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AN E.S.R. INVESTIGATION OF THE REACTIVE INTERMEDIATE GENERATED IN THE REACTION BETWEEN Fe^{II} AND H_2O_2 IN AQUEOUS SOLUTION. DIRECT EVIDENCE FOR THE FORMATION OF THE HYDROXYL RADICAL

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The technique of E.S.R. spectroscopy, when employed in conjunction with a continuous flow system, provides direct evidence for the nature of free radicals formed from organic substrates in the presence of Fe^{II} and H_2O_2 in aqueous solution. It is shown, both via the identification of hydroxyl-radical adducts to alkenes and via the observed site-selectivity of radical attack, that the *hydroxyl radical* is formed as the reactive intermediate in the presence of various chelators (e.g. EDTA, DTPA). This approach also allows the rate constants for the Fe^{II}–H_2O_2 reaction in the presence of the different chelates to be determined; values obtained are in reasonable agreement with most of those measured by other methods. Examples of radical oxidation (by Fe^{III}) and reduction (by Fe^{II}) are revealed.

KEY WORDS: hydroxyl radical, Fenton reaction, E.S.R. spectroscopy, radical oxidation/reduction.

ABBREVIATIONS: DTPA, diethylenetriaminepentaacetic acid; EDDA, ethylenediaminediacetic acid; EDTA, ethylenediaminetetraacetic acid; HEDTA, N-(2-hydroxyethyl)ethylenediaminetriacetic acid; NTA, nitrilotriacetic acid.

INTRODUCTION

It has often been suggested that the hydroxyl radical, formed for example via reactions (1)–(3) in iron–peroxide systems, is a major species involved in the initiation of free-radical-mediated biological damage.[1,2]

$$Fe^{III} + H_2O_2 \longrightarrow Fe^{II} + HO_2^{\cdot} + H^+ \tag{1}$$

$$Fe^{III} + HO_2^{\cdot}/O_2^{\cdot-} \longrightarrow Fe^{II} + O_2 + H^+ \tag{2}$$

$$Fe^{II} + H_2O_2 \longrightarrow Fe^{III} + HO^- + HO\cdot \tag{3}$$

However, the Fenton reaction [reaction (3)] is not necessarily as simple as depicted above and evidence for $\cdot OH$ production from Fe^{II} and hydrogen peroxide is by no means conclusive. Though its generation was first proposed by Haber and Weiss in 1932,[3] Bray and Gorin suggested[4] that the crucial intermediate is FeO^{2+}: qualitative and quantitative evidence has subsequently been offered to support claims that the intermediate is $\cdot OH$,[5-7] FeO^{2+} (Refs. 8–10) and Fe^{II} (H_2O_2).[11]

For example, evidence against the formation of $\cdot OH$ from Fe^{II} and H_2O_2 was provided by Shiga[10] who employed E.S.R. spectroscopy in conjunction with a flow system. He compared the E.S.R. spectra of organic radicals produced under steady-state conditions from the $Ti^{III}-H_2O_2$ redox couple (believed to produce $\cdot OH$) and organic substrates with those from $Fe^{II}EDTA-H_2O_2$ under similar conditions. Results from the two couples were not identical: for example, in the reaction of ethanol the predominant signal was from the α-radical $\cdot CHMeOH$ for $Ti^{III}-H_2O_2$ (as expected for reaction of $\cdot OH$) whereas $\cdot CH_2CH_2OH$ predominated for $Fe^{II}-H_2O_2$ (an observation interpreted as evidence that $\cdot OH$ was not involved).

Rush and Koppenol[8] investigated the $Fe^{II}-H_2O_2$ reaction by comparing the reactivity of the intermediate generated from $Fe^{II}EDTA-H_2O_2$ with that of $\cdot OH$ towards a series of organic substrates. In most cases (e.g. with formate, mannitol and imidazole) the reactivity matched that of $\cdot OH$ but for other substrates (e.g. Bu^tOH, benzoate and acetate anions and arginine) it was concluded that the intermediate formed was less reactive than expected for $\cdot OH$. In a spectroscopic study of the reaction of $Fe^{II}HEDTA$ with H_2O_2 it was concluded[11] that an intermediate complex between $Fe^{II}$and H_2O_2 (which does not give $\cdot OH$) can be scavenged by added substrates.

Walling[5] has suggested that the reactive intermediate from Fe^{II} and H_2O_2 under aqueous conditions is indeed $\cdot OH$; we have also argued[6,7] that discrepancies between E.S.R. results for Ti^{III} and Fe^{II} could be understood if both systems serve as effective methods for generation of $\cdot OH$ but that in the iron-containing system the α-alcohol radicals (e.g. $\cdot CHMeOH$) are destroyed by rapid reaction with Fe^{III}. Similarly, the apparently lower reactivity of the substrate Bu^tOH *may* be explained in terms of reduction of $\cdot CH_2CMe_2OH$ by Fe^{II}: indeed, product studies[12] establish that $CH_2{=}CMe_2$ is formed in this reaction. A small yield of 2-methylpropene has also been detected (Rush and Koppenol, private communication), though it is not believed to be of sufficient concentration to account for the observed stoichieometry of the $Fe^{II}-H_2O_2$ reaction (1.7 : 1).

The reaction of $Fe^{II}HEDTA$ with H_2O_2 in the presence of formate has also been studied by Czapski and his coworkers;[13] these authors dispute the claim made for an intermediate $Fe^{II}-H_2O_2$ complex and assign the absorption detected to $Fe^{III}(HEDTA)CO_2^-$, formed by reaction of $\cdot OH$ with HCO_2^- to give $CO_2^{\cdot-}$ which reacts with $Fe^{III}HEDTA$.

Polarographic and spectroscopic techniques have also been employed to determine the rate constant for the $Fe^{II}-H_2O_2$ reaction, which is found to depend critically upon pH and the nature of the added ligand. For example, reaction of $Fe^{II}EDTA$ and H_2O_2 at pH 7 is believed to have *k ca.* $10^4\,dm^3\,mol^{-1}\,s^{-1}$ (Refs. 6, 7, 11, 14, 15) and HEDTA, NTA, and EDDA have rate constants somewhat greater;[11,14] there is some discrepancy over the values claimed for Fe^{II}–DTPA (from 5×10^2 to $10^4\,dm^3\,mol^{-1}\,s^{-1}$: *cf.* Refs. 6, 7, 11 and 15), but in all cases the rate constants are appreciably greater than that associated with the aquated Fe^{II} complex (*ca.* $75\,dm^3\,mol^{-1}\,s^{-1}$: *cf.* Ref. 5). This may be taken to reflect the increase in the thermodynamic driving force associated with the reduction in E for the redox potential of the complexed ion [compare, for example, the value of 0.71 V for Fe^{III}/Fe^{II} (at low pH 3) with that of 0.12 for $Fe^{III}EDTA/Fe^{II}EDTA$ at pH 7 (Ref. 16); E for $\cdot OH/H_2O_2$ at pH 7 is 0.46 V].

The aim of the work reported here was two-fold: firstly, to employ E.S.R. spectroscopy and continuous-flow techniques under conditions similar to those reported by Shiga[10] but with a range of substances, in an attempt to obtain clear and direct evidence as to the mediation of the hydroxyl radical (or otherwise). Secondly, we intended to employ the E.S.R. technique and steady-state analysis to obtain rate-constants for the generation of the reactive intermediate (to compare with those obtained by more conventional techniques for following the reaction of iron): in this way we hoped to establish unambigously whether the free-radical reaction (monitored by E.S.R. spectroscopy) was indeed the major (or only) reaction pathway.

METHODS AND MATERIALS

Materials

All chemicals purchased were of the highest quality available (i.e. Analar Grade) and used without further purification. Iron(II) was used as its sulphate, iron(III) and titanium(III) as their chlorides (Fisons): hydrogen peroxide was supplied by Fisons as a solution of *ca.* 33% w/v and was titrated against potassium permanganate to determine its exact concentration. Chelates (some as their sodium salts) were obtained from both Aldrich Chemical Company and Sigma Chemical Company.

All experiments were carried out with deionized distilled water.

