

# Formation of Hydroxyl Spin Adducts via Nucleophilic Addition–Oxidation to 5,5-Dimethyl-1-pyrroline *N*-Oxide (DMPO)

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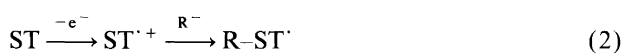
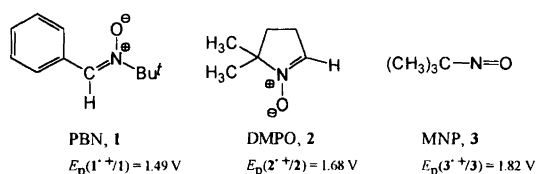
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The formation of the hydroxyl radical spin adduct of DMPO, HO–DMPO<sup>•</sup>, has been studied by EPR spectroscopy under conditions where the hydroxyl radical cannot be involved. One method was the photosensitized oxidation of DMPO to its radical cation, followed by reaction of the latter with water. This procedure worked well with sensitizers Sens (quinones) having triplet states with  $E^{\circ}({}^1\text{Sens}^*/\text{Sens}^{\cdot-}) > 1.7–1.8$  V (SCE).

The second method employed nucleophilic addition of water to DMPO, followed by reaction of the intermediate hydroxylamine by mild thermal oxidants (quinones, Fe<sup>III</sup>) with redox potentials in the range  $-0.5$  to  $0.5$  V. The observation of HO–DMPO<sup>•</sup> in this reaction was dependent on the acidity of the medium, since the relatively slow rate of formation of HO–DMPO<sup>•</sup> must be compensated for by a correspondingly longer half-life,  $\tau_{1/2}$ . This is decreased by increasing acidity, and a lower limit of  $\tau_{1/2}$  was estimated at 1–1.5 min. The use of buffered neutral or slightly alkaline solutions or ethyl acetate as solvent satisfied this requirement.

The nucleophilic mechanism, sometimes denoted the Forrester–Hepburn mechanism, was also applicable to the reaction between normal alcohols and DMPO, giving rise to RO–DMPO<sup>•</sup>. A sterically demanding alcohol (*t*-BuOH) or electrophilic alcohols (2,2,2-trifluoroethanol or 1,1,1,3,3,3-hexafluoropropan-2-ol) required photochemical activation for reaction to occur.

Spin trapping is an often used technique for the detection and characterization of transient radicals in solution.<sup>1</sup> A spin trap ST can add a transient radical R<sup>•</sup> and thereby be converted to a persistent radical [eqn. (1)] which can be identified by its EPR spectrum. The most common spin traps are either nitrones, for example  $\alpha$ -phenyl-*N*-*tert*-butylnitron (1, PBN) or 4,5-dihydro-5,5-dimethyl-1-pyrroline-1-oxide (2, DMPO), or nitroso compounds like 2-methyl-2-nitrosopropane (3, MNP).

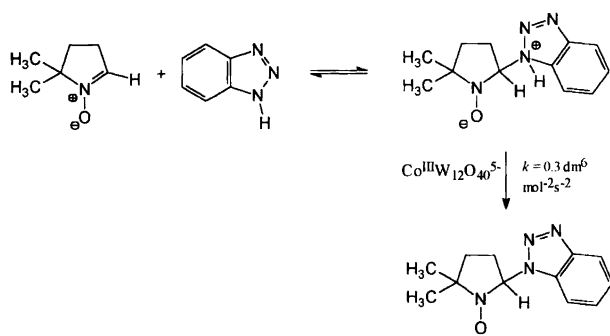


The interpretation of spin trapping experiments is often complicated by other reactivities of the spin traps. Under strongly oxidizing conditions, as for example in most photochemical experiments, the ST might be oxidized to its radical cation ST<sup>•+</sup> and react as such with nucleophile R<sup>-</sup> [eqn. (2)] to give the same spin adduct as in eqn. (1). Because of the inverted electronic situation compared to eqn. (1), the reaction sequence of eqn. (2) has been denoted inverted spin trapping.<sup>2</sup> Clearly, it has no connection with any generation of R<sup>•</sup>. Situations in which inverted spin trapping might occur are recognized by the need for strong oxidants to produce ST<sup>•+</sup> (*cf.* redox potentials of 1–3 above). Usually, the problem of recognizing inverted spin trapping can be handled.<sup>2</sup>

The most serious artifact in spin trapping is related to the Forrester–Hepburn mechanism, which is initiated by addition of a nucleophile to ST [eqn. (3)].<sup>3</sup> This gives rise to a hydroxylamine derivative, either as the anion or as the hydroxylamine itself after protonation. Both these species are easily oxidized, as exemplified by the redox potentials of PhNHO<sup>-</sup> ( $E_{pa} = -0.6$  V) and PhNHOH ( $E_{pa} = 0.7$  V),<sup>4</sup> to give the formal spin adduct [eqn. (4)]. Weak oxidants, such as dioxygen, quinones or

many redox proteins, can accomplish this transformation.<sup>5</sup> Thus acetate ion or fluoride ion can be converted into  $\text{CH}_3\text{COO-ST}^\cdot$  or  $\text{F-ST}^\cdot$  without any difficulty, in spite of the fact that the true trapping of acetoxy radical or fluorine atom is considered to be an impossible reaction.<sup>2,6,7</sup>

While the properties of PBN in inverted spin trapping and the Forrester-Hepburn mechanism have been extensively studied,<sup>2</sup> reports on these aspects of DMPO reactivity are more limited and indicate certain restrictions. First, the redox potential of  $\text{DMPO}^{\cdot+}/\text{DMPO}$  is about 0.2 V higher than that of PBN, thus reducing the yield of spin adduct according to eqn. (2) under otherwise similar conditions or requiring the use of stronger oxidants. Secondly, the UV absorption maximum of DMPO is about 50 nm lower than that of PBN, meaning that the excitation of DMPO is not feasible with the mercury lamps commonly used. Nevertheless, inverted spin trapping has been demonstrated for DMPO with nucleophiles such as carboxylates and carboxylic acids, fluoride ion, tetramethylsuccinimide and triethyl phosphite, employing strong oxidants.<sup>8</sup> With oxidants weak enough to exclude oxidation of DMPO to its radical cation, the Forrester-Hepburn mechanism has been shown for formation of DMPO spin adducts of fluorine atom<sup>8</sup> and certain heteroaromatic NH bases, as shown in detail by EPR/UV spectral and kinetic experiments with benzotriazole (Scheme 1).<sup>9</sup>

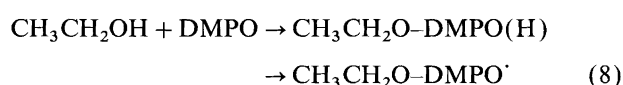
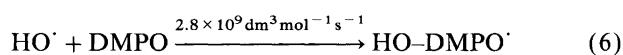
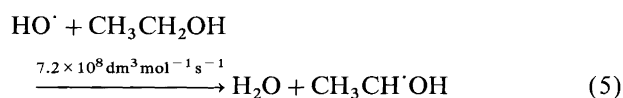


Scheme 1.

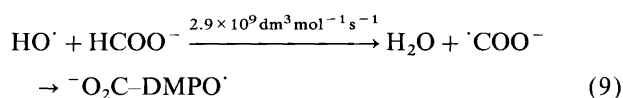
The detection of spin adducts of the hydroxyl radical is probably the most common spin trapping reaction because of its biological implications.<sup>10</sup> If the hydroxyl radical were indeed formed as such in a biological system, its high reactivity might lead to random damage and thus possibly be the cause of long-term biological deterioration processes.<sup>11</sup> On the other hand, the hydroxyl radical is a high-energy species, and it is not obvious that the reaction conditions necessary for its formation are prevalent in biological systems.<sup>5</sup> The Forrester-Hepburn mechanism would then go a long way to explain the frequent sightings of hydroxyl spin adducts.

