Radical cations from nitrone spin-traps: reaction with water to give OH adducts

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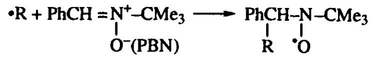
Exposure of dilute solutions of three widely used nitrone spin-traps in dry Freon matrices ($CFCl_3$) at 77 K to ionising radiation gave corresponding radical cations, characterised by their EPR spectra. On melting, these radical-cations were stable and good liquid-phase EPR spectra were obtained, together with spectra for chloride ion adducts. When solutions containing water were used, the same cations were detected at 77 K. However, on melting, isotropic spectra characteristic of the ˙OH radical adducts were obtained in high yield. These results are discussed in terms of the use of nitrone spin-traps to detect ˙OH radicals, especially in biological systems.

The technique of spin-trapping, whereby highly reactive radicals (R˙), that are difficult to detect directly using electron paramagnetic resonance (EPR) spectroscopy, are converted into stable radical 'adducts', as for example in reaction (1), is

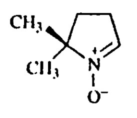


$$\cdot\mathrm{R} + \underset{\mathrm{O^-(PBN)}}{\mathrm{PhCH{=}N^+{-}CMe_3}} \longrightarrow \underset{\mathrm{R\ \ \cdot O}}{\mathrm{PhCH{-}N{-}CMe_3}} \qquad (1)$$

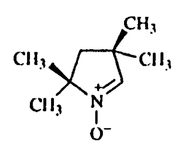
very widely used, especially in biological systems.[1] It is, in particular, useful for detecting radicals, such as ˙OH and $\dot{O}_2^-$, that cannot be detected directly by EPR spectroscopy because the lines are too broad. However, in systems where the rate of generation is small, such that relatively long periods are required to accumulate detectable concentrations of adducts, there is a danger that other reactions may intervene so seriously that no weight can be given to the results. Also, stable radicals such as nitric oxide may not add to such spin traps.

The most serious of these alternative processes are redox reactions, especially for aminoxyl radicals. Whilst these are stable as radicals, and have little tendency to dimerise, they are half way between the stable species R_2NO^+ and R_2NO^-, and are readily converted into these in the presence of electron-donors or -acceptors. Even so, it is possible to detect aminoxyl radicals in biological systems and the radicals can sometimes be 'recovered' by suitable manipulation of redox reagents.[2]

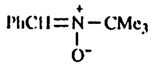
Another problem arises if the spin-traps themselves can be oxidised or reduced, as is illustrated for DMPO (**1**) in eqns. (2)–(5).



1 (DMPO)



2 (TMPO)



3 (PBN)

$$\mathrm{DMPO} - e^- \longrightarrow \mathrm{DMPO}^{\cdot +} \qquad (2)$$

$$\mathrm{DMPO}^{\cdot +} + H_2O \longrightarrow \mathrm{DMPO} - \mathrm{OH}\cdot + (H^+) \qquad (3)$$

$$\mathrm{DMPO} + e^- \longrightarrow \mathrm{DMPO}^{\cdot -} \qquad (4)$$

$$\mathrm{DMPO}^{\cdot -} + (H^+) \longrightarrow \mathrm{DMPO} - \mathrm{H}\cdot \qquad (5)$$

Thus, electron loss may lead to a species identical with the ˙OH radical adduct, whilst electron gain may result in a species identical with the ˙H atom adduct, even though ˙OH and/or ˙H are not involved.

In order to study the former possibility in a direct way, we used Freon ($CFCl_3$) as a solvent, since it is well established that dilute solutions of suitable substrates always give the corresponding radical-cations on irradiation at 77 K (unless these are unimolecularly unstable).[3] Using DMPO (**1**), TMPO (**2**) and PBN (**3**) we obtained EPR features assignable to the parent cations.[4] When water was also added, these cations were converted into the ˙OH adducts on melting, as judged by the EPR spectra. In view of current interest in this field,[5,6] and of its pertinence to studies of ˙OH radicals in biological systems containing redox agents, we now report full details of this work. Zubarev and Brede have subsequently studied the formation of PBN radical-cations formed in this way.[7]

Results and discussion

Formation of radical-cations

Exposure of very dilute solutions of substrates (S) in halo-solvents, such as the Freons ($CFCl_3$ in this study), to ionising radiation at low temperatures (usually 77 K) normally results in the generation of the radical-cations, $S^{\cdot +}$, or unimolecular breakdown products, provided the ionisation potentials of S are less than those of the solvent.[3] This field has been very well established and assignments of spectra to these radical-cations can be made with confidence. This conclusion is accepted in the present work.

