Mechanisms of Peroxide Stabilization. An Investigation of some Reactions of Hydrogen Peroxide in the Presence of Aminophosphonic Acids

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It has been established by continuous-flow studies in conjunction with EPR spectroscopy that the aminophosphonic acids **1**–**4** accelerate significantly the Fenton reaction between Fe^{II} and H_2O_2 in aqueous solution *via* complexation of the metal ion (with values of the rate constant *k* for the generation of the hydroxyl radical up to 2×10^5 dm^3 mol^{-1} s^{-1} at room temperature). To a certain extent this behaviour parallels that of EDTA and some structurally-related aminocarboxylic acids. It is also shown that the *N*-oxides of the aminophosphonic acids **1**–**3** react readily with the hydroxyl radical to give long-lived nitroxides *via* β-scission of first-formed carbon-centred radicals.

Neither of these findings is believed to correspond to the major chemistry which underlies the efficacy of these ligands as peroxide stabilizers. It is suggested instead that the crucial role of these compounds depends upon their ability to stabilize the higher valence state of iron, and hence not only to encourage oxidation of Fe^{II} by $O_2^{\bullet -}$ and H_2O_2 but also to prevent effective reduction of Fe^{III} by $O_2^{\bullet -}$, $HO_2^{\bullet}$ and H_2O_2. However, radical scavenging by *N*-oxides may be a secondary, contributory factor in this stabilizing function, especially in peroxide systems when the sequestrant is added before storage, when slow *N*-oxidation is to be expected.

Haber and Weiss proposed that the decomposition of hydrogen peroxide induced by Fe^{III} occurs *via* a mechanism involving $HO_2^{\bullet}$ [reactions (1)–(3)].[1] Although reactions (2) and (3) are believed to be energetically favourable, and reaction (3) is reported[2] to have a rate constant of *ca.* 10^7 dm^3 mol^{-1} s^{-1}, reaction (2) proceeds only very slowly, if at all.[3] However, some metal ions (including iron) can evidently catalyse reaction (2) *via* reactions (4) and (5) (the so-called Fenton reaction), thus giving an effective chain reaction for decomposition of H_2O_2 involving (*e.g.*) reactions (1), (4) and (5).

$$Fe^{III} + H_2O_2 \longrightarrow Fe^{II} + HO_2^{\bullet} + H^+ \qquad (1)$$

$$HO_2^{\bullet} + H_2O_2 \longrightarrow HO^{\bullet} + H_2O + O_2 \qquad (2)$$

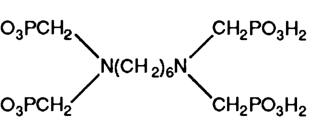
$$HO^{\bullet} + H_2O_2 \longrightarrow HO_2^{\bullet} + H_2O \qquad (3)$$

$$Fe^{III} + HO_2^{\bullet} \longrightarrow Fe^{II} + O_2 + H^+ \qquad (4)$$

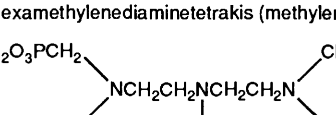
$$Fe^{II} + H_2O_2 \longrightarrow Fe^{III} + HO^- + HO^{\bullet} \qquad (5)$$

A number of aminophosphonic acids and their salts (*e.g.* **1**) which are available under the trade marks "Dequest" and "Briquest"† are examples of compounds used to stabilize solutions of hydrogen peroxide (their carboxylic acid analogues are also known to stabilize peroxides[4,5]). Although little detailed research has been reported, the aminophosphonic acids are known to be readily oxidized by hydrogen peroxide to *N*-oxides which retain their ability to complex metal ions[6] and stabilize peroxides.[7]

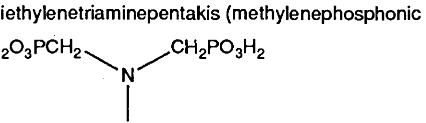
Several mechanisms can be envisaged for the stabilization of peroxide solutions by these compounds. For example, they could act primarily either as metal-ion sequestrants or as inhibitors of radical reactions.[4,5] For instance, stabilization by sequestration of metal ions would occur if the rates of some or all of reactions (1), (4) and (5) are slowed significantly. [This might be expected, for example, if chelation of the metal ion alters the redox potential of the Fe^{III}/Fe^{II} couple such that it is significantly outside the range from −0.33 V (E° for $O_2/O_2^{\bullet -}$) to 0.46 V (E° for $H_2O_2/HO^{\bullet}$, H_2O at pH 7)].[8] Alternatively, stabilization would also occur if the aminophosphonic acids themselves were to scavenge the key intermediates $HO^{\bullet}$ and/or $HO_2^{\bullet}$ to produce relatively unreactive radicals; this would have the effect of preventing the occurrence of reactions (3) and (4).



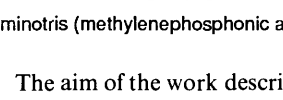
Ethylenediaminetetrakis (methylenephosphonic acid) **1**



Hexamethylenediaminetetrakis (methylenephosphonic acid) **2**



Diethylenetriaminepentakis (methylenephosphonic acid) **3**



Aminotris (methylenephosphonic acid), Nitrilotrismethylenephosphonic acid **4**

The aim of the work described here was to investigate several aspects of the probable rôle(s) of some aminophosphonic acids and their *N*-oxides, as well as some carboxylic acid analogues. In particular, we have employed EPR spectroscopy both to

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† DEQUEST is a trade name used by the manufacturer Monsanto and BRIQUEST is used by Albright and Wilson. [(1), Dequest 2041, Briquest 422; (2) Dequest 2051, Briquest 462; (3) Dequest 2060, Briquest 543; (4) Dequest 2000, Briquest 301].

study the reactions of Fe^{II} with H_2O_2 to give $^{\bullet}OH$ (chosen as a model reaction) in the presence of the ligands and also to investigate some of the radical reactions of the compounds and derivatives.