Two tests are available to distinguish the mechanisms discussed above from a mechanism involving  $\text{HO}^\cdot$ . If ethanol is added in low concentration to the reaction

mixture, it will compete favourably with the trapping reaction for any  $\text{HO}^\cdot$  formed [eqns. (5) and (6)]. The attack on ethanol will take place at an  $\alpha$ -hydrogen, giving  $\alpha$ -hydroxyethyl radical which is trapped by DMPO to give a spin adduct with a characteristic EPR spectrum [eqn. (7)]. If  $\text{HO}^\cdot$  is not involved, nucleophilic addition-oxidation of ethanol to DMPO will take place and the ethoxyl spin adduct with a distinctly different EPR spectrum will be formed [eqn. (8)].<sup>12</sup>



The second test is similar in nature, but formate is the species from which a hydrogen atom can be abstracted [eqn. (9)], in competition with eqn. (6).<sup>13</sup>



Hydroxyl spin adducts are not very persistent, probably because of their solvolytic reactivity in the presence of protons.<sup>14</sup> Only in a basic solvent like pyridine will a hydroxyl spin adduct have appreciable persistency,<sup>15</sup> as exemplified by  $\text{HO-DMPO}^\cdot$ . In an aqueous phosphate buffer at pH 6,  $\text{HO-PBN}^\cdot$  had a half-life of about 90 s,<sup>16</sup> whereas in acetonitrile the half-life was only 10 s.<sup>17</sup> In an aqueous phosphate buffer of pH 6,  $\text{HO-DMPO}^\cdot$  had a half-life of about 25 min.<sup>18</sup>

Previous studies of the photochemical reaction between DMPO and quinones in an aqueous medium led to different interpretations of the chemistry involved.<sup>19</sup> It was earlier suggested<sup>19a</sup> that the excited quinone  $\text{Q}^*$  would abstract a hydrogen atom from  $\text{H}_2\text{O}$  and thus produce  $\text{HO}^\cdot$ . A different version of this mechanism suggested that  $\text{Q}^*$  would oxidize  $\text{H}_2\text{O}$  to its radical cation which would give  $\text{HO}^\cdot$  by loss of a proton.<sup>19c</sup> Finally, the proposal was made that DMPO would be oxidized to its radical cation by  $\text{Q}^*$  and thus undergo inverted spin trapping.<sup>19b</sup>

We now report a study of the formation of hydroxyl and alkoxy spin adducts of DMPO by nonconventional mechanisms, using primarily quinones as photo-oxidants or thermal oxidants. Also other mild ET oxidants were tested, in particular  $\text{Fe}^{\text{III}}$ .

## Results

*Choice of reaction conditions.* For the purpose at hand, it is an advantage to be able to use a weak thermal oxidant which can be converted into a strong oxidant by photochemical excitation. In this respect, quinones afford

a rich variety of choices.<sup>20</sup> Tetrachloro-1,4-benzoquinone (chloranil, Cl<sub>4</sub>Q) is a widely used photochemical oxidant and has earlier<sup>4</sup> been shown to produce RCOO–DMPO<sup>•</sup> by UV irradiation of dichloromethane solutions of DMPO and RCOOH, showing that its triplet state, with  $E^\circ(\text{Cl}_4\text{Q}^*/\text{Cl}_4\text{Q}^{\cdot-})=2.14\text{ V vs. SCE}$ , is capable of oxidizing DMPO to its radical cation [ $E_p(\text{DMPO}^{\cdot+}/\text{DMPO})=1.68\text{ V}$ ]. Its thermal redox potential,  $E^\circ(\text{Cl}_4\text{Q}/\text{Cl}_4\text{Q}^{\cdot-})=0.02\text{ V vs. SCE}$ , is on the other hand of a magnitude which would make Cl<sub>4</sub>Q a feasible oxidant in eqn. (4). Tetrafluoro-1,4-benzoquinone (fluoranil, F<sub>4</sub>Q) has nearly identical redox properties in its ground and triplet state, and was also employed. It has an advantage over Cl<sub>4</sub>Q in that its radical anion did not persist in most experiments performed. With Cl<sub>4</sub>Q, the EPR signal of its radical anion often dominated the EPR spectrum. In most respects, Cl<sub>4</sub>Q and F<sub>4</sub>Q had similar redox properties toward DMPO and could be used interchangeably.

A great deal of experimentation was performed in order to find conditions suitable for generating HO–DMPO<sup>•</sup> according to the Forrester–Hepburn mechanism. Such reactions must be run thermally, and the rate of formation of HO–DMPO<sup>•</sup> must be high enough to compensate for its kinetic instability. The former rate was judged to be less easily manipulated, and ways to increase the persistency of HO–DMPO<sup>•</sup> were accordingly investigated. The photo-oxidants to be employed, F<sub>4</sub>Q or Cl<sub>4</sub>Q, create weakly acidic conditions upon dissolution in water- or alcohol-containing media<sup>21</sup> and thus increase the rate of a proton-catalyzed decomposition of HO–DMPO<sup>•</sup>.

Table 1 shows approximate rate constants for the disappearance of HO–DMPO<sup>•</sup> in acetonitrile–water (7:1), the solvent composition which was eventually chosen for part of the study. The spin adduct was generated photochemically, using first F<sub>4</sub>Q as the photo-oxidant. Without any additive, the half-life of HO–DMPO<sup>•</sup> was only 14 s; a slight increase was noticeable when water was replaced by deuterium oxide. Basic additives increased the half-life by a factor of >10 in the most favourable case. As shown below, such a change was sufficient for the demonstration of the Forrester–Hepburn mechanism. With other photo-oxidants (Cl<sub>4</sub>Q, 2,3-Cl<sub>2</sub>Q and 9,10-dicyanoanthracene) longer half-lives

were observed, especially with 9,10-dicyanoanthracene. This compound does not spontaneously generate acidity in protic media.

A second, efficient way to increase the half-life of HO–DMPO<sup>•</sup> was to use ethyl acetate, saturated with water, as the solvent. It has been shown earlier that spin adducts like HO–DMPO<sup>•</sup>, initially generated in aqueous medium, can be stabilized significantly by extraction into ethyl acetate.<sup>22</sup> However, the spin adducts can equally well be generated directly in ethyl acetate–water, the saturated solution containing 3.0 wt% or 2.7 vol% of water at 23 °C.<sup>23</sup> Under such conditions, F<sub>4</sub>Q could be used as a thermal oxidant and the Forrester–Hepburn mechanism be demonstrated (see below).

*Control experiments involving possibly hydroxyl radical attack on ethyl acetate or acetonitrile.* The hydrogens  $\alpha$  to the ether oxygen of ethyl acetate are potentially abstractable by hydroxyl radical in the same way as for ethanol, and it was therefore necessary to check this possibility. Photolysis for 5 min of 1% hydrogen peroxide with DMPO in ethyl acetate gave as expected EPR spectra of HO–DMPO<sup>•</sup> (30%:  $a^N=1.38$ ,  $a^H=1.11$ ,  $a^H=0.087\text{ mT}$ ) and two closely similar, persistent carbon-centred radicals (25%:  $a^N=1.42$ ,  $a^H=1.90$ ; 45%:  $a^N=1.44$ ,  $a^H=2.06\text{ mT}$ ) to which the structure of the diastereomers of CH<sub>3</sub>COOCH(CH<sub>3</sub>)–DMPO<sup>•</sup> was assigned. Similar photolysis with ethyl acetate as solvent gave the HO–DMPO<sup>•</sup> (50%) spectrum and a spectrum with  $a^N=1.45$ ,  $a^H=2.05\text{ mT}$  (50%), assigned to CH<sub>3</sub>COOCH<sub>2</sub>–DMPO<sup>•</sup>. Photolysis of hydrogen peroxide–DMPO in acetonitrile gave only the HO–DMPO<sup>•</sup> spectrum.