In most previous radiation studies using Freon matrices, only solid-state spectra have been obtained since loss of the radicals generally occurs close to the melting point of the Freon. However, in the present study, well defined, isotropic spectra were obtained at the melting point of the glasses which gave the original solid-state features on rapid re-cooling. Thus, these isotropic spectra are due to the same species that are responsible for the solid-state spectra, which are, we propose, the parent radical-cations.

Interpretation of solid-state spectra

Knowing the isotropic parameters has aided the interpretation of the solid-state spectra, examples of which are shown in Fig. 1. These spectra are complicated by the fact that there are three

Table 1 Magnetic parameters for radical-cations derived from PBN, DMPO and TMPO[a]

Radical	Hyperfine coupling/G				
	$^{14}N_{//}$	$^{14}N_{\perp}$	^{14N}Iso	$^{1}H_{\alpha}(1)$	$^{1}H_{\beta}(2)$
$PBN^{\cdot+}$					
Solid	26			*ca.* 5	—
Liquid	—	—	12.5	*ca.* 4.5	—
$DMPO^{\cdot+}$					
Solid	23.5			*ca.* 5	*ca.* 7
Liquid	—	—	12.8	4.8	6.2
$TMPO^{\cdot+}$					
Solid	23			*ca.* 5	—
Liquid	—	—	12.7	4.5	—

[a] In all cases, g_{max} *ca.* 2.0085, $g_{int.}$ *ca.* 2.0055, g_{min} *ca.* 2.0025, g_{soln} *ca.* 2.0055.

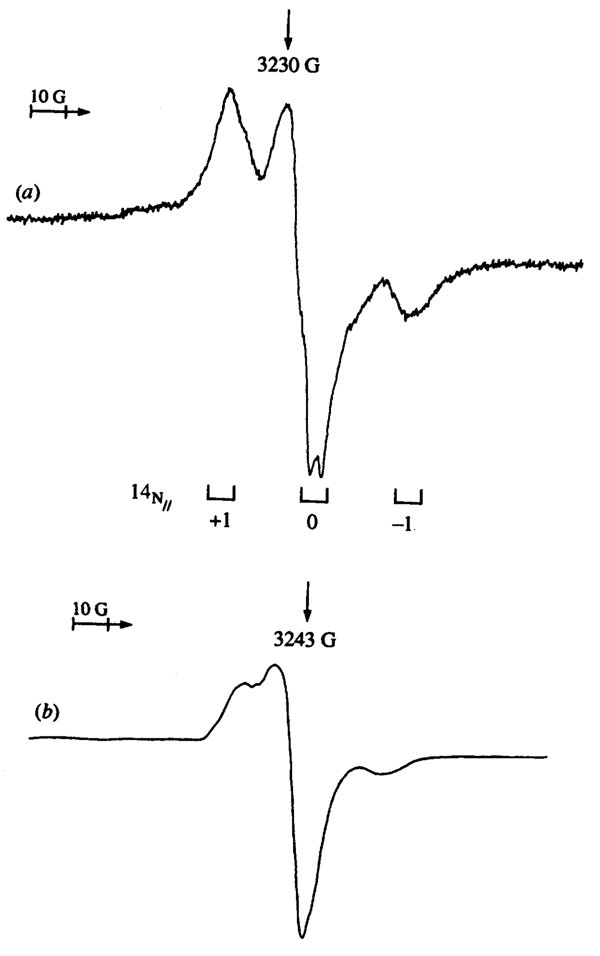


Fig. 1 First derivative X-band EPR spectrum from dilute solutions of (*a*) TMPO and (*b*) PBN in $CFCl_3$ after exposure to ionising radiation at 77 K, showing features assigned to the corresponding radical-cations