Methods

(i) *E.S.R. measurements.* E.S.R. spectra were recorded on a Varian E-104 and a Bruker ESP 300 Spectrometer, each employing 100 kHz modulation and equipped with an X-band klystron. Hyperfine splittings were measured directly from the spectrometer field scan, which was periodically recalibrated with an aqueous solution of Fremy's salt[17] [$a(N)$ 1.309 mT]. All g-values were measured by comparison with that of $\cdot CHMeOH$ (g 2.00321 ± 0.0005)[18] itself compared with Fremy's salt (g 2.00550).[19] Relative concentrations of radicals were determined from peak-height measurements (where line-widths were the same) or by double integration of peaks (using the ESP 300). Absolute radical concentrations were obtained by comparison with the doubly-integrated spectra from $\cdot CH_2CMe_2OH$ (3×10^{-6} mol dm^{-3}) generated under standard conditions.[7]

(ii) *Continuous-flow experiments.* These employed a three-way mixer which allows simultaneous mixing of three reagent streams prior to their passage through a flat aqueous-solution sample cell in the cavity of the E.S.R. spectrometer. Flow was maintained with a Watson-Marlow 502S peristaltic pump positioned on the inlet tubing: the mixing time (in the range 20–50 ms) was controlled by adjusting the flow-rate and was typically *ca.* 30 ms. Measurements of pH were made by use of a Russell pH Ltd glass electrode coupled to a Pye Unicam PW 9410 pH meter: measurements were made of the three separate solutions and of the reaction mixture at the exit from the E.S.R. cell. Adjustment of pH was made by addition of sulphuric acid or ammonia (d 0.880; laboratory grade) to the metal-ion stream. The temperature of the combined solution was measured at the exit from the cell. The reagent streams were prepared in deionized distilled water which had been deoxygenated by passage of nitrogen for *ca.* 40 min; the flow of nitrogen was maintained throughout the experiment.

The reactions of the hydroxyl radical were studied using continuous-mixing experiments involving Ti^{III} and H_2O_2, with the substrate in the third stream: typical concentrations after mixing were [Ti^{III}] 1.7×10^{-3} mol dm^{-3}, [H_2O_2]

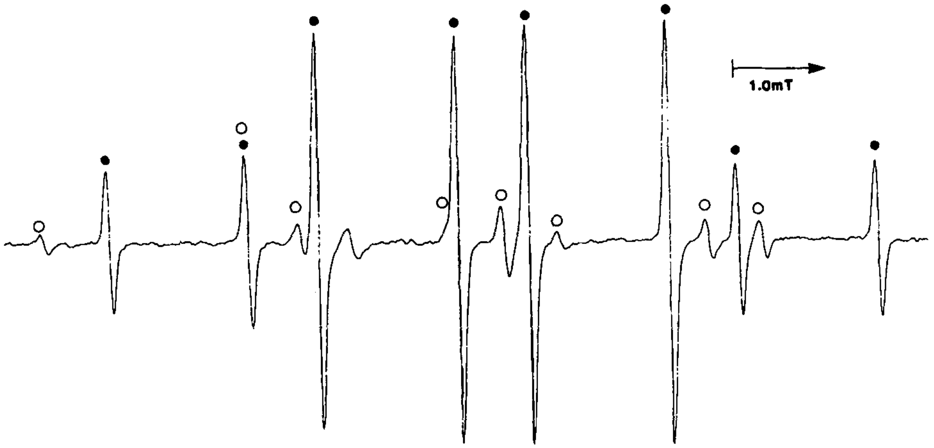

FIGURE 1 E.S.R. spectra of $\cdot CHMeOH$ (●) and $\cdot CH_2CH_2OH$ (○) obtained at pH 1.9 with the Ti^{III}/H_2O_2 redox couple in the presence of ethanol $[Ti^{III}]_0$ 1.7×10^{-3} $[H_2O_2]$ 1.7×10^{-2} $[EtOH]_0$ 0.6 mol dm^{-3}.

1.7×10^{-2} mol dm^{-3} and [substrate] in large excess (usually ~3% v/v for alcohols, 1% v/v for alkenes). At high pH values, Ti^{III} was chelated by the addition of EDTA to the metal stream (to give a final concentration of 3.4×10^{-3} mol dm^{-3}).

Related experiments with iron involved the use of iron(II) sulphate (typically *ca.* 10^{-3} mol dm^{-3} after mixing, with added chelate at double this concentration), hydrogen peroxide (typically in the range 10^{-3}–1.6×10^{-2} mol dm^{-3}) and substrate (in excess, as above).

(iii) *Computer simulations.* Simulations of reaction kinetics were carried out with a program (Simula) provided by Dr T.M.F. Salmon and modified by Dr M.J. Brown for use on a VAX mainframe computer. The E.S.R. spectra simulation program was written by Dr M.F. Chiu and modified to run on the VAX computer.

RESULTS AND DISCUSSION

E.S.R. Studies of the Ti^{III}–H_2O_2 Reaction as a Source of $\cdot OH$

Our initial experiments involved recording E.S.R. spectra of organic radicals in continuous-flow experiments, with mixing time 0.027 s, in which we mixed solutions of Ti^{III} (to a final concentration of 1.7×10^{-3} mol dm^3), H_2O_2 (final concentration 1.7×10^{-2} mol dm^3) and the substrate [in excess, so as to scavenge $\cdot OH$, formed as in reaction (4)], both at pH 2 and 7. Our strategy was to determine selectivities of reaction for both hydrogen-atom abstraction (from alkanols, acids) and addition (to alkenes). The results are illustrated in Figures 1 and 2, which show the excellent signal-to-noise ratios obtained (corresponding to an observed steady-state concentration of organic radicals of *ca.* 3×10^{-6} mol dm^{-3}), and Table I contains details of the hyperfine splittings of the radicals obtained and their assignments (for example to *hydroxyl* adducts of the alkenes).[20] Table II contains selected data on the ratios of radicals formed in experiments to determine the site-selectivity of the intermediate radical (believed to be $\cdot OH$).

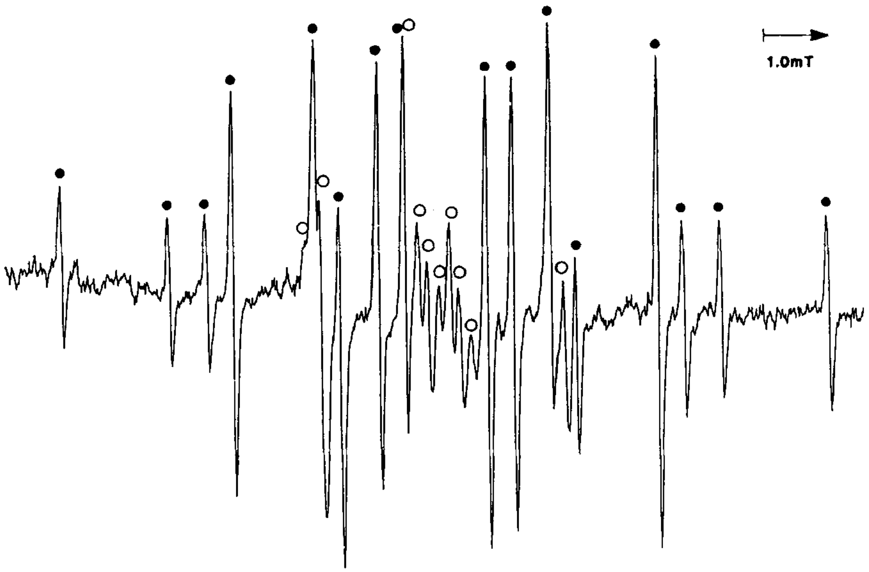

FIGURE 2 E.S.R. spectra of $\cdot CHMeCH(OH)CO_2H$ (●) and $\cdot CH(CO_2H)CHMeOH$ (○) obtained at pH 1.9 with the Ti^{III}/H_2O_2 redox couple and crotonic acid. $[Ti^{III}]_0$ 1.7×10^{-3} $[H_2O_2]$ 1.7×10^{-2} $[MeCH{=}CHCO_2H]$ 1.7×10^{-2} mol dm^{-3}

Several features are worthy of note. For example, methanol and *t*-butyl alcohol react to form solely $\cdot CH_2OH$ and $\cdot CH_2CMe_2OH$, and dimethyl sulphoxide reacts to give a strong signal from the methyl radical [as expected on the basis that $\cdot OH$ is indeed formed: *cf.* reactions (4) and (5)]. When ethanol was used as substrate both α ($\cdot CHMeOH$) and β ($\cdot CH_2CH_2OH$) radicals were detected (see Figure 1), with steady-state ratio $[\alpha]:[\beta]$ *ca.* 7 : 1. This does not reflect *directly* the ratio of the rate constants for attack of $\cdot OH$ at the α- and β-positions of ethanol (13 : 1; see e.g. Ref. 21 and references therein); the apparent discrepancy is attributed to the reaction of the α-radical with H_2O_2 [see reactions (6) and (7), for the latter of which k is *ca.* $10^5\,dm^3\,mol^{-1}\,s^{-1}$][22] which will serve to reduce [$\cdot CHMeOH$] at the concentrations of H_2O_2 employed.[6,7,21]

$$Ti^{III} + H_2O_2 \longrightarrow Ti^{IV} + HO\cdot + OH^- \qquad (4)$$

$$\cdot OH + Me_2SO \longrightarrow [Me_2S(OH)O\cdot] \longrightarrow Me\cdot + MeSO_2H \qquad (5)$$

$$\cdot OH + EtOH \longrightarrow \underset{\alpha}{\cdot CHMeOH} + \underset{\beta}{\cdot CH_2CH_2OH} \qquad (6)$$

$$\cdot CHMeOH + H_2O_2 \longrightarrow \cdot OH + OH^- + {}^+CHMeOH \qquad (7)$$

For propan-2-ol both α- and β-radicals were observed (with the former the major species detected) and for propan-1-ol the α-, β-, and γ-radicals were all detected (in the ratio 2.8 : 1.6 : 1.0) (n.b. in earlier experiments reported by Shiga,[10] only the α-radical was described): again, we argue that reaction of $\cdot OH$ is likely to lead to a considerable predominance of the corresponding α-radical (*cf.* pulse-radiolysis results summarized in Ref. 23) and that further oxidation takes place [*cf.* reaction (7)].