*Alkoxyl spin adducts of DMPO.* In order to explore the thermal and photochemical oxidative ability of F<sub>4</sub>Q and Cl<sub>4</sub>Q in eqn. (4) in situations where persistent spin adducts closely related to HO–DMPO<sup>•</sup> are formed, the reaction between DMPO and alcohols was investigated, using acetonitrile as the solvent. Figure 1 shows the development of EtO–DMPO<sup>•</sup> in acetonitrile from DMPO, ethanol and F<sub>4</sub>Q under dark conditions. The EPR spectrum of EtO–DMPO<sup>•</sup> (see Fig. 1 and Table 2) reached a maximum intensity after about 1 h [ $k_{up}=0.062(7)\text{ min}^{-1}$ ] and then decayed over a period of 20 h.

Table 1. Rate constant  $k_{down}$  for the disappearance of HO–DMPO<sup>•</sup> under various conditions, using acetonitrile–water (7:1) as solvent. The spin adduct was generated by photoexcitation (UV light).

Additives or changes	Sensitizer	$k_{down}/\text{min}^{-1}$	$\tau_{1/2}/\text{s}$
—	F <sub>4</sub> Q	3.0	14
—	Cl <sub>4</sub> Q	1.0	41
—	2,3-Cl <sub>2</sub> Q	0.47	88
—	9,10-(CN) <sub>2</sub> -anthracene	0.040	1035
D <sub>2</sub> O instead of H <sub>2</sub> O	F <sub>4</sub> Q	2.2	19
Satd. with K <sub>2</sub> CO <sub>3</sub>	F <sub>4</sub> Q	1.3	32
2,6-Di- <i>t</i> -Bu-pyridine (60 mmol dm <sup>-3</sup> )	F <sub>4</sub> Q	1.8	23
Et <sub>4</sub> NOH (8.5 mmol dm <sup>-3</sup> )	F <sub>4</sub> Q	0.22	188

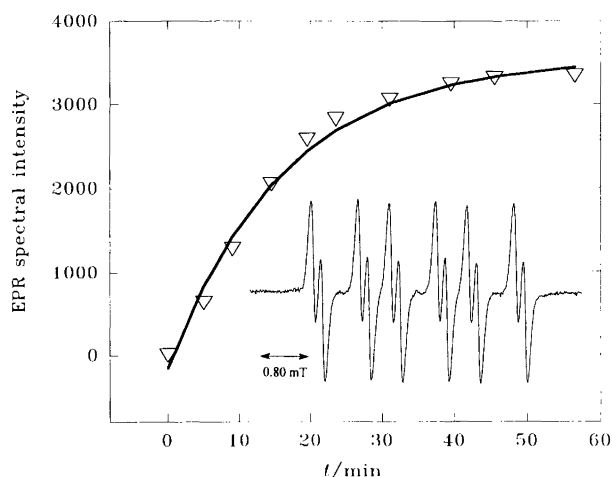


Fig. 1. Growth of the EPR spectrum of EtO-DMPO<sup>•</sup> under dark conditions in a solution of DMPO (50 mmol dm<sup>-3</sup>) and F<sub>4</sub>Q (~50 mmol dm<sup>-3</sup>) in acetonitrile-ethanol (7:1 v/v). The curve corresponds to the best-fitting exponential, with  $k_{up} = 0.062(7) \text{ min}^{-1}$ .

The parameters found agreed well with those published earlier<sup>12</sup> ( $a^N = 1.36$ ,  $a^H = 0.74$ ,  $a^H = 0.17$  mT in neat ethanol). Table 2 also shows the values obtained here in neat ethanol; UV irradiation strongly enhanced the spectral intensity.

Other alcohols of similar structure and steric requirements (methanol, 2-propanol) behaved in the same way as ethanol. 2-Methylpropan-2-ol did not react under dark conditions, presumably for steric reasons, and the more acidic and electrophilic alcohols 2,2,2-trifluoroethanol and 1,1,1,3,3,3-hexafluoropropan-2-ol also required UV irradiation for the alkoxy spin adduct to appear.

Under no conditions employed for the relevant reactions of Table 2 was there any indication of the appearance of the spin adduct derived from trapping the carbon-connected radical possibly resulting from abstracting an  $\alpha$ -H from the alcohol, for example the 1-hydroxyethyl radical from ethanol [eqns. (5) and (7)]. The EPR

spectrum of CH<sub>3</sub>(HO)CH-DMPO<sup>•</sup> would be easily distinguishable in these experiments because of its large  $a^H$  characteristic of a C-connected spin adduct. [ $a^N = 1.58$  and  $a^H = 2.29$  mT in ethanol-water (9:1)];<sup>24</sup> here we obtained  $a^N = 1.51$  and  $a^H = 2.18$  mT in neat ethanol, 1.49 and 2.22 mT in ethanol-acetonitrile (1:7 v/v) and 1.49 and 2.18 mT in ethanol-ethyl acetate (1:7 v/v) by UV photolysis of 0.5% hydrogen peroxide with DMPO in the appropriate solvent.]

From the experiments listed in Table 2 it is clear that the Forrester-Hepburn mechanism [eqns. (3) and (4)] operates as expected with DMPO and unhindered alcohols of normal nucleophilicity. Only with sterically hindered or electrophilic alcohols is it necessary to use the photochemical pathway, i.e. oxidation of DMPO to its radical cation followed by reaction of this very reactive species<sup>25</sup> with the alcohol.

The hydroxyl spin adduct of DMPO, generated by photolysis with F<sub>4</sub>Q or Cl<sub>4</sub>Q. Next the hydroxyl adduct of DMPO was generated by photolysis of DMPO and F<sub>4</sub>Q or Cl<sub>4</sub>Q in a water-containing solvent with no extra additives (Table 3). Only in ethyl acetate or water, buffered with phosphate at pH 6.5, was thermal generation of HO-DMPO<sup>•</sup> detectable (see below). In both ethyl acetate-water and acetonitrile-water (7:1 v/v) an additional coupling constant of 0.094 and 0.082 mT, respectively, to a single hydrogen could be unambiguously resolved, most likely to one of the hydrogens in the  $\gamma$ -position.

The spectrum in pyridine was recorded from a photolyzed solution of DMPO with 0.5% hydrogen peroxide present. In this case the HO-DMPO<sup>•</sup> spectrum was relatively weak, and a (3×2)-line spectrum of unknown origin ( $a^N = 1.44$  and  $a^H = 1.97$  mT) predominated. The latter spectrum was described also by Rezska *et al.*<sup>15</sup> but was not assigned to any structure. A weak spectrum of similar appearance accompanied the HO-DMPO<sup>•</sup> spectrum in most cases listed in Table 3.

Table 2. Alkoxy spin adducts from DMPO, generated by thermal or photochemical oxidation of DMPO and ROH<sup>a</sup> by chloranil or fluoranil in acetonitrile, unless otherwise stated.