We have focussed attention in particular on the DEQUESTS **1**–**4** and their carboxylic acid analogues EDTA (ethylenediaminetetracetic acid), DTPA (diethylenetriaminepentaacetic acid), and NTA (nitrilotriacetic acid). These compounds and their carboxylic analogues are known to be extremely good chelating agents, with very high stability constants for the first transition-metal series (10^{10}–10^{28}).[9] It is also known,[10] for example, that EDTA complexes of Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+} and Cu^{2+} contain on average more water molecules in the first coordination sphere than DTPA analogues (*e.g.* Fe^{III}–EDTA has a hydration number of 1, whereas for Fe^{III}–DTPA it is 0.29):[7] complexes of **1** with several first-row transition metals have a higher hydration number than those of **3**, both of which have larger hydration numbers than their carboxylic analogues (so that the metal ion is not as effectively surrounded by the chelate as the carboxylic acid complexes).[10,11] On the other hand, for Fe^{III} the complexes of **1** and **3** each have one water molecule in the coordination sphere (as for EDTA).[10,11] Little is known about the complexes of **2** and **4**, but Fe^{II}–NTA is believed to form a 1:1 complex with two molecules of water in the first coordination sphere.[12]

Results and Discussion

Determination of the Rate Constants for the Reaction of Fe^{II}-*complexes with Hydrogen Peroxide* via *Steady-state Analysis of EPR Flow Experiments.*—The reaction of Fe^{II} with H_2O_2 (the Fenton reaction) is generally believed to produce the hydroxyl radical[13] [reaction (5)], a finding supported by EPR studies,[14] though it has also been suggested that the reactive intermediate formed is an iron(IV) ferryl species (FeO^{2+}) or an iron–peroxo complex [reaction (6)] (see refs. 15–19). The postulated complex

$$Fe^{II} + H_2O_2 \longrightarrow [Fe^{II}\text{–}HOOH] \longrightarrow [Fe^{IV}{=}O] + H_2O \quad (6)$$

is proposed to have a generally similar reactivity to the hydroxyl radical but it is claimed to be less reactive towards certain substrates, for example 2-methylpropan-2-ol, and acetate and bromide ions (see *e.g.* ref. 17). However, the anomalously low reactivity may reflect in part the fact that Fe^{II} is an effective reductant for certain radicals (*e.g.* $^{\bullet}CH_2CMe_2OH$) and it is perhaps notable therefore that most discrepancies occur with substances that produce radicals which might be expected to be relatively easily reduced.[14,18]

The reaction of uncomplexed iron(II) with hydrogen peroxide is relatively slow, with a rate constant of *ca.* 75 $dm^3\ mol^{-1}\ s^{-1}$ at room temperature in acidic aqueous solution.[13] Complexation of Fe^{II} to EDTA, DTPA or NTA has been found to enhance dramatically the rate constant for this reaction (up to *ca.* $10^4\ dm^3\ mol^{-1}\ s^{-1}$)[12,20,21] while complexation to bipyridyl reduces the rate constant to $10^{-4}\ dm^3\ mol^{-1}\ s^{-1}$.[22]

Studies were first undertaken with Fe^{II} complexes of **1**–**4** in order to determine whether these ligands enhance or retard the rate of reaction of Fe^{II} with H_2O_2. Our approach involved the continuous mixing of Fe^{II} (complexed), H_2O_2, and Bu^tOH [a scavenger for the initially formed hydroxyl radical (or other reactive intermediate)] in the cavity of an EPR spectrometer and monitoring of the concentration of $^{\bullet}CH_2CMe_2OH$ at a point about 30 ms after mixing. It has been shown[23] that under these circumstances a steady-state radical concentration is achieved in the cavity and that suitable kinetic analysis can yield useful information about radical formation and removal. For example, in experiments involving a low-valent metal ion (*e.g.* Fe^{II}, Ti^{III}) and hydrogen peroxide (present in excess), in which $^{\bullet}OH$ is generated and then scavenged by a suitable substrate to give a radical which is removed *via* bimolecular termination [reactions (7)–(9)], then the radical concentration is given by eqn. (10). It follows that a plot of ln $[R^{\bullet}]$ *vs.* *t* (the time after mixing) should be a straight line (*cf.* refs. 23, 24) and that $[R^{\bullet}]$ should vary with H_2O_2 in a manner which gives a maximum ($[R^{\bullet}]_{max}$) at a concentration of peroxide related to k_7 and *t* [eqn. eqn.(11)]. This type of analysis provides a useful method to determine k_7.[14,23–26]

$$M^{n+} + H_2O_2 \xrightarrow{k_7} M^{(n+1)} + HO^{\bullet} + HO^- \quad (7)$$

$$^{\bullet}OH + Bu^tOH \xrightarrow{k_8} H_2O + {}^{\bullet}CH_2CMe_2OH \quad (8)$$

$$2{}^{\bullet}CH_2CMe_2OH \xrightarrow{2k_t} \text{non-radical products} \quad (9)$$

$$[R^{\bullet}]_{ss} = \sqrt{\frac{k_7[M^{n+}]_0[H_2O_2]_0}{2k_t}} \exp\left(-k_7[H_2O_2]t/2\right. \quad (10)$$

$$[H_2O_2] \text{ for } [R^{\bullet}]_{max} = \frac{1}{k_7t} \quad (11)$$

For most of the Fe^{II}–aminophosphonic acid complexes studied, satisfactory signals from $^{\bullet}CH_2CMe_2OH$ could only be obtained when dilute hydrogen peroxide solutions were employed (*e.g.* $[H_2O_2] \leqslant 10^{-3}\ mol\ dm^{-3}$) and with $[Fe^{II}]$ typically $10^{-3}\ mol\ dm^{-3}$ or less): in contrast, signals could be obtained with $H_2O_2 \geqslant 10^{-2}\ mol\ dm^{-3}$ in experiments with Fe^{II} itself or with EDTA.* The effect of variation in $[H_2O_2]$ on $[^{\bullet}CH_2CMe_2OH]$ in experiments with **3** as complexing agent at pH 6 is shown in Fig. 1: though the observation of a 'peak' in $[^{\bullet}CH_2CMe_2OH]$ is in accord with expectation, the rate constant(s) for initiation cannot be obtained reliably *via* the application of the steady-state approach [and eqn. (11)], since the condition $[H_2O_2] \gg [Fe^{II}]$ is not satisfied (more dilute solutions of Fe^{II} could not be reliably employed because these proved to be prone to aerial oxidation). Nevertheless, we can conclude at this stage that the reaction of this Fe^{II} complex with H_2O_2 must be considerably faster than that of, for example, Fe^{II}–EDTA. Use of eqn. (11) for the peroxide concentration which gives the maximum observed radical concentration gives an *estimate* of *ca.* $3 \times 10^4\ dm^3\ mol^{-1}\ s^{-1}$ for k_7 for complex **3** (see Fig. 1).† Since maximum radical concentrations were similarly observed for **1** and **4** with $[H_2O_2]$ *ca.* 7×10^{-4} mol dm^{-3} these chelates clearly further accelerate the reaction to give $^{\bullet}OH$; in contrast, chelate **2** retards it somewhat compared to **3**, a value of *ca.* $1 \times 10^4\ dm^3\ mol^{-1}\ s^{-1}$ being derived from the 'peaking' behaviour.

