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# PHOTOSENSITIZED GENERATION OF SUPEROXIDE RADICAL IN APROTIC SOLVENTS: AN EPR AND SPIN TRAPPING STUDY

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The UV or visible irradiation of pigments such as curcumin, anthralin, benzanthrone, 1,8-dihydroxyanthraquinone, and rose bengal- or eosine-complexes with cationic surfactants in aerated aprotic solvents, such as benzene, toluene, acetone, *n*-heptane, cyclohexane, in the presence of 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) generates EPR spectra with hyperfine splitting constants (hfsc's) $a_N$ = 12.75 G, $a^{\beta}_H$ = 10.50 G, $a^{\gamma}_H$ = 1.26 G in toluene, 12.83 G, 10.64 G, 1.24 G in benzene, 12.75 G, 10.19 G, 1.35 G in acetone and 12.54 G, 10.46 G, 1.38 G in *n*-heptane and cyclohexane. These spectra are similar to those observed when DMPO reacts with 18-crown-6 ether-solubilized $KO_2$ in the respective solvents and suggests that the photoinduced EPR spectra can be safely assigned to the DMPO/superoxide radical adduct (**1**). A correlation between the hfsc's of **1** and solvent parameters, the solvent acceptor number **AN** and the Kosower **Z** value, has been evaluated in terms of its usefulness for the identification of the DMPO/superoxide adduct in organic media.

KEY WORDS: Superoxide radical, photosensitization, aprotic solvent, benzene, toluene, acetone, cyclohexane, *n*-heptane, spin trapping, 5,5-dimethyl-1-pyrroline *N*-oxide.

## INTRODUCTION

EPR spectroscopy in conjunction with the spin trapping technique has become a convenient tool for the detection of the superoxide anion ($O_2^{\cdot -}$), a free radical that is an important intermediate in many biological and chemical processes. The spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) has a significant advantage over other trapping agents because it produces distinct EPR spectral patterns with peroxyl ($RO_2^{\cdot}$), alkoxyl ($RO^{\cdot}$) and carbon-centered ($R^{\cdot}$) radicals.[1] In aqueous systems, DMPO can easily differentiate between superoxide ($O_2^{\cdot -}$) and hydroxyl ($OH^{\cdot}$) radicals due to the unique EPR features of their respective spin adducts.[2] There is, however, one serious limitation of this spin trap; the EPR spectra of adducts **1** and **2**, which derive from superoxide and peroxyl ($ROO^{\cdot}$) radicals respectively, are very similar if not identical in many solvents.[3] In aqueous solutions the presence of $O_2^{\cdot -}$ can be readily verified using the enzyme superoxide dismutase (SOD) which dramatically decreases the concentration of **1** while leaving **2** unchanged.[3]

The question is whether the similarity between **1** and **2** holds also in organic solvents and if so, whether it is possible to differentiate between these two species. Some authors[4–7] have reported hfsc's for **1** in aprotic solvents which are markedly

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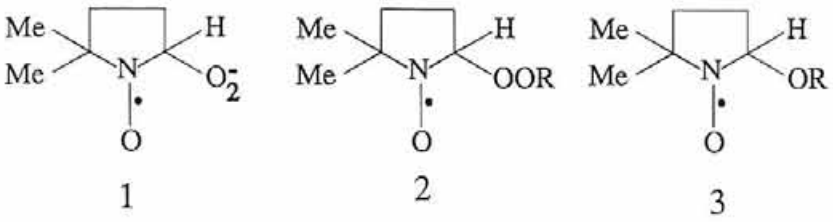



1 2 3

SCHEME 1

different from those of adduct **2**, but which are more characteristic of the adduct **3**. Because in organic media SOD cannot be used it is impossible to verify these assignments. To account for these discrepancies Janzen[8] has proposed that the previously reported EPR spectra of **1** in benzene, acetone and *n*-heptane do not belong to adduct **1**. Alternatively, should the assignments be correct, he suggested that the large differences in hfsc's may be due to different time-averaged conformations of **1**. Because there is no simple procedure for confirming the presence of **1** in aprotic solvents the question remains as to what criteria should be applied to verify assignments and to identify adducts **1** and **2** in these solvents.

We have recently assigned the EPR spectra observed in benzene, toluene, acetone and *n*-heptane produced by reaction of DMPO with 18-crown-6 ether-solubilized $KO_2$ to adduct **1**.[9] In this report we have extended our observations to other systems which could potentially generate the same species. We have employed a photochemical approach using a variety of pigments already known to possess strong photosensitizing properties, such as anthralin, benzanthrone, curcumin, 1,8-dihydroxyanthraquinone (1,8-DHAQ), eosine (Eo) and rose bengal (RB). The primary goal of this study was to generate the DMPO/$O_2^{\cdot-}$ radical by irradiating these pigments in the presence of DMPO in aerated aprotic solvents such as benzene, toluene, acetone, cyclohexane, and *n*-heptane. Identification of adduct **1** was considered positive if the photo-induced EPR spectrum was the same as that produced chemically from $KO_2$ but was different from the spectra of **2** and **3** produced by established procedures in the same solvent. Additionally, to further substantiate the identification of the observed species as adduct **1** we determined how the new hyperfine coupling constants relate to those found in more polar and protic media. We also correlated $a_N$ and $a_H^\beta$ values of **1** with solvent parameters, the solvent acceptor number **AN** and the Kosower **Z** value.