R in ROH	Conditions	$a^N/\text{mT}^b$	$a^H/\text{mT}^b$	$a^H/\text{mT}^b$	Assigned to
Me	F <sub>4</sub> Q, dark	1.321	0.786	0.166	MeO-DMPO <sup>•</sup>
Et	Cl <sub>4</sub> Q, dark <sup>c</sup>	1.353	0.759	0.187	EtO-DMPO <sup>•</sup>
Et	Cl <sub>4</sub> Q, UV <sup>c,d</sup>	1.361	0.757	0.180	EtO-DMPO <sup>•</sup>
Et	F <sub>4</sub> Q, dark	1.340	0.798	0.174	EtO-DMPO <sup>•</sup>
isoPr	F <sub>4</sub> Q, dark	1.343	0.782	0.179	isoPrO-DMPO <sup>•</sup>
isoPr	F <sub>4</sub> Q, UV <sup>e</sup>	1.345	0.782	0.180	isoPrO-DMPO <sup>•</sup>
<i>tert</i> -Bu	F <sub>4</sub> Q, dark	<sup>f</sup>			
<i>tert</i> -Bu	F <sub>4</sub> Q, UV	1.371	1.075	0.141	<i>t</i> -BuO-DMPO <sup>•</sup>
(CF <sub>3</sub> ) <sub>2</sub> CH	Cl <sub>4</sub> Q, dark <sup>c</sup>	<sup>f</sup>			
(CF <sub>3</sub> ) <sub>2</sub> CH	Cl <sub>4</sub> Q, UV <sup>c</sup>	1.396	0.759	0.201	(CF <sub>3</sub> ) <sub>2</sub> CHO-DMPO <sup>•</sup>
CF <sub>3</sub> CH <sub>2</sub>	F <sub>4</sub> Q, UV	1.293	0.789	0.194	CF <sub>3</sub> CH <sub>2</sub> O-DMPO <sup>•</sup>

<sup>a</sup>The acetonitrile-alcohol ratio was 7:1 (v/v). <sup>b</sup>Multiplicity = 1. <sup>c</sup>The reaction was run in the neat alcohol. <sup>d</sup>The spectral intensity went up by a factor of about 30 compared to the dark run. <sup>e</sup>The spectral intensity went up by a factor of about 3 compared to the dark run. <sup>f</sup>No spectrum was detectable in 1 h.

Table 3. EPR spectral parameters of HO-DMPO<sup>•</sup>, photogenerated from DMPO and F<sub>4</sub>Q or Cl<sub>4</sub>Q in a water-containing solvent.

Solvent	[H <sub>2</sub> O](vol%)	$a^N/\text{mT}^a$	$a^H/\text{mT}^a$	$a^{H'}/\text{mT}^a$
CH <sub>3</sub> CN	12.5	1.415	1.256	0.082
CH <sub>3</sub> CN	78	1.446	1.376	
CH <sub>3</sub> CN	83	1.470	1.470	
H <sub>2</sub> O	100	1.478	1.478	
H <sub>2</sub> O	100, pH 6.5 (phosphate buffer) <sup>b</sup>	1.484	1.484	
CH <sub>2</sub> Cl <sub>2</sub>	Satd.	1.408	1.251	
EtOAc	2.7 <sup>b</sup>	1.376	1.095	0.095
EtOAc	2.7 (21.4% <sup>17</sup> O) <sup>b</sup>	1.375	1.091	0.094 <sup>c</sup>
(CF <sub>3</sub> ) <sub>2</sub> CHOH	12.5	1.483	1.483	
Pyridine <sup>d</sup>	1.5	1.390	1.173	0.082

<sup>a</sup>Multiplicity=1. <sup>b</sup>The spectrum appeared also in the dark. <sup>c</sup> $a^{17\text{O}}=0.441$  mT (see also Fig. 4). <sup>d</sup>Photolysis of 0.5% hydrogen peroxide in pyridine (*cf.* Ref. 15).

#### Competitive formation of EtO-DMPO<sup>•</sup> and HO-DMPO<sup>•</sup>.

The dark reaction between DMPO and a mixture of water and ethanol in acetonitrile in the presence of F<sub>4</sub>Q initially gave rise to only EtO-DMPO<sup>•</sup> (Fig. 2a). UV irradiation for 3 min generated a mixture of both EtO-DMPO<sup>•</sup> and HO-DMPO<sup>•</sup> (Fig. 2b); the latter radical then disappeared after the light had been shut off (Fig. 2c). This sequence could be repeated many times. The  $a^N$  1.44,  $a^H$  1.97 mT spectrum mentioned above appeared in low intensity, about 8%, with slightly different coupling constants (see simulation in Fig. 2d).

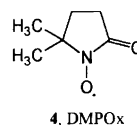
No trace of the EPR spectrum of CH<sub>3</sub>(HO)CH-DMPO<sup>•</sup> was seen in any phase of this experiment. This is a critically important fact, since it excludes the possibility that the hydroxyl radical is involved as an intermediate in the formation of HO-DMPO<sup>•</sup>. Were it involved, its very fast abstraction of the  $\alpha$ -hydrogen of ethanol would compete favourably with its trapping by DMPO in the concentrations employed [eqns. (5) and (6)].

*The thermal generation of HO-DMPO<sup>•</sup> by oxidation of DMPO and water.* Once the proper reactions conditions had been defined, it was possible to generate HO-DMPO<sup>•</sup> by F<sub>4</sub>Q oxidation of DMPO and water in the dark according to eqns. (3) and (4). The first set of conditions involved the use of acetonitrile-water (7:1 v/v) with a low concentration of Et<sub>4</sub>NOH present. Figure 3 (triangles) shows the development of the EPR spectrum of HO-DMPO<sup>•</sup>, the solid curve representing the best-fitting double exponential function with  $k_{\text{up}}=0.030$  min<sup>-1</sup> and  $k_{\text{down}}=0.026$  min<sup>-1</sup>. The second set of conditions used ethyl acetate, saturated with water (3.0 wt% or 2.7 vol%), as the solvent. The EPR spectrum of HO-DMPO<sup>•</sup> then evolved as in Fig. 3 (squares), the solid curve representing the best-fitting double exponential function with  $k_{\text{up}}=0.21$  min<sup>-1</sup> and  $k_{\text{down}}=0.010$  min<sup>-1</sup>.

The latter set of conditions was also used in an experiment in which water containing 21.4 atom% <sup>17</sup>O was the nucleophilic component. Figure 4 shows the experimental EPR spectrum together with a simulation

based on the parameters given in Table 3. The double integration of the relevant peaks in the experimental spectrum (two runs) showed that all HO-DMPO<sup>•</sup> was derived from water.

*Other oxidants in the Forrester-Hepburn mechanism.* A series of weak oxidants, predominantly quinones, with redox potentials in the range -0.9 to 0.5 V was then allowed to react in the dark with DMPO in ethyl acetate-water in order to find the reagent of lowest redox potential still being capable of giving HO-DMPO<sup>•</sup>. Table 4 shows the results. The borderline case was benzoquinone, with  $E^\circ(\text{H}_4\text{Q}/\text{H}_4\text{Q}^{\cdot-})=-0.45$  V. 2,3-Dichloro-4,5-dicyanoquinone (DDQ) was the strongest oxidant employed,  $E^\circ(\text{DDQ}/\text{DDQ}^{\cdot-})=0.51$  V, and did not yield any HO-DMPO<sup>•</sup>; instead a paramagnetic product of further oxidation of HO-DMPO<sup>•</sup>, DMPOx (4), was detectable. Fe<sup>III</sup> and ferricinium ion could also be used.