More accurate rate constants (see Table 1) for reaction of the Fe^{II} complexes with H_2O_2 to give $^{\bullet}OH$ were obtained by computer simulation of the observed steady-state concentrations, as described previously (see *e.g.* refs. 25, 26). The scheme employed reactions (7), (8) and (9), taking[27,28] k_8 as $5.8 \times 10^8\ dm^3\ mol^{-1}\ s^{-1}$ and $2k_t$ for reaction (9) as $1 \times 10^9\ dm^3\ mol^{-1}\ s^{-1}$. The calculated effect of changing the rate constant for the reaction of Fe^{II} and H_2O_2 on the steady-state concentration of

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* Concentrations given in the text are those *after* mixing.

† Under the conditions employed, with low $[Fe^{II}]_0$, no contribution from reduction or oxidation of $^{\bullet}CH_2CMe_2OH$ by iron complexes would be anticipated.[14]

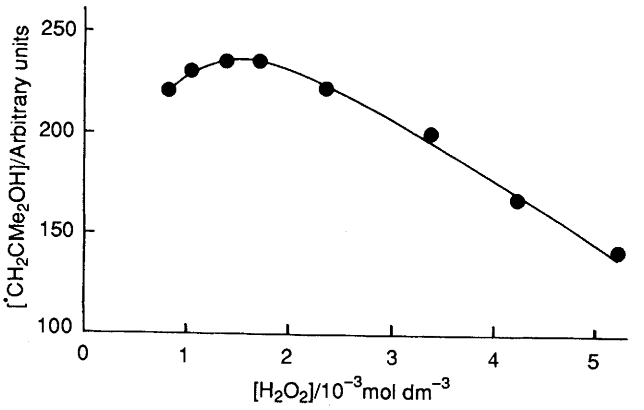

Fig. 1 Variation of [$^{\bullet}CH_2CMe_2OH$] with initial concentration of H_2O_2 ($[H_2O_2]_0$) in steady-state EPR experiments with the Fe^{II} complex of **3** (0.5[**3**] = [Fe^{II}] = 7 × 10^{-4} mol dm^{-3}, Bu^tOH 0.35 mol dm^{-3} at pH 6; mixing time 30 ms)

Table 1 Rate constants for the Fenton reaction between H_2O_2 and Fe^{II} complexes[a]

Complex	$k/dm^3\ mol^{-1}\ s^{-1}$
$Fe^{II}aq$[b]	75
Fe^{II}-EDTA[c]	7 × 10^3
Fe^{II}-DTPA[c]	3 × 10^3
Fe^{II}-NTA[c]	1 × 10^4
Fe^{II}-**1**[d]	2 × 10^5
Fe^{II}-**2**[d]	1 × 10^4
Fe^{II}-**3**[d]	4 × 10^4
Fe^{II}-**4**[d]	2 × 10^5

[a] Values refer to aqueous solution at pH 6–7 and *ca.* 20 °C unless stated otherwise. [b] Acid solution: *cf.* ref. 13. [c] Ref. 21; see also refs. 14, 18. [d] Error estimated as ±15%.

$^{\bullet}CH_2CMe_2OH$ as a function of [H_2O_2] is shown in Fig. 2: as predicted earlier, an increase in k_7 leads to a decrease in the position of the maximum in the variation of $[R^{\bullet}]_{ss}$ with [H_2O_2] as well as to a reduction in $[R^{\bullet}]_{ss}$. Optimum agreement with experiment was obtained (both in terms of the maximum in [$R^{\bullet}$] and the change in overall magnitude) for a rate constant of 4 × 10^4 dm^3 mol^{-1} s^{-1} for chelate **3** and a value of 2 × 10^5 dm^3 mol^{-1} s^{-1} for chelates **1** and **4**. For chelate **2** an alternative approach, involving a plot of ln [$R^{\bullet}$] *vs.* *t* was also employed [for experiments in which the overall flow-rate was varied: see eqn. (10) and Fig. 3]. This confirmed the initiation rate constant for this complex as 1 × 10^4 dm^3 mol^{-1} s^{-1}.

We also employed a kinetic simulation approach to gauge the effect on $[R^{\bullet}]_{ss}$ of the incorporation of the reduction by Fe^{II} of $^{\bullet}CH_2CMe_2OH$ [reaction (12)] (see *e.g.* refs. 14, 21, 29). The

$$Fe^{II} + {}^{\bullet}CH_2CMe_2OH \longrightarrow Fe^{III} + CH_2{=}CMe_2 + OH^- \quad (12)$$

results establish that, as the rate constant of this reaction is increased from 10^3 dm^3 mol^{-1} s^{-1}, there is little effect on the predicted value of the concentration of H_2O_2 which gives $[R^{\bullet}]_{max}$ (see *e.g.* Fig. 4) but that the predicted concentration of $[R^{\bullet}]_{max}$ is very sensitive to k_{12} at higher reduction rates. The observed behaviour of absolute radical concentration of $^{\bullet}CH_2CMe_2OH$ as a function of H_2O_2 in the presence of the ligands **1**–**4** indicates that there is little or no removal of $^{\bullet}CH_2CMe_2OH$ and that the maximum value for k_{12} is in each case *ca.* 10^6 dm^3 mol^{-1} s^{-1}.

Our results establish that the rate constants for the reaction of

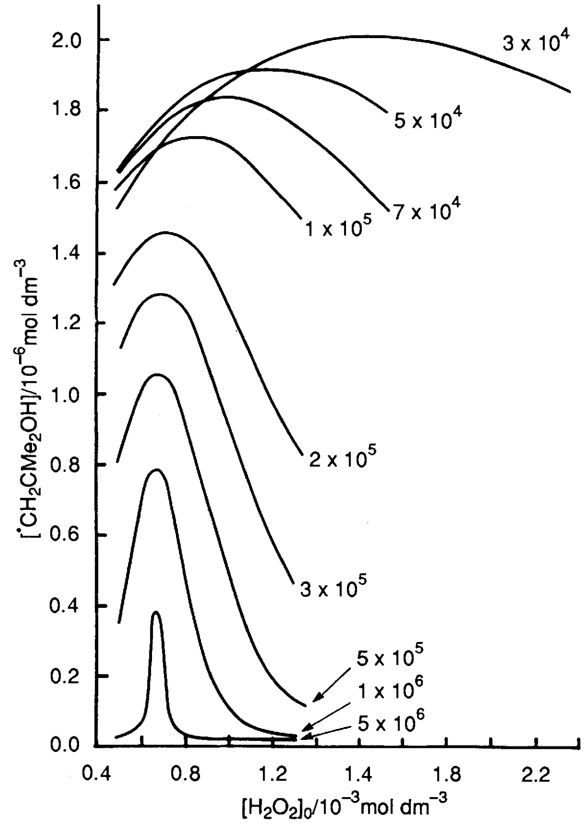

Fig. 2 Simulated variation of [$^{\bullet}CH_2CMe_2OH$] with $[H_2O_2]_0$ as a function of k_7 (the initiation rate) for experiments in which $^{\bullet}OH$, generated from Fe^{II} and H_2O_2, reacts with Bu^tOH to give $^{\bullet}CH_2CMe_2OH$. Input concentrations and mixing time as in Fig. 1; k_8 5.8 × 10^8 dm^3 mol^{-1} s^{-1}, $2k_t$ 10^9 dm^3 mol^{-1} s^{-1}.