## MATERIALS AND METHODS

Potassium superoxide ($KO_2$), rose bengal (RB), eosine (Eo), curcumin (CU), 1,8-dihydroxyanthraquinone (1,8-DHAQ), benzanthrone (BA), benzophenone (BzPh), 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), cetylpyridinium chloride (CPC), dimethyldioctadecylammonium bromide (DB) and 18-crown-6 ether were purchased from Aldrich Chemical Company (Milwaukee, WI). Nitromethane was from Fisher Scientific and all other solvents (spectrophotometric grade) were from Aldrich. Anthralin (An, 1,8-dihydroxy-9[10]-anthracene, Dithranol) was from Sigma (St Louis, MO). 1,8-DHAQ was recrystallized from toluene. DMPO was purified by distillation under reduced pressure and was stored under nitrogen at −21°C. To

TABLE 1
Hyperfine splitting constants (in gauss) of adduct 1 in organic solvents

| Solvent (#) | AN[a] | Z[b] (kcal/mole) | $a_N$ | $a_H^\beta$ | $a_H^\gamma$ |
|---|---|---|---|---|---|
| $C_6H_{12}^c$ (1) | 0 | - | 12.54 | 10.46 | 1.38 |
| *n*-Heptane[c] (2) | 0 | - | 12.54 | 10.46 | 1.38 |
| | | | 12.49[d] | 10.29[d] | 1.2[d] |
| Benzene[e] (3) | 8.2 | 54 | 12.83 | 10.64 | 1.24 |
| | | | 12.63[d] | 10.36[d] | 1.25[d] |
| Toluene[f] (4) | - | - | 12.75 | 10.50 | 1.26 |
| | | | 12.74[d] | 10.45[d] | 1.24[d] |
| Dioxane[g] (5) | 10.8 | - | 12.69 | 10.56 | 1.19 |
| $C_6H_5Cl$[g] (6) | - | - | 12.84 | 10.67 | 1.38 |
| Acetone[h] (7) | 12.5 | 65.7 | 12.75 | 10.19 | 1.35 |
| | | | 12.75[d] | 10.17[d] | 1.38[d] |
| Pyridine[h] (8) | 14.2 | 64 | 12.79 | 10.17 | 1.38 |
| | | | 12.74[d] | 10.17[d] | 1.38[d] |
| DMF[g] (9) | 16.0 | 68.5 | 12.84 | 10.27 | 1.58 |
| DMSO[j] (10) | 19.3 | 71.1 | 12.7 | 10.38 | 1.32 |
| $CH_3CN$[g] (11) | 19.3 | 71.3 | 13.0 | 10.47 | 1.28 |
| $CH_3NO_2$[g] (12) | 20.5 | - | 13.29 | 11.01 | 1.14 |
| *i*-Propanol[g] (13) | 33.5 | 76.3 | 13.14 | 10.47 | 1.29 |
| EtOH[g] (14) | 37.1 | 79.6 | 13.24 | 10.47 | 1.38 |
| MeOH[g] (15) | 41.3 | 83.6 | 13.38 | 10.57 | 1.33 |
| Water[j] (16) | 54.8 | 94.6 | 14.1 | 11.3 | 1.25 |

[a]From Ref. 24; [b]From Ref. 23; [c]$Eo(DB)_2$; [d]using $KO_2$ (Ref. #9); [e]$RB(CPC)_2$, 1,8-DHAQ, BA, CU; [f]$RB(CPC)_2$, $Eo(DB)_2$, CU; [g]CU; [h]CU, BA, An, 1,8-DHAQ; [i]BA; [j]Ref. 4.

render RB and Eo soluble in aprotic solvents their 1:2 complexes (ion pairs) with the cationic detergents CPC and DB were prepared using described procedures.[10] Benzene, toluene, cyclohexane and acetone were dried over molecular sieves (Aldrich; 4A) freshly activated, or over Na-lead alloy (dri-Na, Baker). All other solvents and chemicals were used as received. All solvents used are identified by numbers as shown in Table 1.

