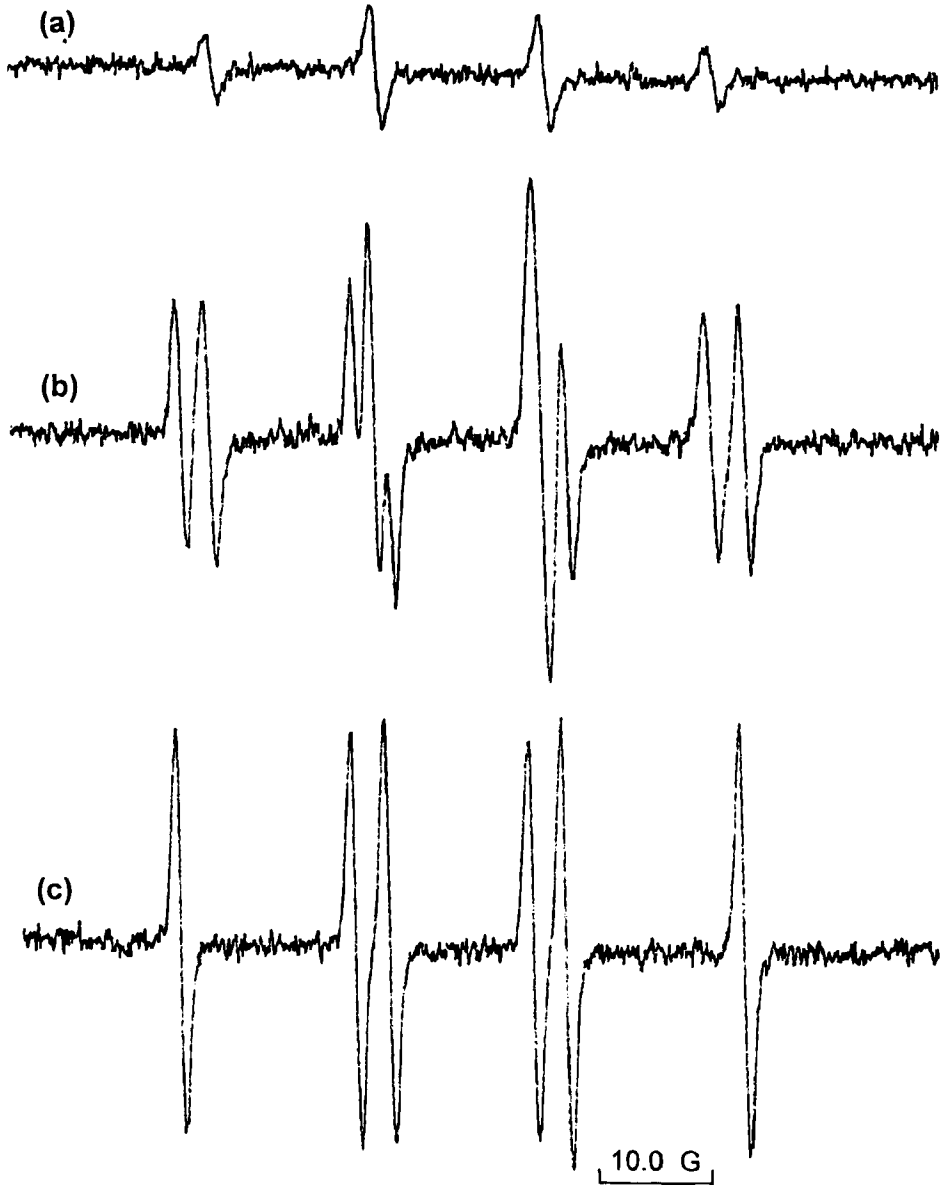

FIGURE 4 ESR spectra for spin adducts from DMPO. In all spectra the DMPO concentration was 10 mM. (**a**) Fe^{II} plus H_2O_2 at pH 2.5. 0.33 mM Fe^{II} was added to 1.0 mM H_2O_2 in 10 mM oxalate (pH 2.5). (**b**) As (a), but pH 4.6. (**c**) Photolysis of Fe^{III} oxalate. A solution containing 0.3 mM Fe^{III} (added as $Fe^{III}Cl_3$) in 10 mM oxalate, pH 4.6, was exposed to diffuse sunlight for 20 min.

In summary, the continuous flow technique has enabled the rate of $^{\bullet}OH$ production to be measured for specified initial concentrations of Fe^{II}, oxalate and H_2O_2. The reaction is much faster than for Fe^{2+}_{aq}. Evidence has also been obtained for oxidation of $Fe^{II}(ox)_2^{2-}$ by dioxygen. Taken together, these findings provide a mechanism for $^{\bullet}OH$ production in aqueous systems in which Fe^{II} is complexed by oxalate. The experiments with DMPO as spin-trap demonstrate that such solutions can in fact generate two radicals at opposite ends of the redox scale: the strongly oxidizing hydroxyl radical, $E^o(^{\bullet}OH/H_2O) = +2.74$ V and the strongly reducing carboxylate radical anion, $E^o(CO_2/CO_2^{\bullet-}) = -1.9$ V.[43,44] The reactions of $^{\bullet}OH$ have been discussed, while two very fast reactions of $CO_2^{\bullet-}$ are reduction of dioxygen to superoxide ($k = 2.4 \times 10^9\ M^{-1}\ s^{-1}$) and reduction of Fe^{III} to Fe^{II} ($k = 1.06 \times 10^9\ M^{-1}\ s^{-1}$ for $Fe^{III}(CN)_6^{3-}$).[45]

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