Tetracyanoethylene (TCNE), with  $E^\circ(\text{TCNE}/\text{TCNE}^{\cdot-})=0.15$  V, underwent a fast dark reaction with DMPO in ethyl acetate-water, as signified by the rapid formation of TCNE<sup>•-</sup>, but no HO-DMPO<sup>•</sup> was detectable. Instead, an intense spectrum of a C-connected spin adduct ( $a^N=1.35$ ,  $a^H=2.06$ ,  $a^{H'}=0.077$  mT) appeared immediately and decayed rapidly (half-life ca. 2 min). The same spectrum also appeared in the absence of water in ethyl acetate (same parameters as above; half-life 1 min), acetonitrile ( $a^N=1.37$ ,  $a^H=2.12$ ,  $a^{H'}=0.077$  mT; half-life 0.9 min) and dichloromethane ( $a^N=1.36$ ,  $a^H=2.08$ ,  $a^{H'}=0.088$  mT; half-life 0.2 min). A control experiment was performed with PBN (1) and TCNE in acetonitrile which showed the slow growth of a (3 × 2)-line spectrum with  $a^N=1.51$ ,  $a^H=0.197$  mT, the same as earlier determined for NC-PBN<sup>•</sup> in acetonitrile ( $a^N=1.51$ ,  $a^H=0.193$  mT).<sup>26</sup>

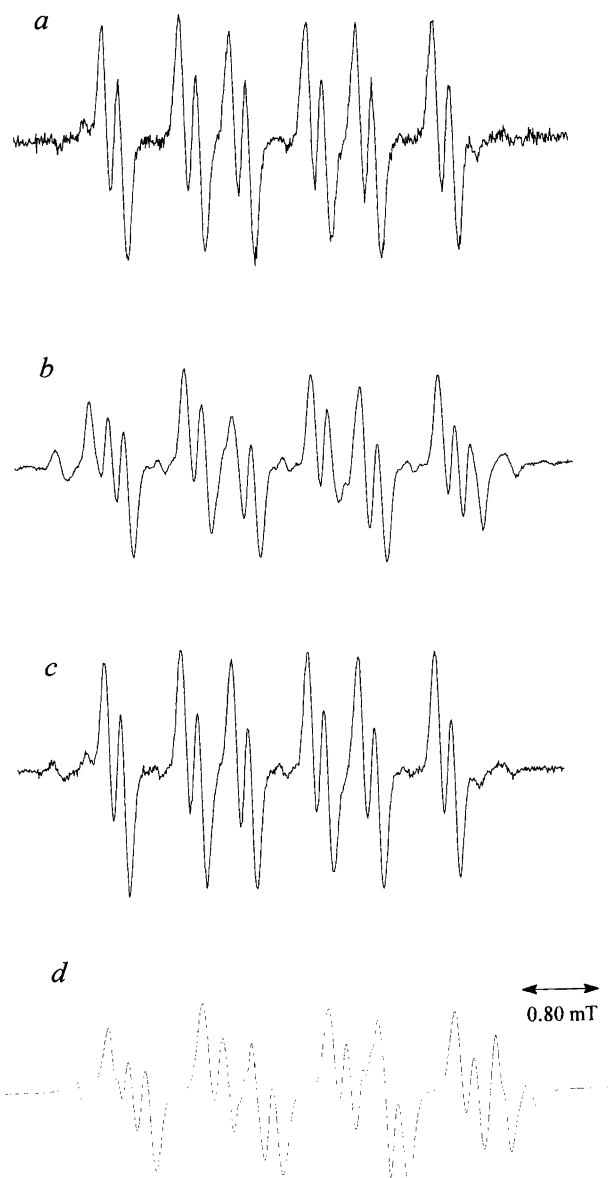


Fig. 2. EPR spectra recorded from a solution of DMPO ( $50 \text{ mmol dm}^{-3}$ ),  $\text{F}_4\text{Q}$  ( $\sim 50 \text{ mmol dm}^{-3}$ ),  $\text{H}_2\text{O}$  ( $2.4 \text{ mol dm}^{-3}$ ) and  $\text{EtOH}$  ( $1.4 \text{ mol dm}^{-3}$ ) in acetonitrile after (a) 10 min in the dark, (b) a further 3 min of UV irradiation and (c) a further 10 min in the dark. A simulation of the spectra of  $\text{EtO-DMPO}\cdot$  (48%) and  $\text{HO-DMPO}\cdot$  (44%) plus a spectrum with  $a^{\text{N}} = 1.42 \text{ mT}$  and  $a^{\text{H}} = 1.94 \text{ mT}$  (8%) is shown in (d). See text and Tables 2 and 3.

*Other photo-oxidants in the inverted spin trapping mechanism.* It was also of interest to elucidate the influence of the triplet state redox potential in the photo-oxidation of DMPO in acetonitrile-water. Table 5 shows results of such experiments. Again, DDQ is too strong a thermal oxidant to allow for the detection of  $\text{HO-DMPO}\cdot$ . In the quinone series, the borderline triplet redox potential between photoactive and photoinactive compounds lies around 1.8 V.

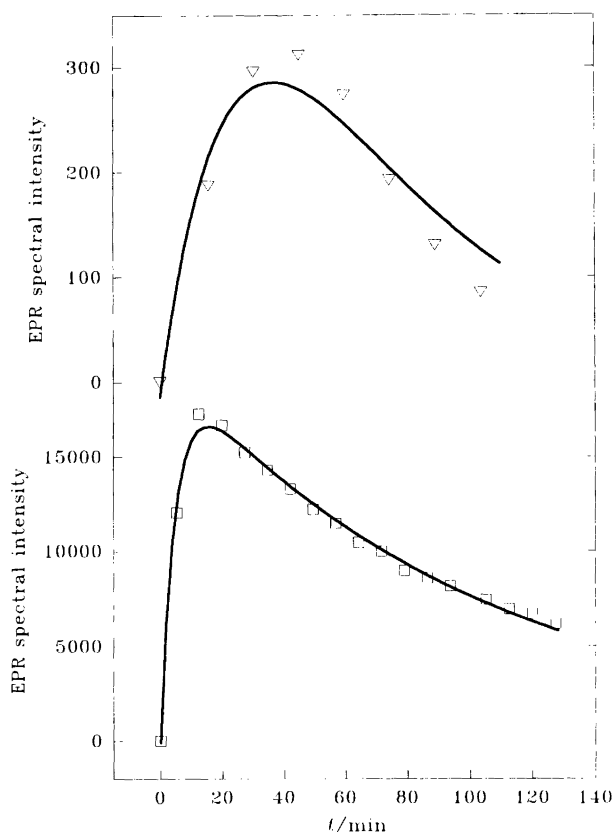


Fig. 3. Development of the EPR spectrum recorded from a solution of DMPO ( $50 \text{ mmol dm}^{-3}$ ),  $\text{Et}_4\text{NOH}$  ( $8.5 \text{ mmol dm}^{-3}$ ) and  $\text{F}_4\text{Q}$  ( $\sim 50 \text{ mmol dm}^{-3}$ ) in acetonitrile-water (7:1 v/v) under dark conditions (triangles, the curve corresponds to the best-fitting four-parameter double exponential with  $k_{\text{up}} = 0.030 \text{ min}^{-1}$  and  $k_{\text{down}} = 0.026 \text{ min}^{-1}$ ), or a solution of DMPO ( $50 \text{ mmol dm}^{-3}$ ) and  $\text{F}_4\text{Q}$  ( $\sim 50 \text{ mmol dm}^{-3}$ ) in ethyl acetate-water (2.7% by volume) under dark conditions (squares, the curve corresponds to the best-fitting four-parameter double exponential with  $k_{\text{up}} = 0.21 \text{ min}^{-1}$  and  $k_{\text{down}} = 0.0010 \text{ min}^{-1}$ ).