EPR spectra were recorded using Varian E-109 Century line EPR spectrometer operating at 9.5 GHz with 100 kHz modulation. Samples consisting of a photosensitizing pigment (~1 mg/ml) and DMPO (80 mM) in a solvent were transferred to an aqueous quartz EPR cell (0.3 mm lightpath) and were gassed briefly (20 sec) with $N_2$ to remove an excess of oxygen and to improve resolution of the spectra. Subsequently the samples were irradiated directly inside the microwave cavity of the spectrometer using 1 kW Xe lamp equipped with Schoeffel grating monochromator at the following wavelengths: 570 nm for $RB(DB)_2$ and $RB(CPC)_2$, 530 nm for $Eo(DB)_2$, 420 nm for CU and BA, 386 for An and 1,8DHAQ. The DMPO/$^{\cdot}O_2^-$ adduct was produced with $KO_2$ as follows. Stock solution of $KO_2$ (ca. 4 mg/mL) was prepared in dry solvent (benzene, toluene or acetone) containing 0.1 M crown ether and the solution was sonicated for 15 sec. An aliquot of the $KO_2$ solution (20 $\mu$L) was added to 500 $\mu$L of the appropriate solvent containing 0.1 M crown ether and 80 mM DMPO. The sample was bubbled with nitrogen for 1 min and then EPR spectra were recorded. Hfsc's were obtained by simulating and optimizing spectra on an IBM PC computer using program developed by Mr David R. Duling.

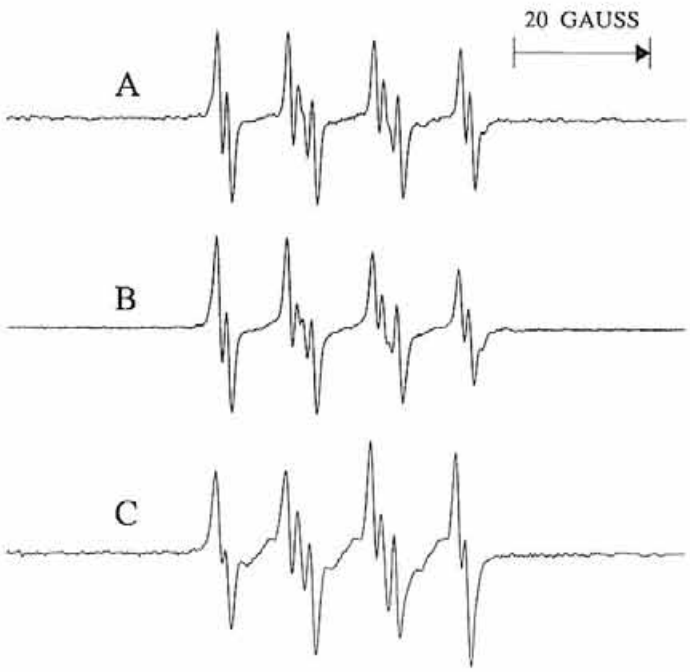



FIGURE 1 EPR spectra of adduct **1** in aprotic solvents. (A) **1** was produced by illumination (530 nm) of $Eo(DB)_2$ in aerated toluene in the presence of DMPO (80 mM); (B) **1** was produced with 18-crown-6 ether-solubilized $KO_2$ in toluene containing DMPO (80 mM); (C) **1** produced by photolysis (530 nm) of $Eo(DB)_2$ in aerated *n*-heptane in the presence of ca. 10 mM DMPO. Experimental settings: microwave power 10 mW, modulation amplitude 0.165 G(A) and 0.33 G (B and C), receiver gain 1.0, 0.32, and $2.0 \times 10^4$ for A, B and C respectively, time constant 0.25 sec (A and C) and 0.128 sec (B), scan rate 4 min.

## RESULTS

Illumination (530 nm) of $Eo(DB)_2$ in toluene in the presence of DMPO (80 mM) generated the EPR spectrum shown in Figure 1A. Similar spectra were observed when toluene solutions of $RB(CPC)_2$ and CU were irradiated (data not shown). Averaged values of the hfsc's, calculated using individual data from all pigments in toluene, are listed in Table 1. Maximum deviations from the average values are small (less than 0.5%, 1.1% and 2.5% for $a_N$, $a_H^\beta$ and $a_H^\gamma$ respectively), indicating that they are independent of the nature of the sensitizing pigment. Thus, it was concluded that all these spectra can be assigned to one and the same species. When the $KO_2$/18-crown-6 ether system reacted with DMPO in toluene the spectrum shown in Figure 1B was obtained. This spectrum has been previously attributed to adduct **1**[6]. Because the photoinduced spectra were identical to that generated using $KO_2$ they were assigned to the same radical **1**.