TABLE I

E.S.R. parameters of radicals detected from the Ti^{III}/H_2O_2 couple at pH 2 and the Ti^{III} EDTA/H_2O_2 and Fe^{II}/H_2O_2 couples at pH 7

			Hyperfine splittings[b] (mT)			
Substrate	pH	Radicals produced[a]	a(α-H)	a(β-H)	a(γ-H)	g[c]
CH_3OH	2, 7	$\cdot CH_2OH$	1.76	–	0.13[d]	2.0031
CH_3CH_2OH	2, 7	$CH_3\dot{C}HOH$	1.51	2.26	–	2.0032
	2, 7	$\cdot CH_2CH_2OH$	2.18	2.78	–	2.0026
$CH_3CH_2CH_2OH$	2, 7	$CH_3CH_2\dot{C}HOH$	1.48	2.13	–	2.0032
	2, 7	$CH_3\dot{C}HCH_2OH$	2.17	1.98(2)	–	2.0026
				2.55(3)	–	
	2, 7	$\cdot CH_2CH_2CH_2OH$	2.18	2.71	–	2.0026
$CH_3CH(OH)CH_3$	2, 7	$\cdot C(CH_3)_2OH$	2.00	–	–	2.0033
	2, 7	$\cdot CH_2CH(OH)CH_3$	2.21	2.35	0.08	2.0026
$CH_3CH_2CO_2H$	2	$CH_3\dot{C}HCO_2H$	2.00	2.49	–	2.0033
	2	$\cdot CH_2CH_2CO_2H$	2.23	2.67	–	2.0027
$CH_3CH_2CO_2^-$	7	$CH_3\dot{C}HCO_2^-$	2.05	2.50	–	2.0033
	7	$\cdot CH_2CH_2CO_2^-$	2.23	2.58	–	2.0025
$(CH_3)_3COH$	2, 7	$\cdot CH_2C(OH)(CH_3)_2$	2.22	–	0.14	2.0024
$(CH_3)_2SO$	2, 7	$\cdot CH_3$	2.27	–	–	2.0025
Z-$HO_2CCH{=}CHCO_2H$	2	$HO_2CCH(OH)\dot{C}HCO_2H$	2.08	1.32	–	2.0037
Z-$^-O_2CCH{=}CHCO_2^-$	7	$^-O_2CCH(OH)\dot{C}HCO_2^-$	2.02	1.56	–	2.0035
$HOCH_2CH{=}CHCH_2OH$	2, 7	$HOCH_2CH(OH)\dot{C}HCH_2OH$	1.78	2.29(2)	–	2.0023
				2.18	–	
	2, 7	$HOCH_2CH{=}CH\dot{C}HOH$	1.37(2)	0.94(2)	–	2.0029
			0.39	–	–	
$H_2C{=}CHCH_2OH$	2, 7	$\dot{C}H(CH_2OH)$	2.02	2.18	–	2.0024
	2, 7	$\cdot CH_2CH(OH)CH_2OH$	2.20	2.50	–	2.0025
	2, 7	$H_2C{=}CH\dot{C}HOH$	1.33(3)	–	–	2.0028
			0.37	–	–	
$(CH_3)_2C{=}CHCH_2OH$	2, 7	$(CH_3)_2\dot{C}CH(OH)CH_2OH$	–	0.98	–	2.0026
			–	2.35(6)	–	
	2	$(CH_3)_2C(OH)\dot{C}HCH_2OH$	2.13	2.37	0.11	2.0023
$CH_3CH{=}CHCO_2H$	2	$CH_3\dot{C}HCH(OH)CO_2H$	2.18	1.62	–	2.0025
				2.58(3)	–	
	2	$CH_3CH(OH)\dot{C}HCO_2H$	2.19	1.57	0.16	2.0033
$CH_3CH{=}CHCO_2^-$	7	$CH_3\dot{C}HCH(OH)CO_2^-$	2.15	1.56	–	2.0027
				2.55(3)	–	
	7	$CH_3CH(OH)\dot{C}HCO_2^-$	2.04	1.57	0.15	2.0031

[a] For comparison of the Ti^{III} and Fe^{II} complexes, see text.

[b] ±0.01.

[c] ±0.0001.

[d] Splitting from the hydrogen of the hydroxyl group.

Steady-state analysis (and exploration of the dependence of the [α]/[β] ratio on H_2O_2) have been reported[6,7,21,24] and will not be repeated here.

Reaction of propanoic acid led to the detection of more or less equimolar concentrations of the α- and β-radicals $\cdot CHMeCO_2H$ and $\cdot CH_2CH_2CO_2H$ respectively, for which the ratio was not found to be sensitive to $[H_2O_2]$. This pattern of behaviour is not unexpected: though $\cdot CHMeCO_2H$ is the more stabilized radical, attack of the

TABLE II

Ratios of steady-state concentrations of radicals ([α]/[β]) detected by E.S.R. spectroscopy in experiments with the Ti^{III}-H_2O_2 and Fe^{II}-H_2O_2 redox couples in aqueous solution

Substrate	Ti^{III} (pH 2)[a]	Ti^{III}EDTA (pH 7)[b]	Fe^{II}EDTA (pH 7)[c]
$\overset{\beta}{C}H_3\overset{\alpha}{C}H_2OH$	7.1 ± 0.1	4.1 ± 0.1	0.4 ± 0.1[d]
$CH_3CH_2CH_2OH$	1.8 ± 0.3	0.7 ± 0.3	–[e]
Me_2CHOH	1.8 ± 0.1	1.7 ± 0.1	–[e]
$\overset{\beta}{C}H_3\overset{\alpha}{C}H_2CO_2H$	0.9 ± 0.3		
$CH_3CH_2CO_2^-$		1.6 ± 0.3	2.5 ± 0.3
$\overset{\alpha}{C}H_2{=}\overset{\beta}{C}HCH_2OH$	1.4 ± 0.8	2.8 ± 0.8	2.2 ± 0.3
$MeCH{=}CHCO_2H$	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.3
$HOCH_2CH{=}\overset{\alpha}{C}H\overset{\beta}{C}H_2OH$	4.2 ± 0.1	4.3 ± 0.1	–[e]

[a] $[Ti^{III}]_0$ 1.7×10^{-3}, $[H_2O_2]_0$ 1.6×10^{-2} mol dm^{-3}.

[b] $[Ti^{III}]_0$ 1.7×10^{-3}, $[EDTA]_0$ 3.4×10^{-2}, $[H_2O_2]_0$ 1.6×10^{-2} mol dm^{-3}.

[c] $[Fe^{II}]$ 6.7×10^{-4}, $[EDTA]_0$ 1.34×10^{-3}, $[H_2O_2]_0$ 1.6×10^{-2} mol dm^{-3}.

[d] As (c) but with $[H_2O_2]_0$ 1.0×10^{-2} mol dm^{-3}.

[e] Only a trace of the oxygen-conjugated radical was detected (see text). Errors ± 1 S.D., $n = 3$.

electrophilic hydroxyl radical on the α-H would be retarded by the electron-withdrawing effect of the carboxyl group (on the assumption that polar effects are important in the transition state).