*The reaction of DMPO with water in the presence of formate.* In a similar way as ethanol, formate ion plays an important role for the identification of mechanisms involving trapping of the hydroxyl radical by DMPO. In the presence of formate, hydroxyl radical will abstract an H atom from formate, thus creating the  $\cdot\text{COO}^-$  radical. The latter radical can be trapped by DMPO to give a spin adduct with a characteristic EPR spectrum ( $a^{\text{N}} 1.54$ ,  $a^{\text{H}} 1.82 \text{ mT}$  in water at pH 7). If no such spin adduct is detected, the hydroxyl radical cannot be involved [eqns. (6) and (9)].

Previously<sup>4</sup> it was shown that the photolysis of DMPO, a carboxylic acid  $\text{RCOOH}$  and  $\text{Cl}_4\text{Q}$  in dichloromethane gave the acyloxy adduct  $\text{RCOO-DMPO}\cdot$  for  $\text{R} = \text{H}$ ,  $\text{CH}_3$ ,  $(\text{CH}_3)_3\text{C}$ ,  $\text{C}_6\text{H}_5$  and  $\text{CF}_3$ . In ethyl acetate, with formic acid as  $\text{RCOOH}$  and  $\text{F}_4\text{Q}$  or  $\text{Cl}_4\text{Q}$  as the photo-oxidant, a weak EPR spectrum with the characteristics of  $\text{HCOO-DMPO}\cdot$  [ $a^{\text{N}} 1.32$ ,  $a^{\text{H}} 1.05$ ,  $a^{\text{H}'} 0.20 \text{ mT}$  (the two remaining  $a^{\text{H}}$  were not resolved); lit.<sup>4</sup> in

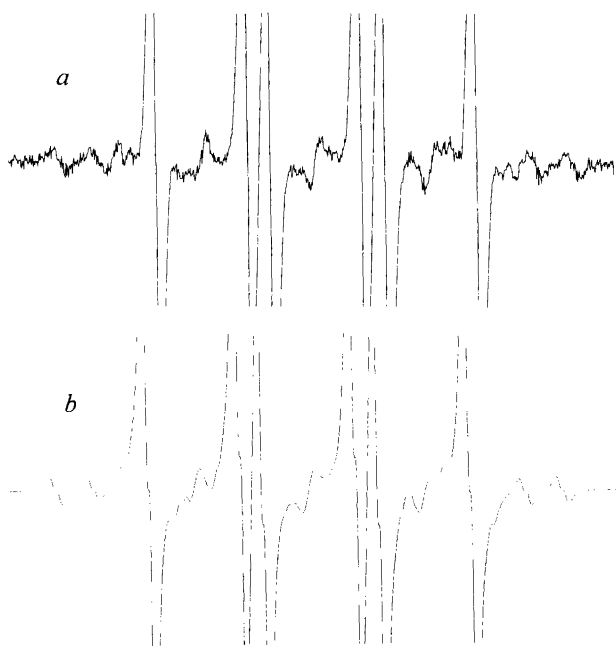


Fig. 4. EPR spectrum recorded from a solution (a) of DMPO ( $50 \text{ mmol dm}^{-3}$ ) and  $\text{F}_4\text{Q}$  ( $\sim 50 \text{ mmol dm}^{-3}$ ) in EtOAc–water (2.7 vol%); the water contained 21.4 atom%  $^{17}\text{O}$ ) after standing in the dark for 20 min. The simulation (b) is based on data given in Table 3. Optimization of the spectrum from an identical run gave the composition 74% unlabelled radical, 26%  $^{17}\text{O}$  labelled, expected 21.4%.

dichloromethane:  $a^{\text{N}}$  1.26,  $a^{\text{H}}$  1.01,  $a^{\text{H}'}$  0.190,  $a^{\text{H}''}$  0.097,  $a^{\text{H}'''}$  0.074 mT] appeared initially. Upon prolonged irradiation, it was gradually replaced by a  $(3 \times 2)$ -line spectrum,  $a^{\text{N}}$  1.40,  $a^{\text{H}}$  1.80 mT.

With tetrabutylammonium formate as the nucleophile and  $\text{F}_4\text{Q}$  as the oxidant, a similar  $(3 \times 2)$ -line spectrum ( $a^{\text{N}}$  1.35,  $a^{\text{H}}$  1.75 mT) appeared and increased in intensity over a period of 1 h (Fig. 5) under dark conditions. This spectrum is sufficiently similar to that of  $^-\text{OOC-DMPO}^{\cdot}$

( $a^{\text{N}}$  1.54,  $a^{\text{H}}$  1.82 mT in water) to warrant a control experiment with  $^{13}\text{C}$ -labelled formate; however, the same spectrum ( $a^{\text{N}}$  1.35,  $a^{\text{H}}$  1.73 mT) developed.

In order to generate the authentic spectrum of  $^-\text{OO}^{13}\text{C-DMPO}^{\cdot}$  for comparison, DMPO and tetrabutylammonium ( $^{13}\text{C}$ )formate were photolyzed with 1% hydrogen peroxide in ethyl acetate. A spectrum of four components appeared. The spectrum of  $^-\text{OO}^{13}\text{C-DMPO}^{\cdot}$  (32%) had  $a^{\text{N}}$  1.46,  $a^{\text{H}}$  1.64 and  $a^{13\text{C}}$  1.16 mT; lit.<sup>27</sup>  $a^{\text{N}}$  1.56,  $a^{\text{H}}$  1.88 and  $a^{13\text{C}}$  1.21 mT in water). The other spectra had  $a^{\text{N}}$  1.42,  $a^{\text{H}}$  1.93 mT (23%),  $a^{\text{N}}$  1.46,  $a^{\text{H}}$  2.10 mT (24%) and  $a^{\text{N}}$  1.38,  $a^{\text{H}}$  1.10,  $a^{\text{H}'}$  0.092 mT (21%), in the order assigned to the two diastereomers of  $\text{CH}_3\text{COOCH}(\text{CH}_3)\text{-DMPO}^{\cdot}$  and  $\text{HO-DMPO}^{\cdot}$  (see above). The spectrum of  $^-\text{OO}^{13}\text{C-DMPO}^{\cdot}$  was also generated by hydrogen peroxide (1%) photolysis in an aqueous phosphate buffer at pH 6.5 ( $a^{\text{N}}$  1.56,  $a^{\text{H}}$  1.87 and  $a^{13\text{C}}$  1.21 mT) together with  $\text{HO-DMPO}^{\cdot}$ .

## Discussion

*Lifetime of the hydroxyl spin adduct of DMPO in various media and its role for EPR spectral detection of HO-DMPO $^{\cdot}$ .* From published results<sup>12–16</sup> and the data in Table 1, it is clear that the lifetime of  $\text{HO-DMPO}^{\cdot}$  is strongly dependent on the medium. In particular, the presence of traces of acid, such as those produced when halogenated quinones are dissolved in a water-containing solvent,<sup>21</sup> decreased the lifetime, whereas buffering in the region of pH 6–8 or the addition of bases increased it. Thus, whatever the mechanism is, the first prerequisite for the observation of an EPR spectrum of  $\text{HO-DMPO}^{\cdot}$  is that the lifetime of the radical is long enough (estimated at  $>1\text{--}1.5$  min) to compensate for a possibly slow mode of generation. As shown earlier,<sup>22</sup> ethyl acetate was a favourable solvent in this respect. The possible disadvantage with using ethyl acetate is its reactivity toward hydroxyl radical which was demonstrated in control

Table 4. Generation of  $\text{HO-DMPO}^{\cdot}$  by the dark reaction of DMPO and a weak oxidant in ethyl acetate–water (2.7 vol%).