Similar photochemical experiments were performed in other solvents using the sensitizers specified in Table 1. In *n*-heptane and cyclohexane only $Eo(DB)_2$ was used because, in contrast to the other pigments, it dissolves readily in these solvents to

form highly colored solutions. An additional advantage of $RB(DB)_2$ and $Eo(DB)_2$ is that they can be photoactivated with low-energy photons corresponding to 570 nm and 530 nm respectively. The EPR spectra observed in these two solvents were identical (Figure 1C). Table 1 gives the respective hfsc's obtained in heptane and cyclohexane. They are close to values obtained using $KO_2$[9] suggesting that they may belong to the same species **1**. We believe that previously reported hfsc's for **1** in *n*-heptane ($a_N$ = 12.9 and $a_H^\beta$ = 6.8 G Ref. #2; $a_N$ = 12.9, $a_H^\beta$ = 6.3, and $a_H^\gamma$ = 1.6 G Ref. #7) are derived from an as yet unidentified DMPO adduct (the values of couplings suggest that it may belong to a DMPO adduct of an alkoxy radical). In addition to toluene, heptane and cyclohexane, we used $KO_2$ to generate adduct **1** in benzene, acetone and pyridine. In all these solvents EPR spectra produced photochemically were very close to those obtained using $KO_2$.

To further substantiate the assignment of the photoinduced spectra to adduct **1** we compared them with spectra of the DMPO adducts of peroxyl and alkoxyl radicals. For these experiments we chose *t*-BuOOH. The reason for this selection was the known photochemistry of this hydroperoxide and the ease with which one can obtain the respective peroxyl and alkoxyl radicals. It has been reported that hydroperoxides quench triplet states of ketone sensitizers (>C=O) and that the quenching is accomplished to a significant extent by hydrogen atom transfer (Eq. 1). Thus, this reaction is an efficient source of the respective peroxyl radical[11,12].

$$>{}^3C{=}O^* + ROOH \rightarrow >C^{\cdot}{-}OH + ROO^{\cdot} \quad (1)$$

Decay of these peroxyl radicals affords alkoxyl radicals, *t*-BuO$^{\cdot}$, whose DMPO adducts have already been characterized by EPR[1,13]. When benzophenone was irradiated (360 nm) in deaerated benzene in the presence of *t*-BuOOH (300 mM) and DMPO (80 mM) the EPR spectrum shown in Figure 2B was observed. It contains contribution from two adducts; the major component is **2** (R = $^{\cdot}$OO$-t$Bu), with a minor contribution from **3** (R = $^{\cdot}$O$-t$Bu). The hfsc's of the former species (Table 2) are close to those found earlier in toluene solution.[13] In the dark the spectrum of **2** slowly decayed away leaving the spectrum of the adduct **3** (Figure 2C). The hfsc's of the latter species are similar to those determined in benzene.[1] As Table 2 shows the hfsc's of **2** and **3** are distinctly different from those attributed to **1** in the same solvent.

## DISCUSSION

Although DMPO has proved useful for the identification of various types of radical species, its ability to differentiate between superoxide/hydroperoxyl and peroxyl

TABLE 2
Hyperfine coupling constants (in gauss) of adducts **2** and **3** in selected aprotic solvents

| Solvent | $a_N$ | $a_H^\beta$ | $a_H^\gamma$ | Ref. # |
|---|---|---|---|---|
| Benzene, *t*-BuOO$^{\cdot}$ | 12.69 | 9.29 | 1.58 | this work |
| Toluene, *t*-BuOO$^{\cdot}$ | 12.72 | 9.36 | 1.44 | 13 |
| Benzene, *t*-BuO$^{\cdot}$ | 13.1 | 8.00 | 1.84 | this work |
| | 13.11 | 7.93 | 1.97 | 1 |
| Methyl oleate ester, LOO$^{\cdot}$ | 12.62 | 10.25 | 1.41 | 20 |