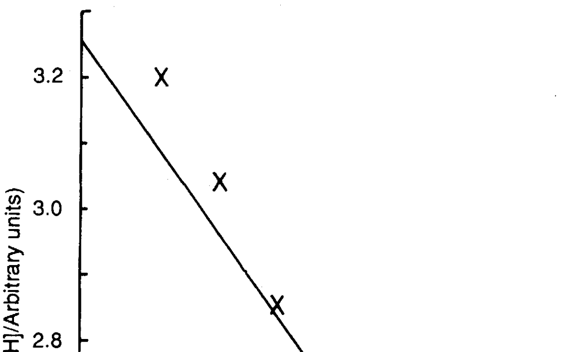

Fig. 3 Variation in ln [$^{\bullet}CH_2CMe_2OH$] with mixing time for an EPR flow-system study on a reaction mixture containing Fe^{II} (with **2** as ligand), H_2O_2, and Bu^tOH ($[Fe^{II}]_0$ = $0.5[\mathbf{2}]_0$ = 7 × 10^{-4} mol dm^{-3}, $[H_2O_2]_0$ = 7.5 × 10^{-3} mol dm^{-3}, $[Bu^tOH]_0$ 0.35 mol dm^{-3}; pH 7)

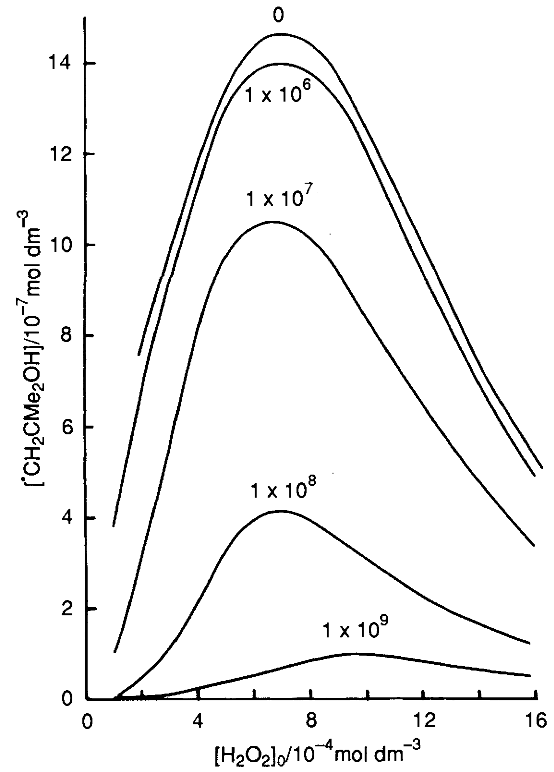

Fig. 4 Simulated variation of [$^{\bullet}CH_2CMe_2OH$] with H_2O_2 as a function of k_{12} (the rate constant for reduction of $^{\bullet}CH_2CMe_2OH$ by Fe^{II}); other input parameters as given in the legends for Figs. 1 and 2

the Fe^{II} complexes with H_2O_2 are considerably higher than for the uncomplexed ion and also higher than for the corresponding aminocarboxylic acid complexes. This may reflect an increase in thermodynamic driving force associated with a reduction in E° of the Fe^{III}/Fe^{II} couple (in an outer-sphere process) or the greater availability of water-bound sites for peroxide attack (in an inner-sphere process); however, it is clear that the aminophosphonic acids do not stabilize hydrogen peroxide by retarding the rate of reaction of Fe^{II} with the peroxide.

Oxidation of Radicals by Fe^{III} *Complexes*.—The oxidation of organic radicals by Fe^{II} [reaction (13)] provides a route, in

$$Fe^{III} + R^{\bullet} \longrightarrow Fe^{II} + R^{+} \qquad (13)$$

addition to reaction (4), for the regeneration of Fe^{II}, and hence it might be expected that the presence of organic substrates might also catalyse the decomposition of peroxides. Experiments were therefore performed to determine whether Fe^{III} complexes of **1**–**4** are effective oxidants of electron-rich carbon-centred radicals.

The reaction of the hydroxyl radical with ethanol is known to produce preferentially the α-radical ($^{\bullet}CHMeOH$) [in *ca.* thirteen times excess over the β-radical, reactions (14) and (15)].[14] However, the presence of powerful oxidants can

$$HO^{\bullet} + MeCH_2OH \longrightarrow {}^{\bullet}CHMeOH + H_2O \qquad (14)$$

$$HO^{\bullet} + MeCH_2OH \longrightarrow {}^{\bullet}CH_2CH_2OH + H_2O \qquad (15)$$

dramatically reduce the concentration of α-radical detected in steady-state EPR experiments. For example, we have shown[14] that when Fe^{II}–EDTA and Fe^{II}–DTPA are employed as initiators in a flow system, [$^{\bullet}CHMeOH$] is typically considerably less than [$^{\bullet}CH_2CH_2OH$] (*ca.* 30%) which is attributed to its oxidation by Fe^{III}–EDTA and Fe^{III}–DTPA [reaction (16) for

$$^{\bullet}CHMeOH + Fe^{III} \longrightarrow Fe^{II} + {}^{+}CHMeOH \qquad (16)$$

which rate constants of 5×10^8 and 1.8×10^9 dm^3 mol^{-1} s^{-1}, respectively, have been estimated[14]].

When ethanol (added in considerable excess) was employed as substrate for the Fe^{II}–H_2O_2 couple in the presence of **1** the observed concentration of the α-radical [$^{\bullet}CHMeOH$] was found to be *ca.* 70% of the observed value of [$^{\bullet}CH_2CH_2OH$] (in experiments with [Fe^{II}] 7×10^{-4} mol dm^{-3}, [H_2O_2] 2×10^{-3} mol dm^{-3} at pH *ca.* 7).* This indicates that the Fe^{III} complex of **1** is a slightly less effective oxidant for $^{\bullet}CHMeOH$ then Fe^{III}–EDTA and Fe^{III}–DTPA: kinetic simulation leads to an estimate of 5×10^7 dm^3 mol^{-1} s^{-1} for reaction (16). For the other complexes the [α]/[β] ratio was typically 0.3 under steady-state conditions, for which an oxidation rate of *ca.* 10^8 dm^3 mol^{-1} s^{-1} is appropriate.