Oxidant	Redox potential	Spectrum from	$a^{\text{N}}/\text{mT}^a$	$a^{\text{H}}/\text{mT}^a$	$a^{\text{H}'}/\text{mT}^a$
2,3- $\text{Cl}_2$ -4,5-( $\text{CN}$ ) $_2\text{Q}$	0.51	DMPOx (4)	0.332	0.339	
$\text{FeCl}_3^b$	0.53	$\text{HO-DMPO}^{\cdot}$	1.36	1.08	0.092
$\text{FcH}^+ \text{CF}_3\text{COO}^-$	0.35	$\text{HO-DMPO}^{\cdot}$	1.37	1.09	Not resolved
TCNE	0.15	$\text{NC-DMPO}^{\cdot c}$	1.36	2.07	0.086
		$\text{NC-DMPO}^{\cdot d}$	1.55	1.89	
$\text{T}_4\text{Q}^e$	0.05	$\text{HO-DMPO}^{\cdot}$	1.36	1.09	Not resolved
$\text{Cl}_4\text{Q}$	0.02	$\text{HO-DMPO}^{\cdot}$	1.370	1.084	Not resolved
$\text{F}_4\text{Q}$	-0.04	$\text{HO-DMPO}^{\cdot}$	1.376	1.095	Not resolved
$\text{Br}_4\text{Q}$	-0.06	$\text{HO-DMPO}^{\cdot}$	1.365	1.091	0.099
2,3- $\text{Cl}_2\text{Q}$	-0.19	$\text{HO-DMPO}^{\cdot}$	1.367	1.094	0.094
$\text{H}_4\text{Q}$	-0.45	$\text{HO-DMPO}^{\cdot}$	1.390	1.114	Not resolved
2,5- $\text{Me}_2\text{Q}$	-0.54	No signal			
AnthraQ-2- $\text{SO}_3^-$	-0.60	No signal			
$\text{Me}_4\text{Q}$	-0.76	No signal			
AnthraQ	-0.94	No signal			

<sup>a</sup>Multiplicity = 1. <sup>b</sup>In the presence of 2,6-di-*tert*-butylpyridine ( $60 \text{ mmol dm}^{-3}$ ); without base, the spectrum was very weak. <sup>c</sup>Half-life ca. 2 min; assignment uncertain (see text). <sup>d</sup>Ref. 35. <sup>e</sup>T = Tetramethyl-*N*-succinimidyl.<sup>38</sup>

Table 5. Generation of HO-DMPO<sup>•</sup> by the photochemical reaction<sup>a</sup> of DMPO and a sensitizer in acetonitrile-water (7:1 v/v).

Sensitizer	Triplet redox potential (SCE)	Spectrum from	$a^N/mT^b$	$a^H/mT^b$	$a^H/mT^b$
2,3-Cl <sub>2</sub> -4,5-(CN) <sub>2</sub> Q (DDQ)	2.65	Radical anion of DDQ			
TPP <sup>+c</sup>	2.29	No signal			
F <sub>4</sub> Q	2.19	HO-DMPO <sup>•</sup>	1.43	1.25	0.078
Cl <sub>4</sub> Q	2.16	HO-DMPO <sup>•</sup>	1.45	1.27	<sup>d</sup>
1,2,4,5-(CN) <sub>4</sub> -benzene	2.15	No signal			
AnthraQ-2-SO <sub>3</sub> <sup>-</sup>	1.80	No signal			
Br <sub>4</sub> Q	1.96	HO-DMPO <sup>•</sup>	1.46	1.25	<sup>d</sup>
2,3-Cl <sub>2</sub> Q	1.95	HO-DMPO <sup>•</sup>	1.46	1.24	<sup>d</sup>
2,3-Cl <sub>2</sub> Q	1.95	HO-DMPO <sup>•</sup>	1.44	1.27	<sup>d</sup>
H <sub>4</sub> Q	1.87	HO-DMPO <sup>•</sup>	1.45	1.26	<sup>d</sup>
2,5-Me <sub>2</sub> Q	1.85	HO-DMPO <sup>•</sup>	1.43	1.25	<sup>d</sup>
AnthraQ	1.77	No signal			
TPA <sup>+e</sup>	1.74	No signal			
2-Ph-benzoxazole	1.73	HO-DMPO <sup>•</sup>	1.45	1.24	<sup>d</sup>
Me <sub>4</sub> Q	1.63	No signal			

<sup>a</sup>Only for 2,3-Cl<sub>2</sub>Q was HO-DMPO<sup>•</sup> detectable also in the dark reaction. <sup>b</sup>Multiplicity=1. <sup>c</sup>2,4,6-Triphenylpyrylium ion. <sup>d</sup>Not measurable owing to line broadening. <sup>e</sup>2,4,6-Tris-(4-methoxyphenyl)pyrylium ion.

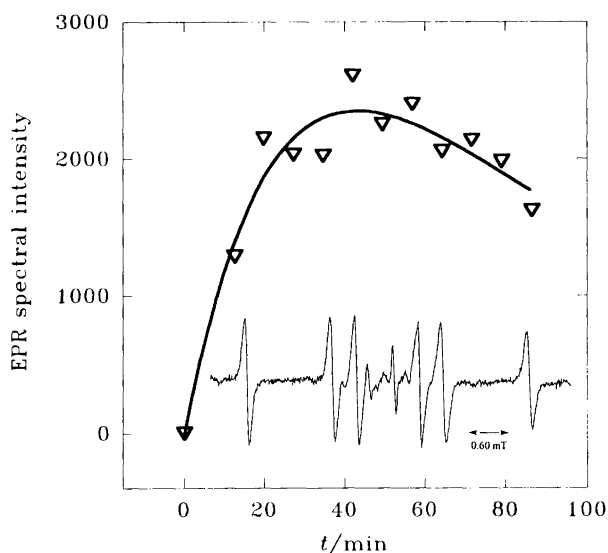


Fig. 5. Development of the EPR spectrum recorded from a solution of DMPO (50 mmol dm<sup>-3</sup>), Bu<sub>4</sub>NHCOO (100 mmol dm<sup>-3</sup>) and F<sub>4</sub>Q (~50 mmol dm<sup>-3</sup>) in ethyl acetate under dark conditions. The curve corresponds to the best-fitting 4-parameter double exponential with  $k_{up}=0.033 \text{ min}^{-1}$  and  $k_{down}=0.016 \text{ min}^{-1}$ . The weak lines in the middle belong to F<sub>4</sub>Q<sup>•-</sup>.

experiments. Diastereomeric spin adducts where the CH<sub>3</sub>COO-CH(CH<sub>3</sub>) radical had been trapped by DMPO were detected. However, such spectra were not observed in the experiments designed to establish the inverted spin trapping or Forrester-Hepburn mechanism.

The Forrester-Hepburn mechanism of eqns. (3) and (4) presumably has the nucleophilic attack as the rate-determining one. Weak nucleophiles, like water and alcohols, will attack DMPO slowly, and therefore the rate of formation of the spin adduct should be slow. Only if the decomposition mode of the latter is relatively slow will it be possible to detect it by EPR spectroscopy.

Once suitable conditions had been found, HO-DMPO<sup>•</sup> could be easily detected in dark reactions (Tables 3 and 4) involving DMPO, water and weak oxidants. As already shown elsewhere<sup>12</sup> by experiments involving <sup>17</sup>O labelled water, the trapped 'hydroxyl radical' was derived entirely from water.