Reaction of Ti^{III} and H_2O_2 in the presence of alkenes led to the detection of the hydroxyl adduct for maleic acid and both adducts for crotonic acid [$\cdot CHMeCH(OH)CO_2H$ and $\cdot CH(CO_2H)CH(OH)Me$ – in the ratio of 1.0 : 1.6]; with but-2-ene-1,4-diol ($HOCH_2CH{=}CHCH_2OH$), E.S.R. signals were detected from both the adduct $\cdot CH(CH_2OH)CH(OH)CH_2OH$ and the radical formed by hydrogen abstraction $\cdot CH(OH)CH{=}CHCH_2OH$ (with the former favoured), and with 3-methyl-but-2-ene-1-ol ($Me_2C{=}CHCH_2OH$) the spectrum was dominated by the adduct $\cdot CMe_2CH(OH)CH_2OH$. Allyl alcohol gave signals from both of the hydroxyl adducts, in the ratio 1.4 : 1.0 [$\cdot CH(CH_2OH)_2$ to $\cdot CH_2CH(OH)CH_2OH$], with a trace of $\cdot CH(OH)CH{=}CH_2$. These results show the generally accepted behaviour of $\cdot OH$ – with its preference for addition rather than abstraction,[25] and, at least for the bulky alkenes, a regioselectivity governed in part by steric effects.

Generally similar results (see Table I) were obtained from experiments at pH 7 (in the presence of EDTA to complex Ti^{III}), but several points are worthy of note. For example, the ratio of α- to β-radicals from ethanol was reduced to 4 : 1, an observation which is believed[21] to reflect the oxidation of the α-radical by Ti^{IV}EDTA [reaction (8)]. The increase in the ratio of α : β radicals from propanoic acid (1.6 : 1) may be explained in terms of the reduction in the electron-withdrawing effect of the carboxyl group upon ionization. Results for the alkenes were almost identical to those observed at pH 2.

$$Ti^{IV}EDTA + \cdot CHMeOH \longrightarrow Ti^{III}EDTA + {}^{+}CHMeOH \qquad (8)$$

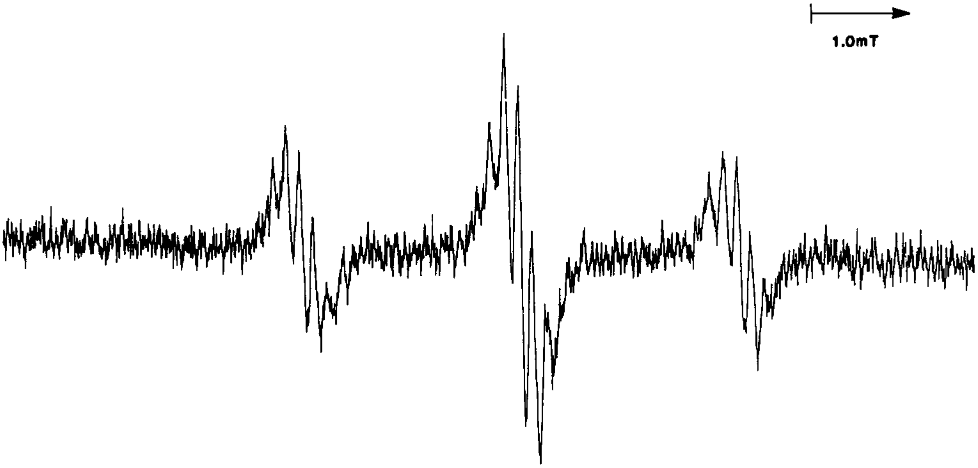

FIGURE 3 E.S.R. spectrum of $\cdot CH_2CMe_2OH$ obtained at pH 7.1 with Fe^{II}EDTA/H_2O_2 and Bu^tOH. $[Fe^{II}]_0$ 1.7×10^{-3}, [EDTA] 3.4×10^{-3}, $[H_2O_2]$ 1.7×10^{-2} $[Bu^tOH]_0$ 0.35 mol dm^{-3}.

Comparison of the $Fe^{II}EDTA/H_2O_2$ and Ti^{III}/H_2O_2 Systems: Site-selectivity of Attack

Initial experiments with the Fe^{II}/H_2O_2 redox couple were carried out using similar conditions and concentrations to those used for the Ti^{III}–H_2O_2 system (with $[H_2O_2]_0$ 1.7×10^{-2} mol dm^{-3}, $[Fe^{II}]_0$ 1.7×10^{-3} mol dm^{-3} and [EDTA] 3.4×10^{-3} mol dm^{-3}), at pH *ca.* 7.

With Bu^tOH as substrate the radical $\cdot CH_2CMe_2OH$ was detected (see Figure 3) with signal-to-noise ratio of *ca.* 20 : 1. A noticeable line-broadening (ΔB 0.1 mT, *cf.* 0.06 mT for Ti^{III}–H_2O_2) reflects the presence of paramagnetic iron complexes: for $[Fe^{II}]_0$ 6.7×10^{-4} mol dm^{-3} a decrease in line-width (0.08 mT) was observed. As $[H_2O_2]_0$ was then varied, the maximum value of the radical concentration was observed for a value of 5×10^{-3} mol dm^{-3}. In the experiments which followed we used $[Fe^{II}]_0$ 6.7×10^{-4} mol dm^{-3}, with $[H_2O_2]_0$ in the range 1.6×10^{-2}–5×10^{-3} mol dm^{-3}.

Methanol gave a relatively weak spectrum of $\cdot CH_2OH$ and ethanol gave signals from both $\cdot CH_2CH_2OH$ and $\cdot CHMeOH$ (n.b. Shiga[10] reports only the β-radical). Improved signal-to-noise ratios were observed at *low* hydrogen peroxide concentrations (see e.g. Figure 4): for $[H_2O_2]_0$ 1.0×10^{-2} mol dm^{-3}, the signal-to-noise ratio was *ca.* 100 with $[\alpha] : [\beta]$ in the ratio 1 : 2.6. Though the corresponding α-radical could not be detected from propan-2-ol, propan-1-ol also gave detectable signals from the α-radical, along with those from the β- and γ-isomers (see Table II) — though, as with ethanol, the contrast between the apparent behaviour of the Ti^{III}/H_2O_2 and Fe^{II}EDTA/H_2O_2 couples is clear.

Reaction with propanoic acid gave both the α-radical ($\cdot CHMeCO_2^-$) and β-radical ($\cdot CH_2CH_2CO_2^-$), in the ratio 2.5 : 1.0, and *hydroxyl-radical adducts* were detected from the alkenes investigated. Maleic acid gave signals from $\cdot CH(CO_2^-)CH(OH)CO_2^-$, crotonic acid gave both $\cdot CH(CO_2^-)CHMeOH$ and $\cdot CHMeCH(OH)CO_2^-$ (ratio 1 : 1.3), and allyl alcohol gave both $\cdot CH(CH_2OH)_2$ and $\cdot CH_2CH(OH)CH_2OH$ in the ratio 2.2 : 1 [no trace of $\cdot CH(OH)CH{=}CH_2$ was seen until $[H_2O_2]_0$ was lowered