As the overall rate of oxidation of $^{\bullet}CHMeOH$ by these Fe^{III} complexes is still fast (though somewhat less than for EDTA and DTPA), it seems unlikely that the role of these ligands in peroxide stabilization is to prevent the regeneration of low-valent metal ions (*e.g.* Fe^{II}) *via* oxidation of organic radicals.

Investigation of the Reaction of the Aminophosphonic Acids, their N*-Oxides, and Related Compounds as Radical Inhibitors*.—(a) *Reaction of* $^{\bullet}OH$ *with aminophosphonic acids and their carboxyl analogues*. When EDTA was employed as the substrate for $^{\bullet}OH$, generated with the Ti^{III}/H_2O_2 couple, EPR signals were detected, as noted before, from the radical $^{-}O_2C\dot{C}HN(CH_2CO_2^{-})CH_2CH_2N(CH_2CO_2^{-})_2$: the signals were found to be strongest in mildly basic solution (pH 7–8).[30] The mechanism of reaction is believed to be either direct hydrogen-atom abstraction or electron-transfer from nitrogen (to give a radical-cation) followed by rapid deprotonation.[31] The fact that only weak signals were detected in acid presumably reflects the nitrogen's protonation, and the detection of only $^{-}O_2C\dot{C}HNR_2$, rather than the carbon-centred radical derived from attack at the ethylene bridge, may reflect in part the expected greater (capto-dative) stability of the radical with +M and −M substituents;[32] oxidative destruction by H_2O_2 of the amino-substituted radical [reaction (17)] would be expected to be very rapid.[14,30]

$$R^1{}_2N\dot{C}HR^2 + H_2O_2 \longrightarrow R^1{}_2\overset{+}{N}{=}CHR^2 + HO^{\bullet} + HO^{-} \qquad (17)$$

The reaction of $^{\bullet}OH$ with NTA led to the detection of $^{-}O_2C\dot{C}HN(CH_2CO_2^{-})_2$, in accord with earlier studies.[33] No signals were observed with DTPA under these conditions: as has been established previously, this reflects a dramatic reduction in the rate of generation of $^{\bullet}OH$ by the reaction of H_2O_2 with the Ti^{III}–DTPA complex.[14]

No signals could be detected in a variety of experiments in the pH range 2–10 with Ti^{III} and H_2O_2 in the presence of the substrates **1**–**4** (added in considerable excess, so that they act as ligands and substrates). This may reflect a significant retardation of the rate of initiation of the Ti^{III}–H_2O_2 reaction: on the other hand it seems more likely that, as with the Fe^{II} complexes, the initiation reactions are fast (possibly too fast for convenient study) and that radical destruction [*e.g. via* reaction (17)] is also effective. These possibilities were not explored further; however,

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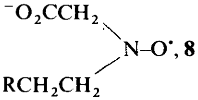
* In experiments at higher pH (7–8), as with other complexes, the overall signal intensity was reduced, but the α/β ratio increased. This may indicate that under these conditions the initiation rate is increased, as the complexes are ionized, but with a reduction in the rate(s) of oxidation. These effects were not further explored.

Table 2 EPR parameters (a/mT, g) for nitroxides detected in aqueous solution during the reaction between $^\bullet$OH and some *N*-oxides[a,b]

Substrate	Nitroxide	a(N)	a(β-H)	$a(^{31}P)$	g
EDTA *N,N'*-dioxide	$(^-O_2CCH_2)_2NO^\bullet$, **5**	1.53	0.83 (4H)	—	2.0056
N,N dioxide of **1**	$(^-O_3PCH_2)_2NO^\bullet$, **6**	1.58	0.95 (4H)	4.50 (2P)	2.0055
DPTA *N,N',N''*-trioxide	 $(^-O_2CCH_2)(RCH_2CH_2)N{-}O^\bullet$, **8** $[R = N(O)(CH_2CO_2^-)_2]$ and **5**	1.57	1.17 (2H) 0.77 (2H)	—	2.0058
N,N',N''-trioxide of **3**	**6**				

[a] For conditions, see text. [b] Splittings ± 0.01 mT, g ± 0.0001.

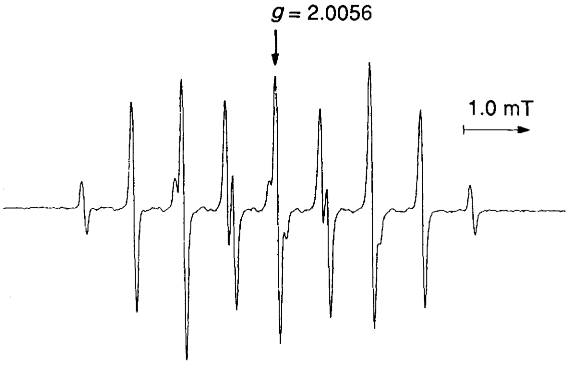


Fig. 5 EPR spectrum from the nitroxide $(^-O_2CCH_2)_2NO^\bullet$ **5**, obtained from the reaction between Ti^{III} and H_2O_2 in the presence of the *N,N'*-dioxide of EDTA at pH 8

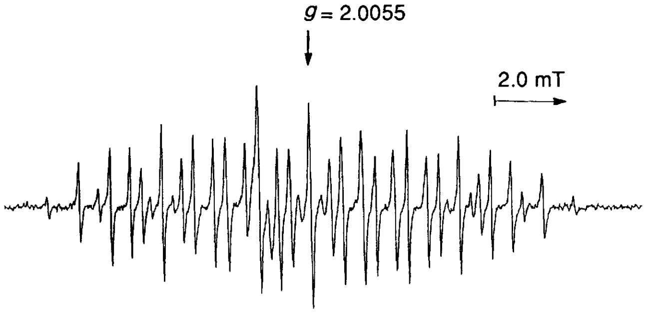


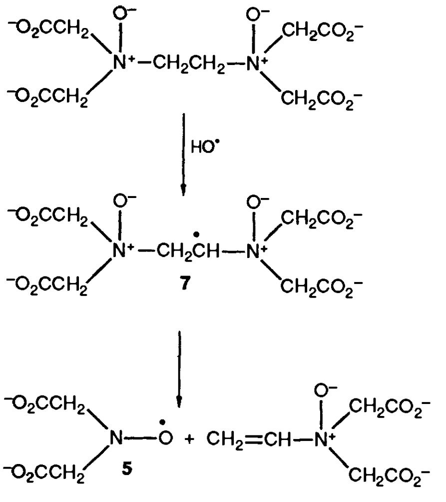
Fig. 6 EPR spectrum from the nitroxide $(^{2-}O_3PCH_2)_2NO^\bullet$ **6**, obtained from the reaction between Ti^{III} and H_2O_2 in the presence of the *N,N'*-dioxide of **1** at pH 8

it seems highly likely that compounds **1–4** share with their carboxylic acid analogues the property of being very reactive towards $^\bullet$OH but also of promoting, rather than inhibiting, subsequent peroxide decomposition; we therefore turned our attention to the reactions of the *N*-oxides of the ligands **1–4** and their carboxylic counterparts.