Alkoxy spin adducts, RO-DMPO<sup>•</sup>, were formed with great ease from nucleophilic, non-hindered alcohols in dark reactions, presumably because of their much longer lifetime (Fig. 1). A sterically hindered alcohol (*t*-BuOH), as well as the electrophilic trifluoroethanol and hexafluoropropan-2-ol, required photolysis for detection of the corresponding spin adducts which then presumably were formed via inverted spin trapping [eqns. (1) and (2)], DMPO being oxidized to its radical cation by the triplet state of the quinone.<sup>28</sup>

*The nature of the oxidant in the dark reaction.* Quinones acted as thermal oxidants in the water-DMPO reaction in ethyl acetate, as long as the redox potential was > ca. -0.5 V vs. SCE. Iron(III)-based reagents also worked well, as shown before.<sup>12</sup> TCNE was also tried, but no EPR spectrum of HO-DMPO<sup>•</sup> was detected (see below).

The behaviour of Fe<sup>III</sup>-based oxidants is of interest in connection with the use of the Fenton reagent for the generation of hydroxyl radical.<sup>12,29</sup> The problem is that Fe<sup>III</sup> is formed in the Fenton reaction, and could be shown<sup>12</sup> to be involved as the oxidant in the Forrester-Hepburn mechanism, competing with addition of the hydroxyl radical. As demonstrated here and earlier,<sup>12</sup> Fe<sup>III</sup> can oxidize the intermediate hydroxylamine, and reactions involving Fe<sup>II</sup>, hydrogen peroxide, spin traps and water therefore must be subjected to careful control experiments if claims about the involvement of the hydroxyl radical are to be reliable.

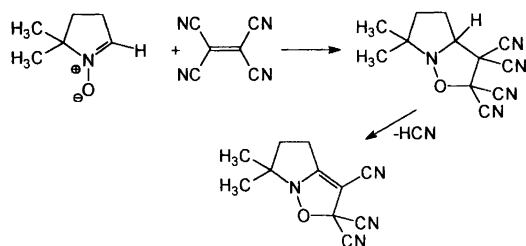
*The nature of the mechanism in the photolyzed reaction.* In acetonitrile-water (7:1 v/v), the lifetime of HO-



DMPO $\cdot$  is short, and only for a few oxidants can one detect this spin adduct in the dark. It was therefore possible to verify (Table 5) that only photosensitizers with a triplet redox potential  $>1.7$ – $1.8$  V (SCE) could affect the one-electron oxidation of DMPO [ $E_p(\text{DMPO}^+/\text{DMPO})=1.68$  V] under these conditions. These potential differences correspond to high electron transfer rates, as predicted by the Marcus theory.<sup>30</sup>

*Tetracyanoethylene as an oxidant; is possibly NC-DMPO $\cdot$  formed?* No spectrum of HO-DMPO $\cdot$  was obtained upon treatment of DMPO–water in ethyl acetate by TCNE, an ET oxidant of somewhat higher reactivity than F<sub>4</sub>Q. Instead the spectrum of a spin adduct with a C-connected radical developed. It had  $a^N=1.36$ ,  $a^H=2.07$  and  $a^I=0.086$  mT and had a short lifetime, of the order of 2 min in ethyl acetate and as low as 0.2 min in dichloromethane. Since a similar reaction with PBN gave rise to the well known<sup>26</sup> EPR spectrum of NC-PBN $\cdot$ , the structure of NC-DMPO $\cdot$  was assigned to the radical above. For a C-connected spin adduct, NC-PBN $\cdot$  has been shown to be curiously reactive under various conditions, as for example indicated by a half-life of 23 min in hexane.

Two plausible mechanisms of formation of NC-DMPO $\cdot$  involve the intermediacy of HCN, known to add to nitrones with ease.<sup>31</sup> One mode of HCN formation might involve protonation of TCNE $\cdot^-$ , followed by elimination of HCN,<sup>32</sup> but this is less likely in view of the large rate difference between the reactions of DMPO and PBN and the fact that the reactions run equally well in nominally nonaqueous media as in aqueous ones. A second mechanism might involve cycloaddition of TCNE to the nitron,<sup>33,34</sup> shown for DMPO in Scheme 2. HCN might then be generated in an elimination reaction. The large rate difference between the two spin traps is then explained by the lower reactivity of PBN caused by steric hindrance from the large *N-tert*-butyl group.

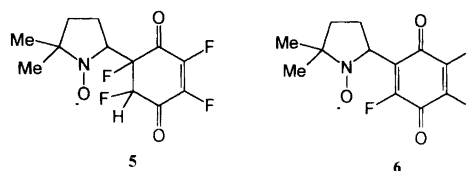


Scheme 2.

EPR spectral data<sup>35</sup> in water have been given for NC-DMPO $\cdot$  as  $a^N=1.55$  and  $a^H=1.89$  mT. Experiments with  $^{13}\text{C}\text{N}^-$  as the source of the cyano group showed that the  $^{13}\text{C}$  atom was connected to the  $\alpha$ -C of DMPO. The difference from the h.f.s. constants in ethyl acetate and other organic solvents of differing polarity is not explicable on the basis of solvent polarity. Either the assignment of the spectrum here is wrong, or the published spectrum in water is derived from a more persistent

radical formed by further chemical transformation of the cyano group of NC-DMPO $\cdot$ .

*An unknown C-connected DMPO spin adduct.* The reaction of formate or  $^{13}\text{C}$ -formate, DMPO and F<sub>4</sub>Q in ethyl acetate under dark conditions gave rise to a new spectrum ( $a^N=1.35$  and  $a^H=1.75$  mT). These are parameters characteristic of a C-connected spin adduct, and the relatively small  $a^H$  is reminiscent of carbonyl-connected adducts, like for example CH<sub>3</sub>CO-DMPO $\cdot$ .<sup>36</sup> Activated olefins have been shown<sup>34</sup> to give reductive coupling products with a nitron such as PBN **1** and since quinones can be considered to be activated olefins, it may be that this spectrum should be assigned to the reductive coupling product **5** or, more likely, the product **6** of further elimination of HF under the slightly basic conditions. This aspect will be the subject of further study.



## Experimental

*Methods.* EPR spectra were recorded by the Upgrade Version ESP 3220-200SH of a Bruker ER-200D spectrometer. Photolyses were performed in the photolysis cavity (ER 4104 OR), using light from the 50 W high-pressure Hg lamp from Bruker (Osram HBO50/AC, L2). The EPR experiments were performed as described earlier (100 kHz modulation frequency, microwave effect 0.4–1.6 mW, modulation amplitude 0.01–0.04 mT). Simulations were carried out by the public domain programme WINSIM.<sup>37</sup>

*Materials.* PBN and DMPO were obtained from Aldrich or Lancaster. Acetonitrile and ethyl acetate were of UVASOL quality and dichloromethane of SupraSolv quality. ( $^{13}\text{C}$ )Formic acid ( $>99\%$   $^{13}\text{C}$ ) and ( $^{17}\text{O}$ )water (21.4%  $^{17}\text{O}$ ) was from Dr Glaser AG, Switzerland. 2,4,6-Tris(4-methoxyphenyl)pyrylium tetrafluoroborate (TPA $^+$ ) and 2,4,6-triphenylpyrylium tetrafluoroborate (TPP $^+$ ) was a gift from Professor Dr. Eberhard Steckhan, University of Bonn. Tetrabutylammonium formate was made by neutralization of a solution of Bu<sub>4</sub>NOH in methanol (Aldrich) by formic acid or ( $^{13}\text{C}$ )formic acid, followed by evaporation of the solvent, toward the end in oil pump vacuum. All other chemicals were of highest commercial quality available.

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