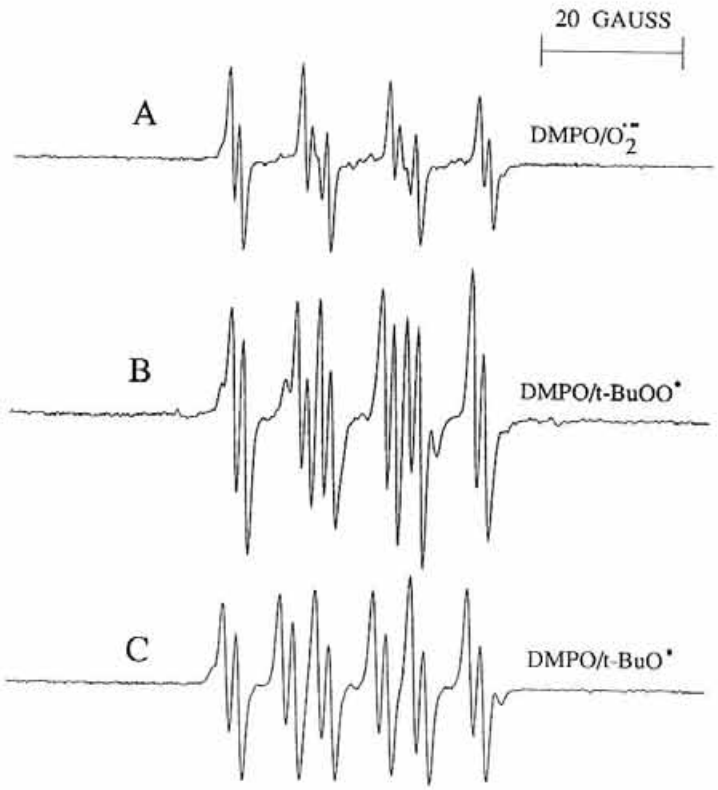



FIGURE 2 EPR spectra of DMPO adducts in benzene. (A) Adduct **1** produced by photolysis (570 nm) of $RB(CPC)_2$ in the presence of DMPO (80 mM); (B) adduct **2** (R = *t*-BuOO$^\cdot$) observed during irradiation (360 nm) of BzPh (10 mM), *t*-BuOOH (300 mM) and DMPO (80 mM) in deaerated benzene; (C) adduct **3** (R = *t*-BuO$^\cdot$) observed after ca. 0.5 hour incubation of a sample prepared as in B. Experimental settings: microwave power 10 mW, modulation amplitude 0.33 G, gain 4.0, 10.0 and $6.3 \times 10^3$ for A, B and C respectively, time constants 0.128 sec (A) and 0.25 sec (B, C), scan rate 4 min.

radicals in organic solvents is limited. This is because in organic solvents there is no convenient test procedure, similar to that of SOD for aqueous solutions, nor are there any clear spectroscopic criteria that allow identification based on EPR spectral parameters alone.

The difficulties encountered in the interpretation of EPR spectra of nitrone spin adducts are partially due to the relative insensitivity of the EPR spectral parameters to even significant differences in the structure of the trapped radicals.[1,14] Moreover, the values of the hfsc's of identical adducts obtained using different methods, or under different experimental conditions in different laboratories also show small variations.[14,15] SOD makes it possible to differentiate between adducts **1** and **2** in water despite the similarity of their EPR spectra in this solvent.[3] It was anticipated that in organic solvents, in which SOD cannot be used, the ambiguity could be resolved by applying an established procedure of superoxide generation, such as the use of 18-crown-6 ether-solubilized $KO_2$. However, in our earlier work we demonstrated that this approach has severe limitations because the EPR spectra generated

in aprotic solvents depend on $KO_2$ concentration.[9] It has been reported that DMPO undergoes degradation under oxidative conditions created by high concentrations of $KO_2$.[16] This may result in the formation of secondary, DMPO-derived, free radical species which may react with the spin trap, to yield artifactual EPR signals. Such DMPO degradation processes are probably a consequence of the markedly enhanced reactivity of superoxide radicals in aprotic *versus* protic solvents[17], although oxidative degradation and DMPO ring opening have also been observed in aqueous solutions.[8,18,19] Thus, extreme caution must be exercised when $KO_2$ is employed to produce the DMPO/superoxide adduct in aprotic media. These side reactions may be the reason why other researchers, who used phthalocyanines, CdS and ZnO as the photoactive elements or $KO_2$ in the presence of DMPO to generate adduct **1**, observed EPR spectra which we believe are not attributable to **1**.[4,7]

In the present work we generated a series of DMPO adducts with oxyl-radicals by illuminating a variety of pigments and DMPO in aerated aprotic solvents and compared their EPR spectra to those obtained from well established sources of superoxide, peroxyl and alkoxyl radicals. It was assumed that the EPR spectrum of **1** should fulfill the following criteria: (i) hfsc's constants should be in the range characteristic of **2**, but at the same time they should differ from those of a DMPO adduct with a simple alkyl peroxyl radical such as *t*-BuOO$^{\cdot}$; (ii) the EPR spectra of photochemically-generated **1** should match the EPR spectrum obtained using $KO_2$/18-crown-6 as the source of the superoxide radical; (iii) the hf couplings of **1** may depend on solvent but not on photosensitizing pigment. We have shown that EPR spectra generated photochemically using several structurally-different sensitizers fulfill all these criteria and therefore they are attributed to adduct **1**.

The DMPO/peroxyl radical (**2**) is chemically very similar to adduct **1**. The formation of **2** (with R derived from either sensitizing pigment or from DMPO itself) in aprotic solvents cannot be excluded. It is unlikely however, that adduct **2** will produce EPR spectra identical to that of **1***. For example, the EPR spectra of DMPO adducts with t-BuOO$^{\cdot}$ (this work and ref. 13) and with peroxyl radicals from arachidonic acid, linoleic acid, or linolenic acid[13] are quite different from those attributed to **1**. Nevertheless Borg and Shaich[20] have reported that the EPR spectrum of DMPO/$^{\cdot}$OOR (R-methyl linoleate ester) has hfsc's ($a_N$ = 12.4 G, $a_H^{\beta}$ = 10.4 G, $a_H^{\gamma}$ = 1.7 G) which are quite close to those assigned in this work to adduct **1** (Table 1). Clearly there is a need for an independent and reliable method for identifying **1** that could be applied to organic solvents.