anisotropic components, the *g*-tensor, the ^{14}N and the $^{1}H(\alpha)$ hyperfine tensors. (Hyperfine coupling to the β-protons in the DMPO radical-cation is expected to be almost isotropic.) These only share one axis (*z* in Fig. 3). The *x* and *y* axes of the *g*-tensors are expected to be controlled by the C–O bond direction, with g_{max} close to this direction. Similarly, the α-proton coupling is controlled by the C–H$^{\alpha}$ bond direction, whereas A_x (^{14}N) is expected to be similar to A_y (^{14}N). Unfortunately, the solid-state spectra were all poorly resolved. Nevertheless, the results given in Table 1 gave reasonable fits for the spectra, and are self-consistent.

The ^{14}N hyperfine data can be used to give approximate orbital populations in the 2s and 2p(π) orbitals on nitrogen by comparison with the isotropic and anisotropic coupling constants, in the usual way.[8,9] This gives a 2p(π) population of *ca.* 30% with no significant 2s population, as expected for planar systems with a π-type SOMO. The ^{1}H coupling constants can be used to give approximate values for the 2p(π) population on carbon.[6] Both the α- and β-hyperfine data lead to about 22% on carbon. This leaves *ca.* 48% on oxygen, which accounts for the rather large shift for g_{max} (Table 1).

Formation of aminoxyl radicals

In the absence of added water the major aminoxyl radical formed from the parent radical-cations on melting the irradiated solids seems to be the chloride ion adduct, conversion being rapid and extensive for the PBN derivative (Fig. 2). However, for DMPO and TMPO only broad, poorly defined features were obtained prior to loss of signal. We have previously argued that the product of electron addition to Freon ($CFCl_3^{\cdot-}$), readily dissociates to give Cl^- + $^{\cdot}CFCl_2$ radicals.[10] Also, we have shown that these Cl^- ions can react with suitable radical-cations to give Cl-adducts, suggesting that cations and anions are formed and trapped in close proximity in $CFCl_3$ glasses. The Cl-adduct for PBN is well known, and our data agree well with those of others.[7,11] However, we have found no record of data for the Cl-adducts of DMPO or TMPO.

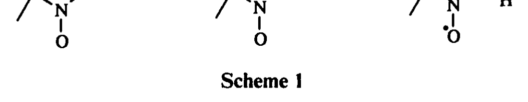
When the solvent was saturated with water prior to irradiation, formation of the Cl-adduct was suppressed, and spectra characteristic of OH adducts grew in on annealing (Fig. 2). For PBN both the Cl- and OH-centres were formed together, but with DMPO and TMPO systems, water addition completely dominated, and only the OH adducts were detected on annealing. We therefore conclude that reaction (6) is important, even when water is present in very low concentration. Hence in water, radical-cations formed by redox processes should be converted into OH adducts with very high efficiency.[4,6] This confirms that care must be taken in inferring the involvement of $^{\cdot}OH$ radicals, especially for systems containing powerful oxidising agents.

Makino *et al.* suggested that when aqueous Fe^{3+} ions react with the spin-trap DMPO, OH adducts are formed by nucleophilic attack of water on the DMPO.[12] This has been nicely confirmed by Mason and his co-workers,[13] using H_2O labelled with ^{17}O. For simple hydrated Fe^{3+} ions, the DMPO–OH adduct was formed from the water. Furthermore, the same adduct was formed as one of the products using Fe^{2+} (aquated) and H_2O_2. However, in the presence of good ligands this process was suppressed, and, using H_2O_2, only the non-labelled OH adduct was detected.