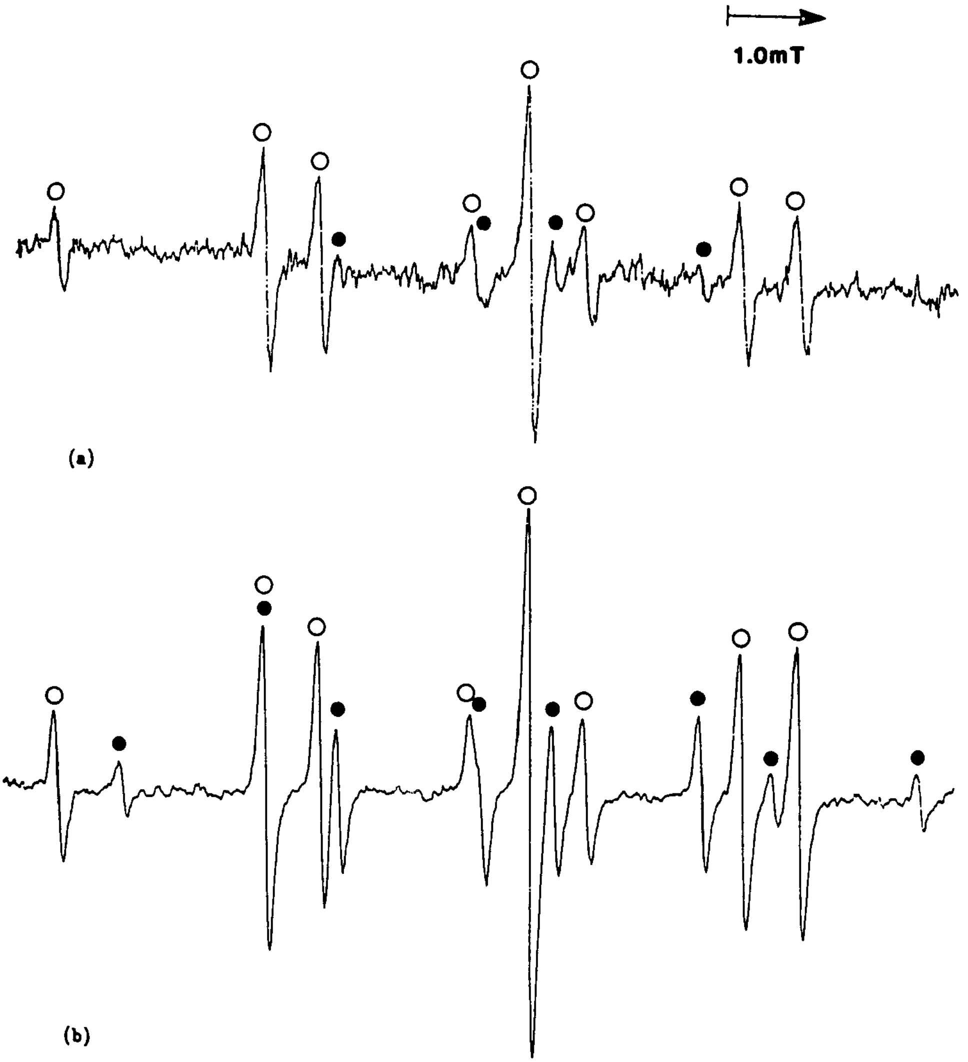

FIGURE 4 E.S.R. spectra of $\cdot CHMeOH$ (●) and $\cdot CH_2CH_2OH$ (○) obtained at pH 7 with the $Fe^{II}EDTA/H_2O_2$ couple and ethanol. $[Fe^{II}]_0$ 6.7 × 10^{-4} $[EDTA]_0$ 1.3 × 10^{-3} $[EtOH]_0$ 0.6. (a) $[H_2O_2]$ 1.7 × 10^{-2}, (b) $[H_2O_2]$ 1.0 × 10^{-2} mol dm^{-3}.

to 5.0 × 10^{-3} mol dm^{-3}]. But-2-ene-1,4-diol gave dominant signals from $\cdot CH(CH_2OH)CH(OH)CH_2OH$ with traces of $\cdot CH(OH)CH{=}CHCH_2OH$, and $Me_2C{=}CHCH_2OH$ gave almost entirely $\cdot CMe_2CH(OH)CH_2OH$.

Dimethyl sulphoxide gave a strong E.S.R. spectrum of the methyl radical, as with Ti^{III}.

The production of *hydroxyl-radical* adducts (and $\cdot Me$ from DMSO) in the $Fe^{II}EDTA/H_2O_2$ system strongly suggests that the *hydroxyl* radical is indeed formed as the crucial *radical* intermediate during the $Fe^{II}EDTA{-}H_2O_2$ reaction. In further support of this assertion is the observation that the site selectivity of the reactive intermediate is essentially the same as that in the Ti^{III}EDTA system (i.e. $\cdot OH$): it seems highly unlikely that these observations could reflect the involvement of a complex or metal-centred intermediate in the oxidations of organic molecules.

The clear differences in $[\alpha]/[\beta]$ ratio observed for the alcohols studied in Ti^{III} and Fe^{II}-based systems can then be effectively explained if it is assumed that Fe^{III}EDTA selectively removes (oxidises) the α-radicals [see reactions (9)–(11)]. Preliminary kinetic simulations of the effect on the observed $[\alpha]:[\beta]$ ratio with the inclusion of reaction (11) in the mechanistic scheme provide confirmation of the consistency of this argument and suggests a value of *ca.* $10^9\,dm^3\,mol^{-1}\,s^{-1}$ for the rate constant for reaction (11).[6] This aspect of our work has been extended through more detailed kinetic calculations described below.

$$Fe^{II}EDTA + H_2O_2 \longrightarrow Fe^{III}EDTA + HO\cdot + {}^{-}OH \tag{9}$$

$$HO\cdot + EtOH \longrightarrow \cdot CHMeOH + \cdot CH_2CH_2OH \tag{10}$$

$$\cdot CHMeOH + Fe^{III}EDTA \longrightarrow {}^{+}CHMeOH + Fe^{II}EDTA \tag{11}$$

The increase in the proportion of α-radical from the propanoate anion in the Fe^{II}EDTA system compared with Ti^{III}-EDTA suggests that $\cdot CH_2CH_2CO_2^-$ might be reduced relatively readily by Fe^{II}EDTA (*cf.* the reaction of $\cdot CH_2CMe_2OH$ described earlier).

Selectivity of the Intermediate Formed in Other Fe^{II}–H_2O_2 Reactions

The experiments described above, with propanoic acid and crotonic acids at pH *ca.* 7, were repeated for the Fe^{II}–H_2O_2 reaction in the presence of the chelates HEDTA, DTPA, NTA and EDDA. We employed conditions as follows: Fe^{II} 6.7×10^{-4}, H_2O_2 $5.3 \times 10^{-3}\,mol\,dm^{-3}$ (with $[L] = 2[Fe^{II}]$ for HEDTA and DTPA; $[L] = 3[Fe^{II}]$ for NTA and EDDA). In each case the ratios of the steady-state concentrations of radicals $CH_3\dot{C}HCO_2^-$ and $\cdot CH_2CH_2CO_2^-$ (from propanoic acid) and $\cdot CHMeCH(OH)CO_2^-$ and $\cdot CH(CO_2^-)CHMeOH$ (from crotonic acid) were, within experimental error, identical to those observed with Fe^{II}EDTA (see Table II). This provides very firm evidence indeed to support our claim that a unique intermediate — the hydroxyl radical — participates as the crucial radical species in the reaction of H_2O_2 with Fe^{II} complexed by these ligands.

Kinetic Analysis of the Fe^{II}–H_2O_2 Reactions

Despite the clear evidence that E.S.R. spectroscopy supports the involvement of $\cdot OH$ in the Fe^{II}EDTA reaction there remains the distinct possibility (not always addressed) that the radicals detected are not those on the main reaction pathway, but rather those formed in a minor reaction. To answer this potential criticism we therefore carried out a full kinetic analysis of the predicted behaviour of the concentrations of radicals detected as a function of experimental variables (e.g. concentrations of reagents) — in an attempt to match the rate of disappearance of Fe^{II} to the generation of $\cdot OH$ and to obtain the corresponding rate constant of the radical-generating process.

The steps which occur in a system containing Fe^{II}, H_2O_2, and organic substrate RH are believed to be reactions (3) and (12)–(15) (ligands for the metal ions are omitted for simplicity). In a typical flow-system study such as that presented here it is believed[26] that · OH and R · are present in steady-state concentrations (i.e. that the radicals detected are those formed *in* the cavity); appropriate kinetic analysis for the system, under conditions with $[H_2O_2]_0 \gg [Fe^{II}]_0$, and with an excess of substrate [to scavenge · OH via reaction (12)] shows that two crucial equations can then be derived. These are eq. (16), which relates the radical concentration (R ·) to initial concentrations of reagents and also the time between mixing and observation (t) and eq. (17) which leads to the prediction that as $[H_2O_2]_0$ is varied, the concentration of [R ·] gives a "peak" with maximum at a value of H_2O_2 as indicated. These relationships provide an opportunity for verification of the predicted behaviour and for determination of k_3.

$$Fe^{II} + H_2O_2 \longrightarrow Fe^{III} + HO\cdot + {}^{-}OH \tag{3}$$

$$\cdot OH + RH \longrightarrow H_2O + R\cdot \tag{12}$$

$$R\cdot + R\cdot \longrightarrow \text{non-radical products} \tag{13}$$

$$Fe^{II} + \cdot OH \longrightarrow Fe^{III} + {}^{-}OH \tag{14}$$

$$H_2O_2 + \cdot OH \longrightarrow HO_2\cdot + H_2O \tag{15}$$

$$[R\cdot] = \left(\frac{k_3[Fe^{II}]_0[H_2O_2]_0}{2k_{13}}\right)^{1/2} \exp - \left(\frac{k_3[H_2O_2]_0 t}{2}\right) \tag{16}$$

$$[H_2O_2]_{R_{max}} = \frac{1}{k_3 t} \tag{17}$$

Confirmation of the validity of the kinetic analysis was first attempted via study of the reaction of · OH (from Fe^{II}EDTA and H_2O_2) with Bu^{t}OH, employing $[Fe^{II}]_0$ 6.7×10^{-4} mol dm^{-3}, $[EDTA]_0$ 1.34×10^{-3} mol dm^{-3} [Bu^{t}OH] 0.35 mol dm^{-3}, pH 7 (measured at the exit from the cavity) and t 0.027 s. The concentration of $[H_2O_2]_0$ was varied over the range 10^{-2}–10^{-3} mol dm^{-3}; as expected on the basis of Eq. (17) the predicted maximum in [· CH_2CMe_2OH] is obtained (Figure 5), and a value of $(7.6 \pm 0.9) \times 10^3$ dm^3 mol^{-1} s^{-1} derived from the value of $[H_2O_2]_{R_{max}}$ and t. In a further set of experiments, with $[H_2O_2]_0$ 5.3×10^{-3} mol dm^{-3} we explored the dependence of [R ·] upon t. As predicted, a plot of ln [· CH_2CMe_2OH] vs. t is a straight line (Figure 6) from which, via analysis according to Eq. (16), k_3 is calculated as $(7.1 \pm 0.4) \times 10^3$ dm^3 mol^{-1} s^{-1}, i.e. within experimental error the same as that calculated via Eq. (16).