If the hfsc's of **1** could be related to some solvent property then it might be possible to use such a relationship to identify **1** in any solvent. Janzen[8] has reported that $a_N$ and $a_H^{\beta}$ parameters of **1** measured in $H_2O$, ethylene glycol, methanol, ethanol, acetonitrile, DMSO and DMF, are related by equation 2. Unfortunately this relationship did not hold for acetone,

$$a_H^{\beta} = a_N - 2.7 \qquad (2)$$

benzene, and *n*-heptane.[8] Figure 3 shows $a_H^{\beta}$ plotted against $a_N$ for all solvents used in this work. It is apparent that even when the newly determined $a_N$ and $a_H^{\beta}$ values

*While it is premature to indicate the exact mechanism of the formation of the superoxide radical it may be suggested that the process is initiated by the photoinduced electron transfer within aggregated pigment molecules, or in the case of $RB(CPC)_2$, $RB(DB)_2$, $Eo(DB)_2$, within the dye/surfactant complexes, followed by oxidation of radical anions by oxygen to give superoxide radical.

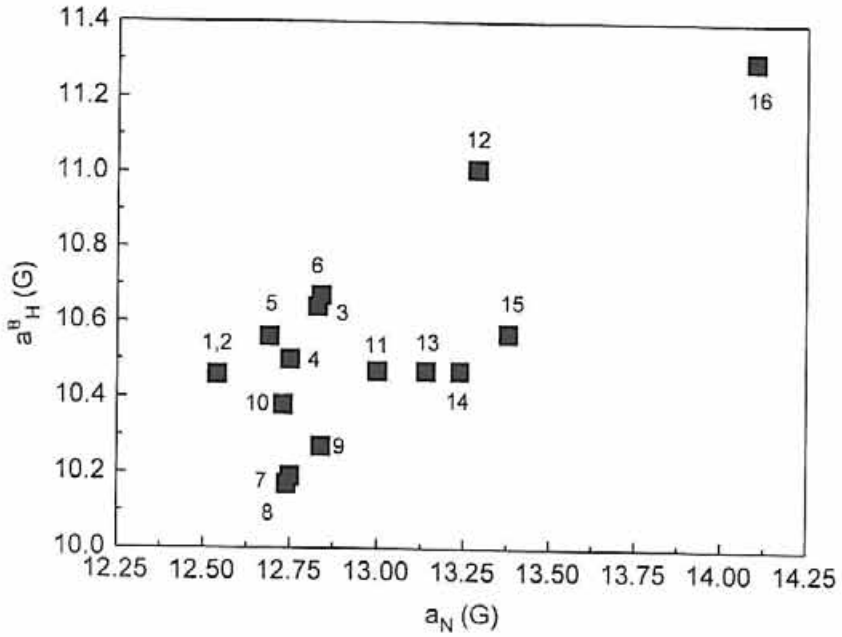



FIGURE 3 Plot of $a^{\beta}_{H}$ versus $a_N$ for **1**. Numbers indicate solvents as given in Table 1.

for **1** are employed, and data from a larger number of solvents are included, no linear relationship, encompassing all types of solvents exists.

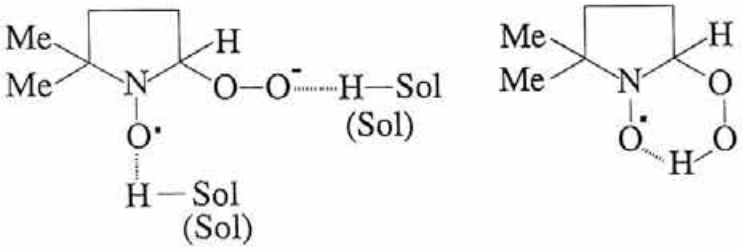
The failure to find a simple correlation between EPR parameters for **1** obtained in solvents of different polarity and proticity is a reflection of the complexity of intermolecular interactions between the DMPO adduct and solvent molecules. Analysis of the role of solvent on $a_N$ for the structurally related stable nitroxide radicals has shown[21,22] that $a_N$ increases when solvent polarity increases. One interpretation of this observation is that highly polar solvents induce redistribution of electrons in the nitroxide moiety ($>N-O^{\cdot}$) to achieve a new state, characterized by higher electron density on oxygen and higher spin density on the nitrogen atom (Eq. 3) causing larger nitrogen splitting.

$$>N-O^{\cdot}\,(\text{non-polar media}) \rightleftarrows >N^{\cdot+}-O^{-}\,(\text{highly polar media}) \qquad (3)$$

It is believed that solvent parameters which describe most adequately such donor-acceptor interactions should correlate best with the hfsc's of nitroxide radicals.

The DMPO/$O_2^{\cdot-}$ adduct has two possible centers of interaction with solvent; these are the nitroxide and the $-O-O^{-}$ groups. Both may form intermolecular hydrogen bonds in protic solvents, while in polar and aprotic environment these centers will be solvated. These two situations are depicted in structure **4**.