It is interesting that yet another mechanism is proposed to explain the former effect. In this case it is suggested that an initial 'nucleophilic' attack on DMPO occurs and this is then followed by loss of hydrogen [reaction (6)].

$$\mathrm{DMPO} + H_2O \longrightarrow \mathrm{DMPO}(OH_2) \xrightarrow[-H^+]{-e} \mathrm{DMPO} - OH^{\cdot} \quad (6)$$

Our results show that *initial* electron-loss to give $DMPO^{\cdot+}$ followed by addition of water (Scheme 1) is a reasonable



Scheme 1

alternative to reaction (6). In other words, the oxidation may precede the addition of water. It seems probable that the spin-trap can coordinate to the metal ions, in which case some

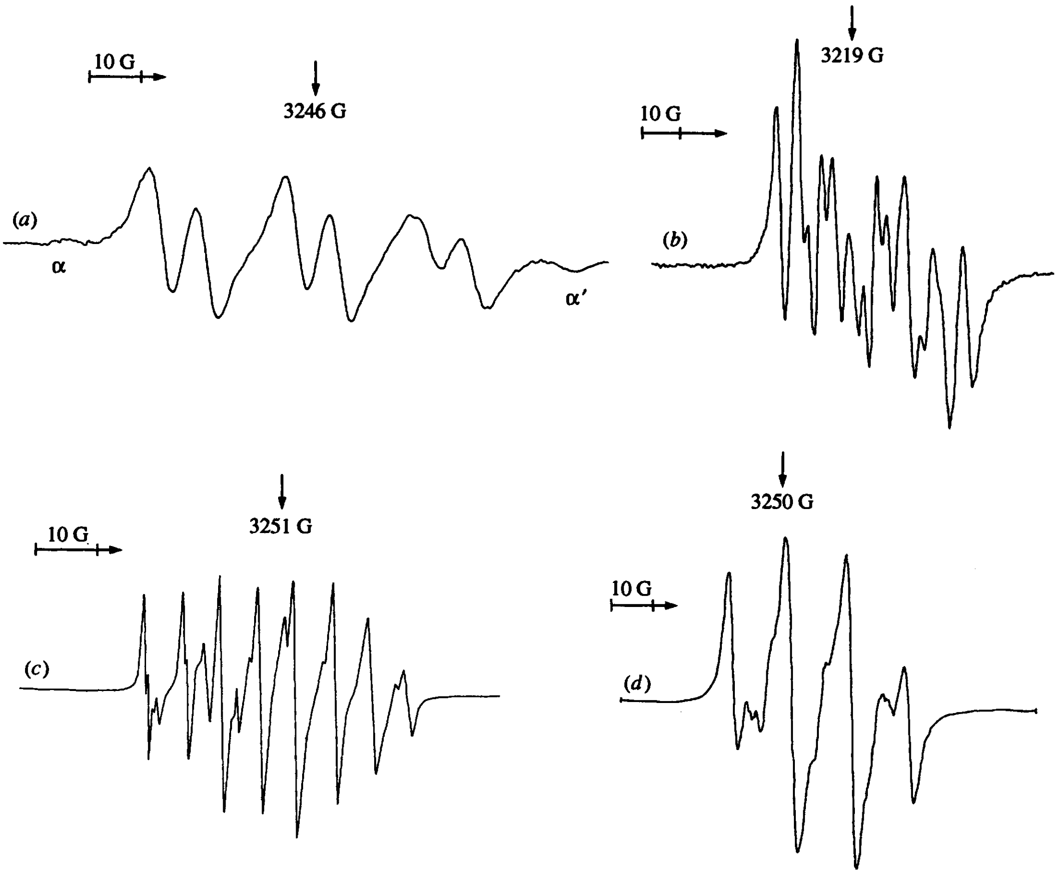


Fig. 2 As for Fig. 1, after warming to just above the melting points of the solutions, showing features assigned to (*a*) the radical-cation of TMPO, (*b*) the radical-cation of DMPO, (*c*) the chloride ion addition product with the PBN cation and (*d*) the addition product between water and the DMPO cation. [The outer features (α,α′) in (*a*) are tentatively assigned to the –$CFCl_2$ adduct of TMPO.]