The value for the rate constant for generation of · OH (k_3) from Fe^{II}EDTA and H_2O_2 is encouragingly close to those obtained by Rush and Koppenol[8] and Sutton and Winterbourn[15] (see Table III) which strongly suggests that the hydroxyl-radical generation pathway represents the dominant, if not sole, mechanism of reaction. The value of k_3 is somewhat lower than that obtained in our preliminary investigation[6,7] (reported as 1.4×10^4 dm^3 mol^{-1} s^{-1}), an apparent discrepancy which is believed to reflect the use of 1 : 1 ratio of Fe^{II} to EDTA in the earlier work (since Fe^{II}EDTA is known to form a 1 : 1 complex).[27]

Thus, via a set of subsequent experiments we have shown that k_3 is especially sensitive to the relative concentrations of Fe^{II} and EDTA when the latter is approximately the same as the former but insensitive to the ratio when the latter is in

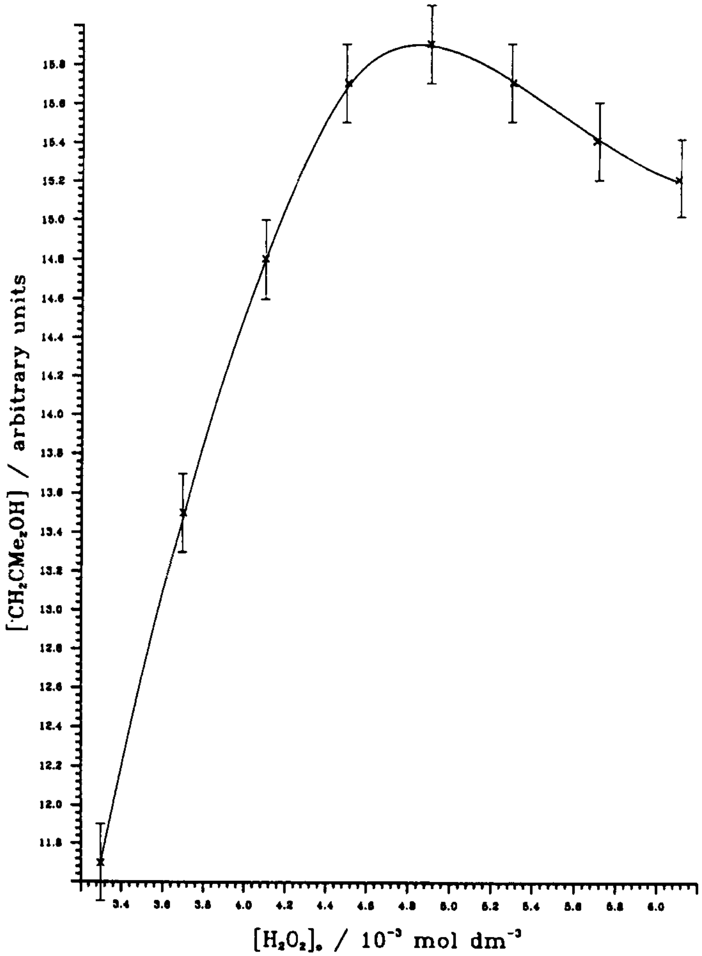

FIGURE 5 Variation of [$\cdot$ CH_2CMe_2OH] with $[H_2O_2]_0$ in experiment with the Fe^{II}EDTA/H_2O_2/Bu^tOH reaction, at pH 7.1, 296 K and with mixing time 27 ms.

significant excess (e.g. in *ca.* 2:1 ratio). Our experiments also confirm k_3 as $(1.0 \pm 0.1) \times 10^4 \, dm^3 mol^{-1} s^{-1}$ for *equimolar* concentrations with measured *exit* pH as 7.0, as noted before. The reason for this difference is that for the 1:1 ratio there is a significant change in pH between mixing and the point at which pH is measured *after* the cell [on account of the production of OH^- in reaction (3)]; indeed for an experiment with a measured final pH of *ca.* 7 an *initial* pH of 4.0 is calculated. In contrast, the addition of an excess of EDTA (e.g. with ratio *ca.* 2:1) provides an effective *buffering* action so that the measured final concentration is then approximately the same as that on mixing. Thus we believe that the appropriate value of k_3 at pH 7.1 is indeed $(7.1 \pm 0.4) \times 10^3 \, dm^3 mol^{-1} s^{-1}$ and that at pH *ca.* 4 the higher value (*ca.* $10^4 \, dm^3 mol^{-1} s^{-1}$) is appropriate. This was confirmed by experiments with Fe^{II}, EDTA (in two-fold excess) and a final pH of 4.0; consistent with this observation

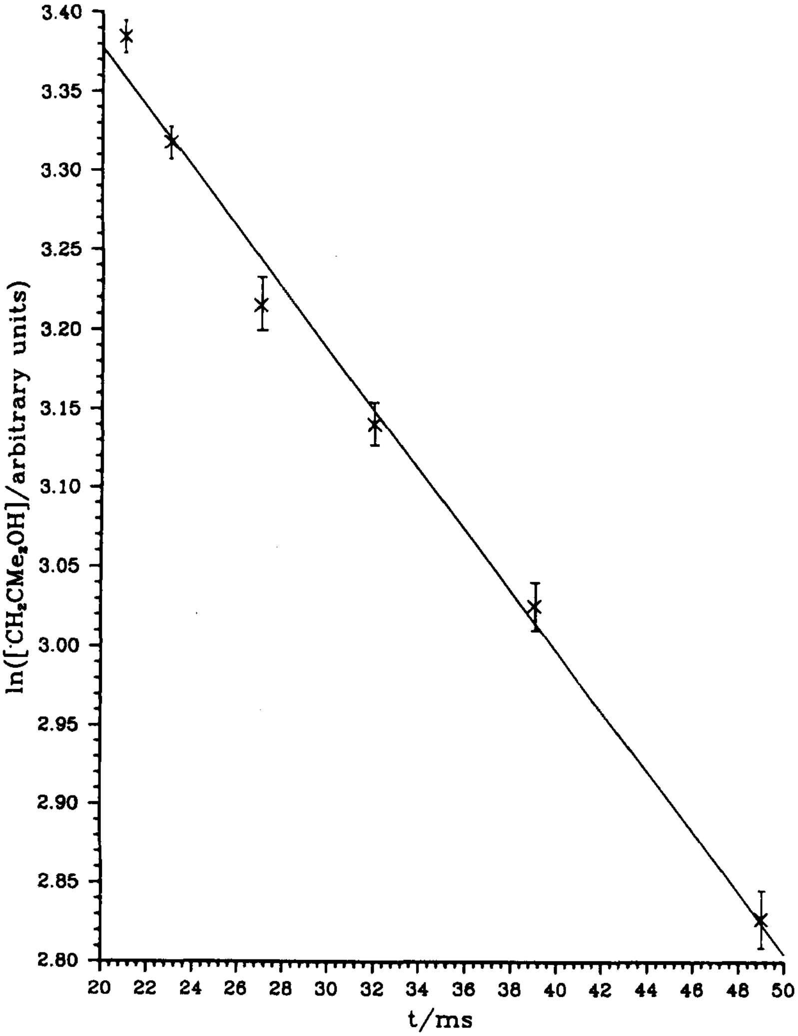

FIGURE 6 Variation of ln[$\cdot$ CH_2CMe_2OH] with time after mixing (*t*) in the $Fe^{II}EDTA/H_2O_2/Bu^tOH$ system at pH 7.3 and 296 K. $[Fe^{II}]_0$ 6.7×10^{-4}, [EDTA] 1.34×10^{-3}, $[H_2O_2]$ 5.3×10^{-3} $[Bu^tOH]$ 0.35 mol dm^{-3}.

it has been shown that the rate constant of the reaction of Fe^{II}EDTA and H_2O_2 increases with decrease in pH.[14]

All subsequent experiments were carried out under conditions which ensured an excess of chelate (with ratio at least 2 : 1) to buffer the reaction system. In this way we studied the reaction of $\cdot$OH with Bu^tOH in the presence of a variety of chelates of Fe^{II} (HEDTA, EDDA, DTPA, NTA, citrate and tartrate). In each case good kinetic behaviour [according to Eq. (16)] was observed; the resulting values of k_3 are shown in Table III.