(b) *Reaction of* N-*oxides with the hydroxyl radical.* When a solution of EDTA *N,N'*-dioxide was allowed to react with Ti^{III} and H_2O_2 in the flow system, exceptionally strong signals were detected from a radical which has a life-time of the order of hours: the spectrum, shown in Fig. 5, is assigned to the nitroxide **5** $(^-O_2CCH_2)_2NO^\bullet$, with parameters as shown in Table 2 (see also ref. 34). In a series of experiments in which the pH was monitored it was found that maximum nitroxide concentration was obtained at pH 9. No signals could be detected in the absence of the metal.

In related experiments with the aminophosphonic analogue, the *N,N'*-dioxide of **1**, strong signals were similarly obtained from a relatively stable phosphorus-containing nitroxide assigned the structure **6** (see Table 2 and Fig. 6). Maximum signal intensity was obtained at pH 9.5. For both *N,N'*-dioxides (and others described below) the strong signals from the same nitroxides were also observed when the hydroxyl radical was generated photolytically *in situ* by UV-irradiation of H_2O_2 in the absence of the metal ion.

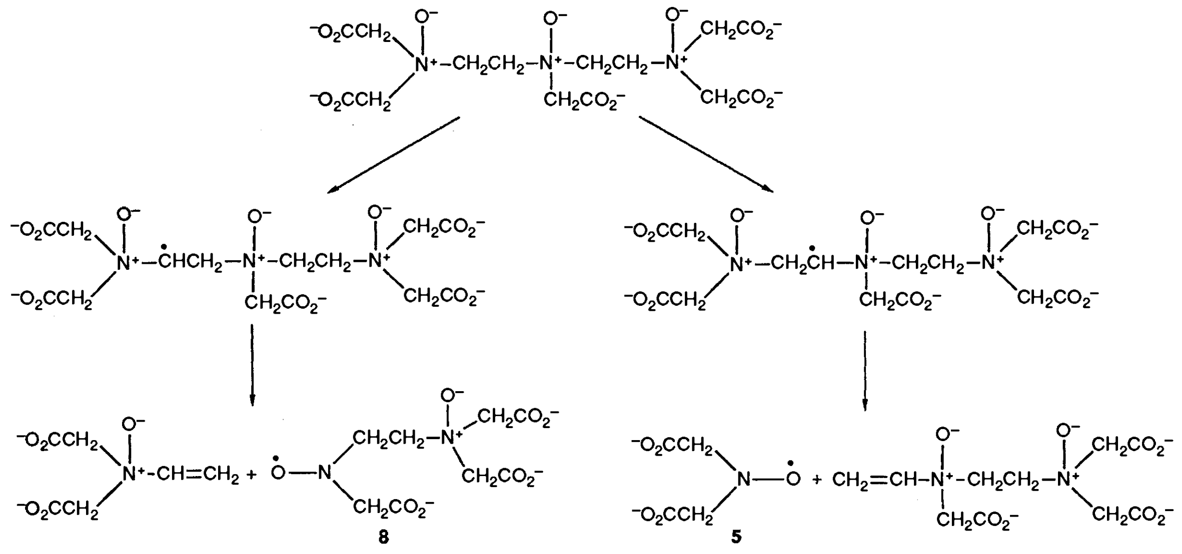
We suggest that the reaction sequence involves attack of $^\bullet$OH primarily at the C–H atoms in the ethylene bridge of EDTA *N,N'*-dioxide and its analogue (see Scheme 1). Note that in the alternative radical of the type $^-O_2C\dot{C}HN^+(O^-)R_2$ there will be no capto-dative stabilization following quaternization of nitrogen; the electrophilic hydroxyl radical may well thus be reluctant to attack a position deactivated by both the carboxylate group and a positively-charged nitrogen. Rapid fragmentation of **7** *via* β-scission would lead to the (stabilized) nitroxide and alkene.



Scheme 1

To provide support for this interpretation we also studied the reactions of DTPA and NTA *N*-oxides and their phosphonic acid analogues. Thus DTPA *N,N',N''*-trioxide was found to react with $^\bullet$OH in the pH range 7–11 to give two long-lived nitroxides (see Fig. 7 and Table 2) namely **5**, as from EDTA, and that assigned the structure **8**: despite the obvious differences in signal height, apparent in Figure 7, measurement of relative radical concentrations *via* double integration established that the concentrations of the two nitroxides were approximately equal. This observation adds further support to the mechanism proposed above: thus if initial attack by $^\bullet$OH is at the ethylene-bridge C–H groups then a 1:1 ratio of possible nitroxides is indeed expected (see Scheme 2).

Reaction of the *N,N',N''*-trioxide of **3**, under similar circumstances led to the detection of a strong signal from only one nitroxide, **6** (*cf.* the *N*-oxide of **1** and the behaviour of EDTA *N,N'*-dioxide). Our failure to detect the phosphorus-containing analogue of **8** may reflect a further increase in line-width



Scheme 2

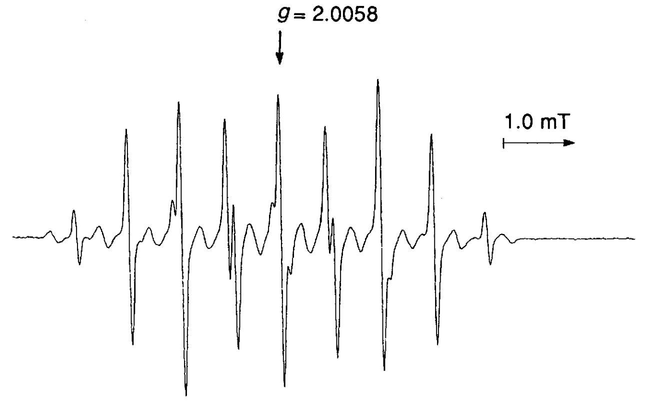


Fig. 7 EPR spectra obtained in a flow experiment involving Ti^{III} and H_2O_2 in the presence of DTPA *N,N'N''*-trioxide, assigned to $(^{-}O_2CCH_2)_2NO^{\bullet}$ **5** and $^{\bullet}ON(CH_2CO_2^{-})CH_2CH_2N(O)(CH_2CO_2^{-})_2$, **8** (see Scheme 2)

compared with **8** (as noted above) which itself may reflect line-broadening associated with hindered conformational motion of phosphonate-substituted methylene groups.