In non-polar aprotic media the adduct may exist in form **5**, with intramolecular hydrogen bond involving the nitroxide oxygen and hydrogen atom of the $-O-OH$ moiety. The possibility that DMPO/$^{\cdot}O_2H$ might exist in such a conformation has been suggested by Janzen[8] by analogy to hydroxyalkyl spin adducts which form a similar structure.[1] The superoxide radical is known to behave as an extremely strong




4 5

H-Sol - protic solvent

Sol - aprotic, polar solvent

SCHEME 2

base in an aprotic environment[17] abstracting protons from many organic donors. One cannot exclude the possibility that in solvents such as benzene or cyclohexane, $O_2^{\cdot-}$ could abstract $H^+$ from solutes (DMPO, photosensitizing pigments) to form the neutral $HO_2^{\cdot}$ radical. Then, after the additon to DMPO the cyclic structure **5** could be formed. One may speculate that in solvents that favor structure **5** for the DMPO/$^{\cdot}O_2H$ adduct, the interaction of the radical with solvent will be very weak and there should be little, if any, dependence of the hf splittings on solvent properties. In contrast to this, solvents which promote the open structure **4** may have a significant influence on the hf splittings.

To test this hypothesis we used two frequently applied solvent parameters; the Kosower **Z** value[23] and the solvent acceptor number, **AN**[24]. Values of these parameters for solvents used in this work are given in Table 1. Earlier Harbour and Hair[4] used the **Z** parameter and related it to hfsc's of **1** in several organic solvents. They found that the nitrogen coupling of **1** decreases slightly on going from aqueous solution to organic solvents and then remains constant for solvents with $\mathbf{Z} < 71$ kcal/mole (aprotic solvents; see Table 1). In contrast to the nitrogen coupling, the $\beta$-hydrogen coupling of **1** showed very strong dependence on **Z**, decreasing sharply for solvents with $\mathbf{Z} < 71$ kcal/mole. If this observation were correct, it might be helpful in identifying the radical in organic solvents. However, when $a_N$ and $a_H^{\beta}$, determined in the present work are plotted versus **Z** (Figure 4) the strong decrease of $a_H^{\beta}$ with **Z** is not observed. Both the nitrogen and hydrogen splittings generate lines that are, roughly, parallel to each other (solid lines in Figure 4). Initially, on going from water (#16) to alcohols (#15–13) the splittings decrease significantly and then their values remain almost constant. A similar type of dependence of the hf splittings on **Z** has been observed for **1** produced using $KO_2$.[9] This lack of dependence on **Z** is particularly evident for $a_N$. On the other hand, fitting all data points to a one single straight line using linear regression, appears to be inadequate (dashed lines in Figure 4) indicating that all these solvents cannot be treated as a single uniform group (correlation coefficients 0.8878 and 0.6559 for $a_N$ and $a_H^{\beta}$, respectively).

We tried to correlate the hfsc's of **1** with another solvent parameter, the solvent acceptor number, **AN**. This parameter is a dimensionless number related to the relative chemical shift of $^{31}P$ in triethylphosphine oxide ($Et_3PO$) in the particular

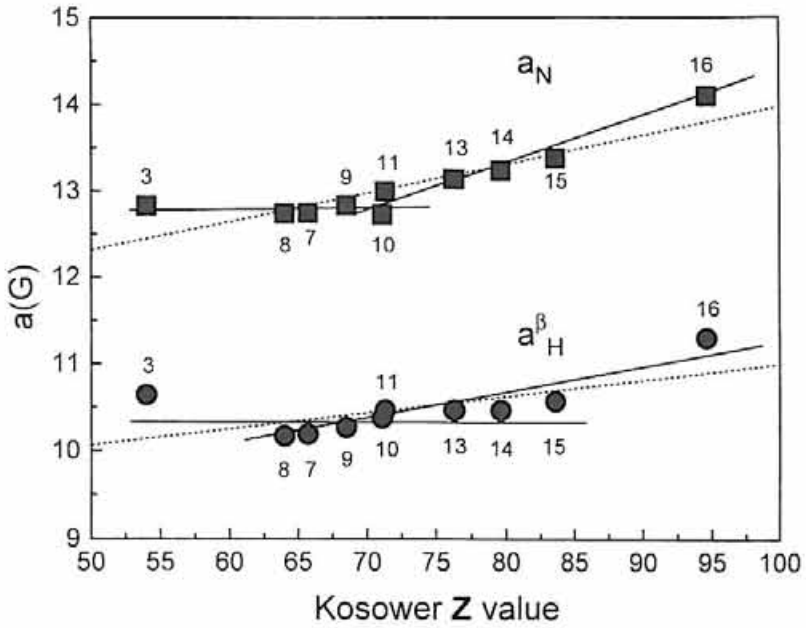



FIGURE 4 Relationship between hfsc's $a_N$, $a^{\beta}_H$ of **1** and the Kosower solvent parameter **Z**. The solid lines were obtained grouping solvents according to their influence on the hfsc's: strong effect (solvents #16–13) and weak effect (solvents #1–11). The dashed lines were obtained by applying a linear regression analysis to data from all solvents treated as one group.