TABLE III

Rate constants for the reaction between Fe^{II} and H_2O_2 in the presence of a variety of chelating agents at pH 7.2 (±0.1) and 296 (±2) K[a]

Chelate	$k/10^3\,dm^3\,mol^{-1}\,s^{-1}$	Lit values, $k/10^3\,dm^3\,mol^{-1}\,s^{-1}$
EDTA pH 7.2	7.1 ± 0.4	7,[11,15] 9,[14] 14[31]
pH 4	10 ± 1	[39 ($FeEDTA^-$)[14]]
HEDTA	10 ± 1	17,[14] 42[11]
EDDA[b]	23 ± 2	78[32]
DTPA	2.7 ± 0.6	0.4,[31] 0.5,[11] 0.8,[15] 1.3[9]
NTA[b]	9.7 ± 0.7	10,[15] 18,[14] 30[32]
Citrate	4.9 ± 0.2	4.9
Tartrate[b]	5.3 ± 0.8	

[a] Except where stated otherwise $[L]:[Fe^{II}]$ 2:1. Literature values refer to pH *ca.* 7 except where stated otherwise. Errors ± 1 S.D.

[b] $[L]:[Fe^{II}]$ 3:1.

Oxidation and Reduction of Radicals by Iron Complexes

The possibility that alkyl radicals containing electron-withdrawing groups (e.g. $\cdot CH_2CMe_2OH$) can be reduced by Fe^{II}EDTA [reaction (18); *cf.* Ref. 12] was explored via kinetic E.S.R. experiments in which the variation of $[R\cdot]$ ($\cdot CH_2CMe_2OH$) with Fe^{II} was explored. We first established that at relatively low concentrations of Fe^{II} compared with H_2O_2 [conditions under which Eq. (16) can be applied for kinetic analysis], the steady-state concentration was indeed proportional to $[Fe^{II}]_0^{1/2}$ as shown in Figure 7 (deviation from linearity in experiments of this type has been used previously to establish[28] the rapid reduction of α-keto conjugated radicals by Ti^{III} and Fe^{II}). Experiments were then conducted at low concentrations of H_2O_2 ($1.3 \times 10^{-3}\,mol\,dm^{-3}$) and a range of comparable Fe^{II} concentrations (6.7×10^{-4}–$5.4 \times 10^{-3}\,mol\,dm^{-3}$) in order to increase the rate of effective radical reduction; since Eq. (16) no longer holds under these conditions, we used a kinetic simulation approach to studying the variation of $\cdot CH_2CMe_2OH$ with $[Fe^{II}]_0$.

$$Fe^{II}EDTA + \cdot CH_2CMe_2OH \longrightarrow Fe^{III}EDTA + [^-CH_2CMe_2OH] \longrightarrow \text{products} \tag{18}$$

In a series of experiments with Fe^{II}EDTA and H_2O_2 with large excess of Bu^tOH (to scavenge $\cdot OH$) and mixing time 27 ms, we found that the concentration of $\cdot CH_2CMe_2OH$ increased with increasing $[Fe^{II}]_0$ up to a maximum at $[Fe^{II}]_0$ $2.7 \times 10^{-3}\,mol\,dm^{-3}$ (see Figure 8). Computer simulations were then carried out with the inclusion of the reactions between Fe^{II}EDTA and H_2O_2 (k $7.1 \times 10^3\,dm^3\,mol^{-1}\,s^{-1}$), $HO\cdot$ and Bu^tOH (k $5.8 \times 10^8\,dm^3\,mol^{-1}\,s^{-1}$)[23] the self-termination of $\cdot CH_2CMe_2OH$ (k $1.0 \times 10^9\,dm^3\,mol^{-1}\,s^{-1}$)[29] and different values of k_{18}: all other parameters employed were the same as those used in the experiments. As can be seen from Figure 8, the predicted variation in $[R\cdot]$ as $[Fe^{II}]_0$ is increased does indeed show a decrease after the initial build-up, for higher values of the rate constant for reduction. Comparison of experimental results and the computer simulation suggests that the rate constant for reaction (18) is *ca.* $2 \times 10^6\,dm^3\,mol^{-1}\,s^{-1}$ in aqueous solution at room temperature. This result and calculation, relevant to conditions under which $[Fe^{II}]_0 \geqslant [H_2O_2]$ adds further weight to our proposal that

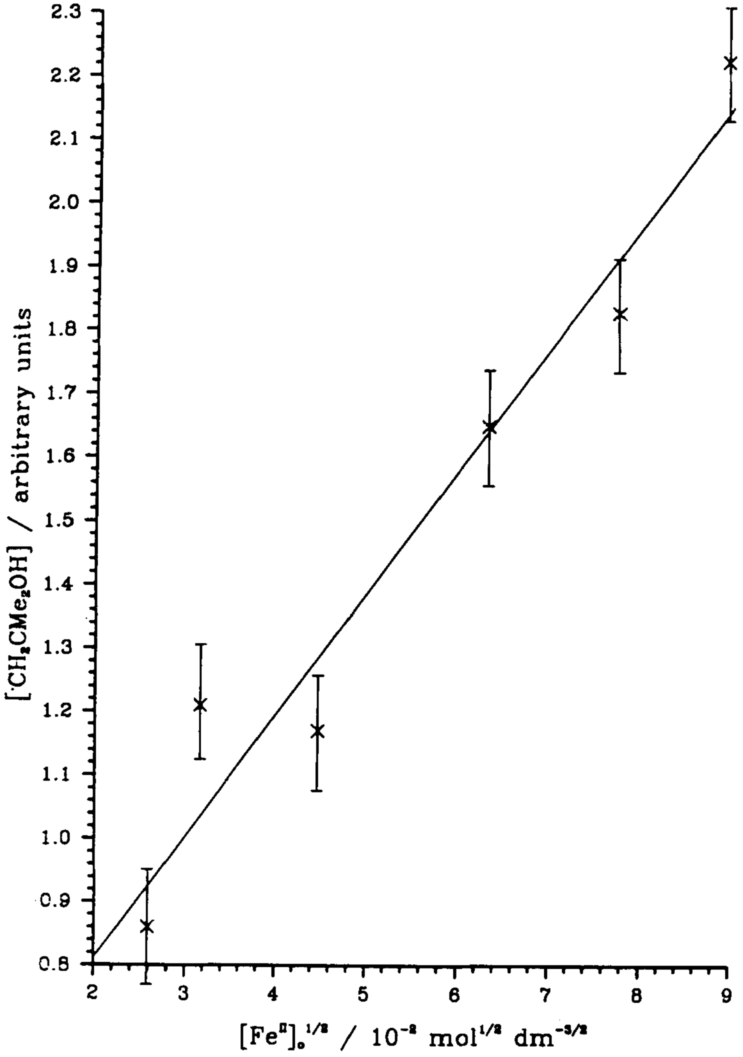

FIGURE 7 Variation of [· CH$_2$CMe$_2$OH] with [Fe^{II}]$_0^{1/2}$ in the Fe^{II}EDTA/H$_2$O$_2$/Bu^{t}OH system at pH 7.2 and 296 K, with mixing time 27 ms. 2[Fe^{II}]$_0$ = [EDTA]$_0$, [H$_2$O$_2$] 1.0 × 10^{-2}, [Bu^{t}OH] 0.35 mol dm^{-3}.

· OH is indeed formed but that interaction of Fe^{II} with · CH$_2$CMe$_2$OH, at least, can account for some of the anomalies previously reported.