Observation of strong signals from the radical $^{\bullet}ON(CH_2PO_3^{2-})_2$ **6** from the *N,N'*-dioxide of **2** can also be rationalized in terms of the occurrence of rapid fragmentation following attack at the methylene groups in the chain adjacent to nitrogen: we believe that attack at *all* the bridge-methylene groups would be expected, though signals from the resulting short-lived radicals (which might well be expected to be relatively weak, and certainly overlapping) could not be unambiguously detected.

In contrast with the strong signal from nitroxides described above, only very weak signals [from $(^{-}O_2CCH_2)_2NO^{\bullet}$] could be detected from NTA *N*-oxide under comparable conditions, and no nitroxide could be detected from the *N*-oxide from the corresponding phosphonic acid **4**. In both cases weak signals were observed from the Ti^{III}–$HO_2^{\bullet}$ complexes referred to as S_1 and S_2 (with g 2.0132 and g 2.0118 respectively) which is characteristic of reaction of a substrate of low reactivity towards $^{\bullet}OH$. Our failure to detect significant concentrations of nitroxides here lends support to our proposal that the nitroxides detected earlier have precursors in which the radical centre is a methylene group bonded to nitrogen but not carboxyl (a feature absent in NTA and its analogue).

(c) *Other radical reactions of* N-*oxides*. A series of experiments was performed employing the Ti^{III}/H_2O_2 couple to generate $^{\bullet}CHMeOH$ (from $^{\bullet}OH$ and ethanol) in the presence of EDTA *N,N'*-dioxide as a model substrate; in a mixed substrate system, the concentration of the *N,N'*-dioxide (0.01 mol dm^{-3}) was considerably lower than that employed earlier, and the effect of variation in the ethanol concentration was explored. By comparison with the experiments involving only the *N,N'*-dioxide, the effect of addition of ethanol up to 0.1 mol dm^{-3} was to bring about a reduction in the intensity of the nitroxide **5**, presumably on account of the competition between the reaction of $^{\bullet}OH$ with EtOH and the *N,N'*-dioxide; only weak signals from $^{\bullet}CHMeOH$ could be observed. On increasing the concentration of ethanol, no significant change in the intensity of signals from either radical was observed, even up to the highest concentration of EtOH achieved.

These observations suggest that the *N,N'*-dioxide reacts with $^{\bullet}CHMeOH$ (and/or $^{\bullet}CH_2CH_2OH$), formed *via* scavenging of the hydroxyl radical, in a manner which leads to the eventual production of the nitroxide **5** [reaction (18)]. Whilst the

$$\text{EDTA } N,N'\text{-dioxide} + R^{\bullet} \longrightarrow RH + (^{-}O_2CCH_2)_2NO^{\bullet} \quad (18)$$

$$(R = {}^{\bullet}CHMeOH, {}^{\bullet}CH_2CH_2OH) \quad \mathbf{5}$$

mechanistic and kinetic details of this reaction were not further explored, it nevertheless appears that *N*-oxides are capable of scavenging organic radicals, as well as $^{\bullet}OH$, to form nitroxide radicals.

(d) *Reactions of* Ti^{III}–(N-*oxide*) *complexes with* H_2O_2. In the flow experiments described earlier, the final pH after mixing was typically *ca.* 9, yet no precipitate from Ti^{III} was formed. This suggests that the *N*-oxides themselves retain the ability to complex the metal ion(s), in which case it seems possible that the rate of generation of the hydroxyl radical from Ti^{III} and H_2O_2 is significantly affected (and that this should be taken into account in kinetic and/or mechanistic analysis).

Detailed and reliable measurements of the appropriate rate constants proved difficult because of the presence of hydrogen peroxide as an impurity in the samples of *N*-oxides and also because the *N*-oxides are themselves reactive towards $^{\bullet}OH$. However, an estimate for the rate constant for reaction between H_2O_2 and the Ti^{III} complex of EDTA *N,N'*-dioxide was made in a study employing Bu^tOH as substrate. By monitoring the effect of $[H_2O_2]$ and flow rate on $[^{\bullet}CH_2CMe_2OH]$ in experiments with Ti^{III}, together with an equimolar concentration of EDTA *N,N'*-dioxide (1.3×10^{-3}), and Bu^tOH (0.35

mol dm^{-3}), it was estimated by the methods described earlier that the rate constant for initiation is *ca.* 4×10^3 dm^3 mol^{-1} s^{-1} [*cf.*[14] *k* 4.5×10^3 dm^3 mol^{-1} s^{-1} for Ti$^{\text{III}}$–EDTA/H_2O_2].

Conclusions

The observation that the *N*-oxides of the aminophosphonic acids **1**, **2** and **3**—like those of EDTA and DTPA—react with $^\bullet$OH to give stable nitroxides suggests that the *N*-oxides are good inhibitors of radical reactions. However, we believe that this scavenging of $^\bullet$OH is not the primary rôle of aminophosphonic acids in stabilizing peroxides (*e.g.* hydrogen peroxide) for two reasons. Firstly, only dilute solutions of the stabilizers are normally employed; it is believed that under these conditions the hydroxyl radical would preferentially react with H_2O_2 to give $HO_2^\bullet$ [reaction (3); *k* 1×10^7 dm^3 mol^{-1} s^{-1}].* Secondly, the *N*-oxide of **4** does not apparently react with $^\bullet$OH to give a nitroxide radical (since there is no hydrogen atom β to the nitrogen), whereas this species also stabilizes peroxides. We have also shown that the aminophosphonic acids do not stabilize hydrogen peroxide by preventing or retarding the Fenton reaction to generate $^\bullet$OH: indeed the reactions between the Fe$^{\text{II}}$–Dequest complexes and hydrogen peroxide are considerably faster than the reaction of hydrated Fe$^{\text{II}}$ with H_2O_2 (and faster even than the corresponding reaction of Fe$^{\text{II}}$ in the presence of EDTA and DTPA).

The increased rate constants of this reaction are believed to be due, at least in part, to the ability of the aminophosphonic acids to stabilize the higher oxidation state of iron (and hence lower E° for Fe$^{\text{III}}$ Fe$^{\text{II}}$), as noted for EDTA and related ligands. Thus it is perhaps significant that although EDTA accelerates the Fenton reaction and effectively catalyses the Haber–Weiss cycle (*via* the regeneration of Fe$^{\text{II}}$ from Fe$^{\text{III}}$ by reaction with $HO_2^\bullet$ or $O_2^{\bullet -}$), the ligand DTPA catalyses the Fenton reaction (Fe$^{\text{II}}$/H_2O_2) but not the Haber–Weiss cycle, evidently since reaction of Fe$^{\text{III}}$ with $HO_2^\bullet/O_2^{\bullet -}$ is retarded (see refs. 35–37). Thus for the reaction of $O_2^{\bullet -}$ with Fe$^{\text{III}}$EDTA$^-$ in the pH range 6–8, values of the rate constant in the range 10^5–6×10^6 dm^3 mol^{-1} s^{-1} have been reported (see refs. 36, 37) whereas for Fe$^{\text{III}}$DTPA^{2-} at pH 6–8, the rate constant is $< 10^4$ dm^3 mol^{-1} s^{-1}. It should also be noted that the reaction of Fe$^{\text{II}}$ with $O_2^{\bullet -}$ to give Fe$^{\text{III}}$ is considerably faster with DTPA as ligand than EDTA (2×10^7 compared with 3×10^6 dm^3 mol^{-1} s^{-1}: *cf.* ref. 38).