solvent.[24] The interaction of the solvent with the oxygen atom in $Et_3PO$ causes the electron density on the phosphorus atom to decrease which influences the chemical shift of $^{31}P$. Other researchers have found that the $a_N$ hyperfine coupling of stable nitroxide radicals in solvents of different polarity correlates most favorably with **AN**.[22] In Figure 5, the $a_N$ and $a^{\beta}_H$ values of adduct **1** are plotted *versus* **AN**. In this representation both the nitrogen and hydrogen splittings can also be divided into two groups (solid lines in Figure 5): flrst group includes spectra measured in polar and protic solvents (#16–13) in which a significant decrease in the splittings is observed as **AN** decreases; the second group of data contains couplings obtained in aprotic solvents (#1–11), where relatively small changes occur. (Nitromethane, #12, clearly does not fit into either group). When all these points are treated as a single group and it is assumed that they are linearly dependent on **AN**, straight lines

$$a_N = 12.47 + 0.02*\mathbf{AN} \quad (4)$$

$$a^{\beta}_H = 10.29 + 0.01*\mathbf{AN} \quad (5)$$

(Figure 5, dashed lines), described by equations 4 and 5 are obtained (correlation coefficients 0.919 and 0.546 for $a_N$ and $a^{\beta}_H$, respectively).

Thus, the desired global relationship between hfsc's and solvent parameters, **Z** and **AN**, for adduct **1** in all types of solvents does not exist. However, hfsc's in similar solvents may sufficiently narrow the identification choices. This, together with chemical criteria may lead to successful identification of different DMPO/oxyl-

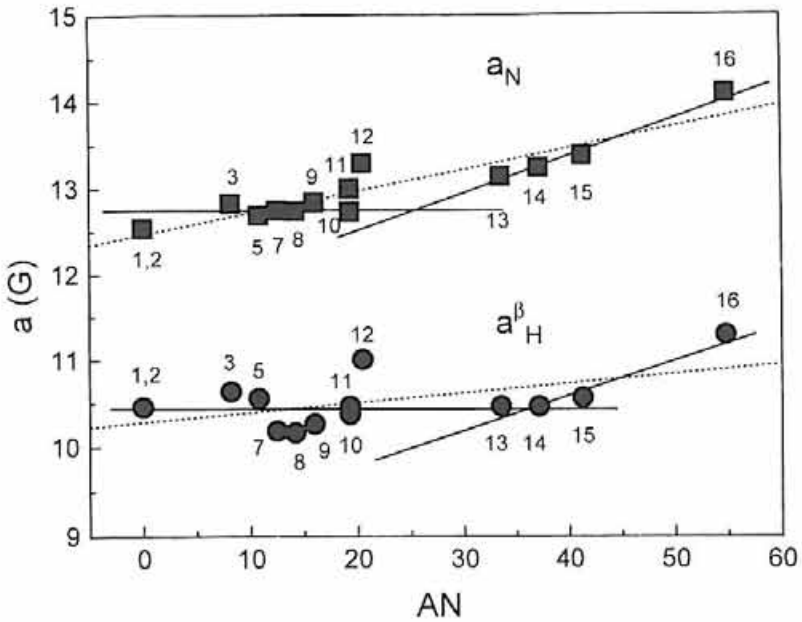



FIGURE 5 Relationship between hfsc's $a_N$ and $a^{\beta}_H$ of **1** and the solvent acceptor number **AN**. Solid and dashed lines have the same meaning as in Figure 4.

radical spin adducts. The analysis of the dependence of hfsc's of **1** on **Z** and **AN** given in Figures 4 and 5 may be considered as supporting evidence for existence of the adduct in form of proposed structure **4**, in protic and polar environments, and structure **5** in aprotic and less polar media.

In conclusion we have found that irradiation of photoactive pigments curcumin, benzanthrone, anthralin, 1,8-dihydroxyanthraquinone and Rose Bengal and eosine/detergent ion pairs is a convenient source of superoxide radicals in aprotic media. We have identified the DMPO/superoxide(hydroperoxyl) adduct by applying spectroscopic criteria and comparing the photoinduced spectra with those generated using 18-crown-6 ether-solubilized $KO_2$ in the same solvents. Additionally, we re-examined the previously proposed empirical correlation of hyperfine constants with solvent parameters. We found that simple linear equations relating the hfsc's to **Z** and **AN** do not describe satisfactorily the experimentally-determined relationship between these parameters unless the analysis is restricted to some selected types of solvents. We postulate that in aprotic nonpolar solvents the DMPO/$^{\cdot}$OOH adduct may exist in cyclic form **5**, which should be less susceptible to solvent polarity. This suggestion is supported by the observation that hfsc's of the radical in such solvents depend weakly on solvent parameters.

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