We also employed a related approach to model the reaction of Fe^{II}EDTA and H$_2$O$_2$ in the presence of EtOH. In a series of experiments at pH 7 with [H$_2$O$_2$]$_0$ 5 × 10^{-3} mol dm^{-3}, [EtOH]$_0$ 0.6 mol dm^{-3} and t 0.027 s we monitored the steady state concentration of the α and β radicals, · CHMeOH and · CH$_2$CH$_2$OH respectively, as a function of [Fe^{II}]$_0$ in the range 3–9 × 10^{-4} mol dm^{-3}. As can be seen from Figure 9, there is reasonable agreement between the absolute value of this ratio and its dependence on [Fe^{II}]$_0$ and the calculations which incorporated the following kinetic

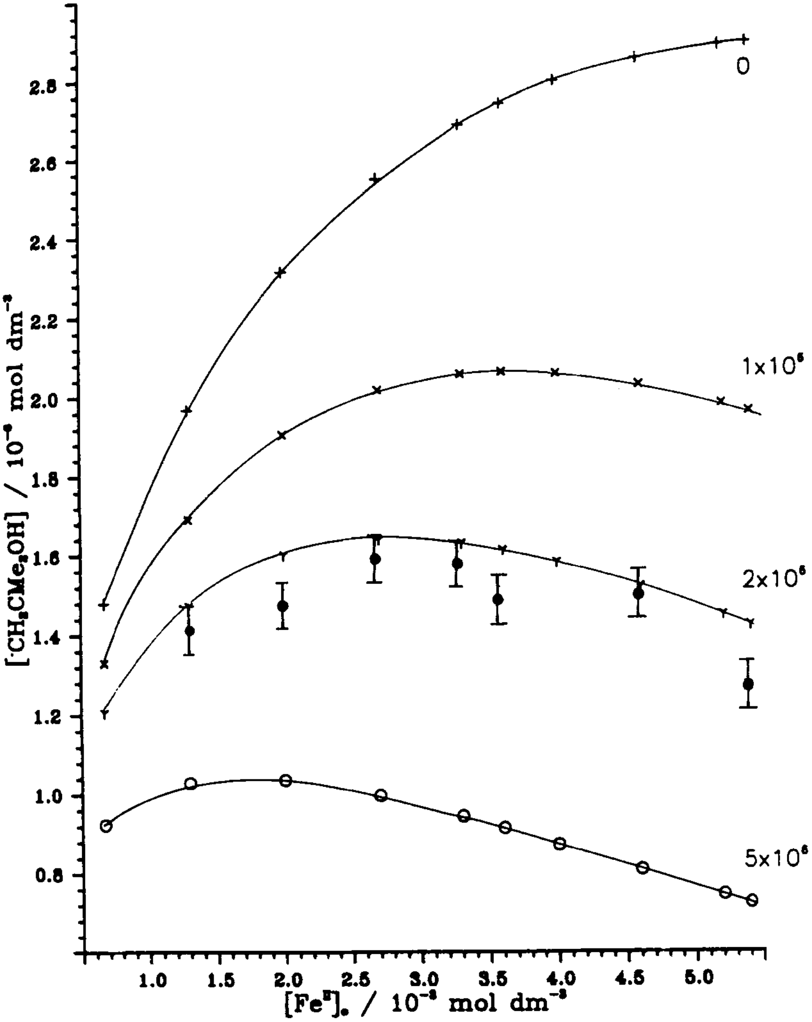

FIGURE 8 Kinetic simulations of the predicted variation of [$\cdot CH_2CMe_2OH$]$_0$ with [Fe^{II}]$_0$ for different values of k_{18} in the $Fe^{II}EDTA/H_2O_2/Bu^tOH$ system, together with experimental results. For [H_2O_2]$_0$ 1.3×10^{-3}, [Bu^tOH]$_0$ 0.35 mol dm,$^{-3}$, mixing time 27 ms.

parameters (k): for $Fe^{II}EDTA + H_2O_2$ 7.1×10^3, $HO\cdot + EtOH$ $1.9 \times 10^9 (\alpha)$, $1.5 \times 10^8 (\beta)$,[6] $\cdot CHMeOH + H_2O_2$ [reaction (7)] 1.5×10^5, $\cdot CH_2CH_2OH + Fe^{II}EDTA$ 2×10^6 (see earlier) and radical termination $1 \times 10^9 dm^3 mol^{-1} s^{-1}$ together with a rate constant for oxidation of $\cdot CHMeOH$ by Fe^{III} [reaction (11)] of the order of $10^9 dm^3 mol^{-1} s^{-1}$. We believe that our experiments confirm that the "anomalous" $\alpha : \beta$ ratio observed in the E.S.R. experiment simply reflects the occurrence of oxidation of the electron-rich α-radical by Fe^{III} and that the rate constant is in the range $1.0–1.5 \times 10^9 dm^3 mol^{-1} s^{-1}$. A similar analysis has recently been

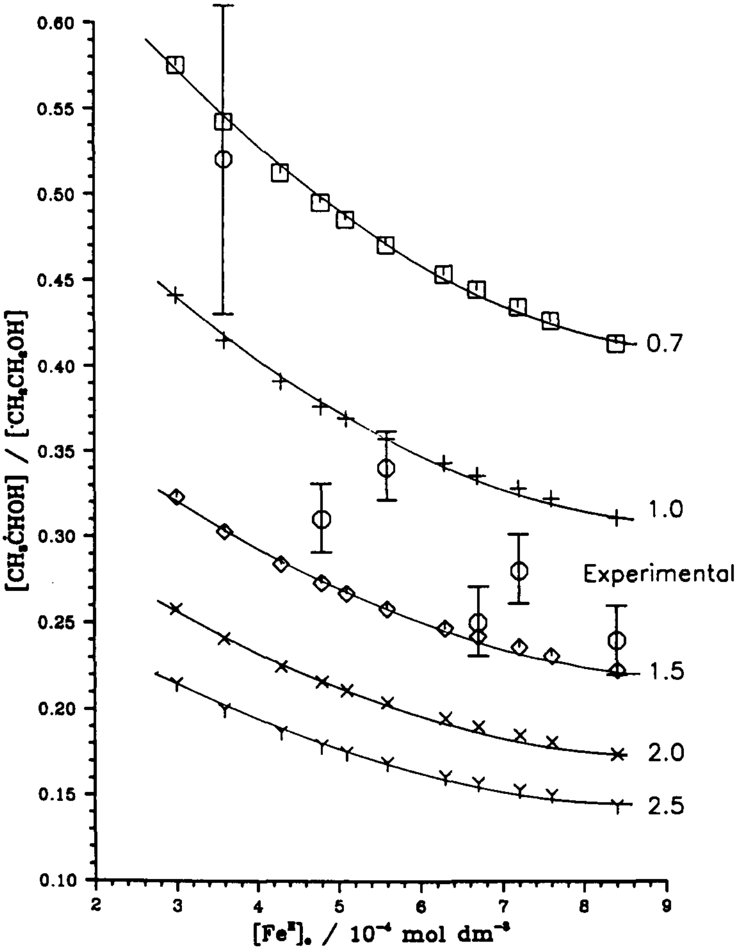

FIGURE 9 Experimental dependence of $[\cdot CHMeOH]/[\cdot CH_2CH_2OH]$ on $[Fe^{II}]_0$ in experiments with pH 7.1, 296 K, $[H_2O_2]_0$ 5.0×10^{-3}, $[EtOH]_0$ 0.6 mol dm^{-3}, t 27 ms, $[EDTA] = 2 \times [Fe^{II}]_0$, together with simulations employing these conditions, different rate constants for the oxidation of $\cdot CHMeOH$ by Fe^{III} ($k/10^9$ dm^3 mol^{-1} s^{-1}) and other rate constants given in the text.

employed by us to obtain rate constants of radical oxidation and reduction by a number of iron complexes of polyaminophosphonic acids.[30]

CONCLUSIONS

We believe that a consistent picture emerges from the results of E.S.R. experiments on the Fenton reaction between $Fe^{II}EDTA$ (and some related ligands) and H_2O_2 in the presence of organic substrates. The crucial intermediate produced under the conditions employed (mostly with $[H_2O_2] > [Fe^{II}]$) is indeed the hydroxyl radical (effectively "free" rather than in "bound" or complexed form).

Kinetic analysis of the formation and reaction of the intermediate formed from Fe^{II}

and H_2O_2 (and which reacts to give organic radicals) is entirely as expected if the hydroxyl radical is indeed formed as the sole transient; further, it is encouraging to note that the rate constants derived from the Fenton reaction in aqueous solution at ambient temperature broadly match those reported using other techniques, including recent spin-trapping experiments[31] (see Table III). For results at pH 7 there is best agreement between other results and those of Borggaard[14] and Sutton and Winterbourn.[15]

In comparing the rate constants for the different ligands we note that the order $Fe^{II}aq < Fe^{II}$ citrate $< Fe^{II}$EDTA is as expected if, in an outer-sphere reaction, the rate constant is governed largely by thermodynamic factors (E values of 0.71,[16] 0.6,[33] and 0.12,[16] respectively); however, the observation of a relatively low rate constant for Fe^{II}DTPA (for which E is 0.03)[16] indicates that an inner sphere process is probably involved.

Kinetic analysis of steady state experiments with certain substrates (e.g. EtOH, Bu^tOH) provides further evidence that reactions of Fe^{III} and Fe^{II} with some intermediate radicals (e.g. $\cdot CHMeOH$ and $\cdot CH_2CMe_2OH$, respectively) must be taken into account if a full and detailed understanding of the reaction system is to be obtained.

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