It seems very likely therefore that a role similar to that for DTPA exists for the aminophosphonic acid complexes of iron. That is, stabilization of the lower valent state (with an anticipated reduction in E°) encourages the very rapid reaction of Fe$^{\text{II}}$ with H_2O_2 to give HO$^\bullet$ and hence $HO_2^\bullet$ (and similarly for the reaction of Fe$^{\text{II}}$ with $O_2^{\bullet -}$); concurrently, reduction of Fe$^{\text{III}}$ to regenerate Fe$^{\text{II}}$ by $O_2^{\bullet -}$ or $^\bullet O_2H$ may well be then retarded either for electronic reasons (*via* reduction in E°) or *via* steric retardation (which would by implication suggest an inner-sphere mechanism for the reaction between Fe$^{\text{III}}$ and $O_2^{\bullet -}$ and $^\bullet O_2H$). More detailed conclusions require kinetic measurements on the reaction of $O_2^{\bullet -}$ and $^\bullet$OH with a range of iron complexes, as well as reliable data on E° for the latter.

Experimental

EPR spectra were recorded on a Varian E-104 and a Bruker ESP-300 spectrometer, each equipped with an X-band Klystron and 100 kHz modulation. Hyperfine splittings were measured directly from the spectrometer field scan: with the E-104 this was calibrated with an aqueous solution of Fremy's salt [*a*(N) 1.309 mT[39]] and with the ESP-300 by determination with an NMR gaussmeter (ER 035M). *g*-Values were determined by comparison with that from $^\bullet$CHMeOH ($g = 2.003\,21 \pm 0.000\,05$[40]), itself checked by comparison with Fremy's salt ($g = 2.005\,50$).[41]

Relative radical concentrations were obtained from measurements of peak-height (where appropriate line-widths were the same) or by double integration using the ESP-300. Absolute radical concentrations were obtained by comparison of the doubly integrated EPR signal with either that from vanadyl sulfate (*ca.* 10^{-4} mol dm^{-3} $VOSO_4 \cdot H_2O$, standardized by titration with potassium permanganate solution) or with that from $^\bullet CH_2CMe_2OH$ obtained from the Ti$^{\text{III}}$/H_2O_2/Me_3COH reaction in a flow system, for which previous measurements (supported by computer simulations) indicate the steady-state radical concentration to be 3.3×10^{-6} mol dm^{-3} for *t* 30 ms.

In the continuous-flow EPR experiments a 3-way mixer was employed which allowed simultaneous mixing of 3 reagent streams, approximately 30 ms before their passage through the cell in the EPR cavity. The overall flow-rate, typically 2.5 cm^3 s^{-1}, was maintained with a Watson Marlow 502 peristaltic pump, positioned on the inlet tubing. pH measurements were made using a Pye-Unicam pH meter PW9410, with the glass electrode positioned in the effluent stream. Calibration of the pH meter was achieved using commercially available buffer solutions (pH 4 and pH 7). The solutions to be mixed were made up in distilled or de-ionized water and were deoxygenated prior to, and during use, by purging with oxygen-free nitrogen. The conditions typically employed for generating the hydroxyl radical in Ti$^{\text{III}}$/H_2O_2 experiments were as follows: the first stream contained titanium(III) chloride (typically 0.004 mol dm^{-3}), the second stream contained hydrogen peroxide (up to 0.05 mol dm^{-3}) and the substrate was in the third stream (with a concentration up to 1.5 mol dm^{-3}). In experiments conducted above pH 2.5 the di-sodium salt of EDTA (or other ligand) was added to the titanium stream usually in concentration equal to that of Ti$^{\text{III}}$. Kinetic experiments with iron similarly involved the use of iron(II) sulfate (typically *ca.* 10^{-3} mol dm^{-3} after mixing, with added chelate at double this concentration), hydrogen peroxide (typically *ca.* 10^{-3} mol dm^{-3}), and substrate (up to 1.5 mol dm^{-3}).

Aqueous solutions of the *N*-oxides of the aminophosphonic acids and their carboxylic acid analogues were prepared by reacting the neutralized acid with a stoichiometric amount of H_2O_2; solid samples were not prepared. The procedure for the preparation of EDTA *N*,*N*′-dioxide, based on that described in ref. 5, was as follows. The di-sodium salt of EDTA (80 g) was dissolved in 50 cm^3 of NaOH solution (12 mol dm^{-3}) and 100 cm^3 of water at 0 °C (with ice-water cooling). The pH of the solution was adjusted to pH 9. Hydrogen peroxide (67 g, 27.5% solution) was then added dropwise with ice-water cooling, and the reaction mixture was then heated to 60 °C for 2–4 h. The 'available oxygen content' (AvOx) of the reaction mixture was determined at various stages during the reaction, by reacting samples with an excess of acidified potassium iodide and titrating the liberated iodine with sodium thiosulfate. After 2 h, 2 moles of H_2O_2 has been consumed per mole of EDTA. The solution of EDTA *N*,*N*′-dioxide thus prepared was used without further purification. The ^{13}C NMR spectrum of the product showed that EDTA had been completely converted into the *N*,*N*′-dioxide, with δ (at pH 8) 60.30 (CH_2), 67.56 (CH_2, bridge) and 170.49 (CO_2^-). DTPA *N*,*N*′,*N*″-trioxide, prepared similarly had δ 171.58 (CO_2^-), 170.49 (CO_2^-), 69.02, 68.54, 61.00 and 60.47 (CH_2). Aqueous solutions of the *N*-oxides of the aminophosphonic acids were prepared similarly: detailed analysis was not attempted.

Solutions of hydrogen peroxide were prepared by dilution of 27.5% (100 vol) H_2O_2 solutions (Fisons); titanium(III) chloride

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* Subsequent scavenging of $HO_2^\bullet$ by the aminophosphonic acid would also be expected to occur to some extent.

(12.5% w/v in dilute HCl) and iron(II) sulfate were obtained from Fisons, and the aminophosphonic acids **1**–**4** were obtained from Interox Chemicals Ltd (compound **2** as the hexapotassium salt). EDTA (as the disodium salt) and DTPA (as the free acid) were obtained from Aldrich.

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