Table 2 Magnetic parameters for OH and Cl adducts

	Isotropic hyperfine coupling/G			
Radical	^{14}N	^{1}H (α)	^{35}Cl	^{37}Cl
PBN–Cl	12.3	0.8	6.2	5.1
PBN–OH	*ca.* 14	*ca.* 2.8	—	—
DMPO–OH	*ca.*[a] 14–15	*ca.* 14–15	—	—
TMPO–OH	*ca.*[a] 14–15	*ca.* 14–15	—	—
PBN–Cl (ref. 11)	12.7	0.89	6.20	5.12

[a] ^{14}N and ^{1}H data ranged between 14 and 15 G as a function of temperature.

form of concerted reaction probably occurs during the lifetime of the complex and the timing of separate events becomes obscure.

One aspect of our results is that the isotropic coupling to ^{14}N for the OH adduct was unusually dependent on the temperature of measurement, ranging reversibly between *ca.* 14 and 15 G, and decreasing with increase in temperature (Table 2). It is well established that $A(^{14}N)$ increases when the oxygen of the $\geq$ NO group is hydrogen-bonded.[14] We also expect that solvation by water will be strongly favoured on cooling, and tentatively infer that this is a major source of the temperature sensitivity for the ^{14}N coupling.

This result helps to explain the very efficient addition of water to the radical cations. Water is very sparingly soluble in $CFCl_3$, and is probably largely present as monomers. These will tend to form hydrogen-bonds to oxygen of the nitrones so that they will be close to the reactive carbon of the radical-cations that are formed.

Finally, it is interesting to recall that, in our related study of the radical-cations of nitrosobenzene, and of 2-methyl-2-nitrosopropane (Me_3CNO),[15] we also found well defined spectra for their primary radical-cations. The former were of interest because the cation clearly had a SOMO that had σ-character on nitrogen, with a large isotropic contribution to the hyperfine coupling. This shows that there is a switch from the π-SOMO found in the present study to the in-plane σ-type SOMO for $(Ph\text{–}NO)^{\cdot+}$.

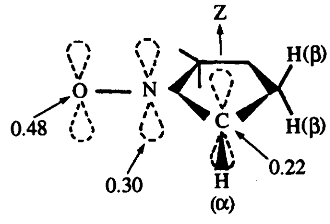


Fig. 3 Suggested structure for the radical-cation of DMPO

Conclusions

We conclude that nitrones form well defined radical-cations on exposure to ionising radiation in dilute solutions in $CFCl_3$. These have the expected π-structure with considerable spin-densities on oxygen, nitrogen and carbon (Fig. 3).

In the absence of water, these are sufficiently stable to be detected in fluid solutions, but they react in part with Cl^- ions formed from the solvent. The $^{\cdot}CFCl_2$ radicals also formed from the solvent may in part add to the spin-traps to give aminoxyl radicals, but these were not clearly defined [*cf.* Fig. 2(*a*)].

In the presence of water in very low concentrations the radical-cations were almost quantitatively converted into the normal OH adducts on melting. These adducts were relatively stable. These results help to reinforce the view that care is needed in interpreting the mechanism of formation of such OH adducts in any system that contains strong oxidising agents.

Experimental

The spin-traps, DMPO, TMPO and PBN were the highest grades available and were used as supplied. Freon ($CFCl_3$) was purified using an alumina column, dried over CaH_2 and deoxygenated. Exposure of the purified solvent to ionising radiation at doses used in the present studies (up to *ca.* 10^3 Gy) gave no significant yields of radicals in the $g = 2$ region. When water was added, no extra signals were obtained provided ice crystallites were not formed on freezing: if they were, then normal ˙OH radical spectra[16] were obtained.

Solutions were *ca.* 10^{-3} mole fraction in spin-trap. Water was used in approximately equivalent concentrations. Samples were frozen as small spherical beads in liquid nitrogen directly prior to irradiation at 77 K. Doses were in the 10^2–10^3 Gy range, and results were independent of dose.

EPR spectra were obtained using an X-band Varian E109 spectrometer. Microwave powers were in the 0.1–1.0 mW range, and, when required, spectra were accumulated and simulated using an Archimedes computer and in-house programs.

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