Oxygen 2001

Sunrise Free Radical School

November 16-19, 2001
Research Triangle Park, NC

“Peroxidases in Biology and Medicine”

Free Radical Basics: Concepts and Considerations

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I. Introduction

A. What is a Free Radical?

"It is an atom or group of atoms possessing <u>one or more unpaired</u> electrons" [1].

The word "free" in front of "radical" is considered unnecessary [2,3].

B. Historically, radical and free radical had slightly different meanings. For example, Linus Pauling defined it as [4]:

> ***"Free Radicals. An atom or group of atoms with one or more unshared electrons, which may enter into chemical-bond formation, is called a free radical. (The same group in a molecule is called a radical; for example, the methyl radical in methyl cyanide or other molecules.)"***

Thus, when reading older literature be aware of this nuance in meaning.

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[1] Leigh GJ. Ed. (1990) *Nomenclature of inorganic chemistry. Recommendations 1990.* Oxford: Blackwell Scientific Publications.

[2] Koppenol WH. (1990) What is in a name? Rules for radicals. *Free Radic. Biol. Med.* **9**:225-227.

[3] Trynham JG. (1986) A short guide to nomenclature of radicals, radical ions, iron-oxygen complexes and polycyclic aromatic hydrocarbons. *Adv. Free Radic. Biol. Med.* **2:**191-209.

[4] Pauling L. (1964) *College Chemistry* WH Freeman and Co., San Francisco pp331-332.

II. Notation & Nomenclature [5]

A. Superscript dot to the right, usually

B. Examples (Note: dot, then charge)

$H^{\cdot}$ $O_2^{\cdot-}$ $CO_2^{\cdot-}$

$HO^{\cdot}$ ${}^{\cdot}CH_3$

$O_2^{\cdot\cdot}$ or $O_2^{2\cdot}$

C. Noncommittal

$(HO)^{\cdot}$ $(CH_3)^{\cdot}$

D. Common Notations and Abbreviations:

Species	Systematic IUPAC Name	Alternative and Comments
$O^{\cdot-}$	oxide(1-)	not found in the 1990 recommendations
$O_2^{\cdot-}$	dioxide(1-)	hyperoxide and superoxide
O_3	trioxygen	ozone
$O_3^{\cdot-}$	trioxide(1-)	ozonide
$HO^{\cdot}$	hydroxyl	not hydroxy, hydroxide is restricted to OH^-
$HO_2^{\cdot}$	hydrogen dioxide	hydrodioxyl, hydroperoxyl is allowed, but perhydroxyl does not make sense
HO_2^-	hydrogen dioxide(1-)	hydrogenperoxide(1-); hydroperoxide is not recommended
H_2O_2	hydrogen peroxide	
$RO^{\cdot}$	alkoxyl	not alkoxy
$ROO^{\cdot}$	alkyldioxyl	alkylperoxyl
ROOH	alkyl hydroperoxide	
$O{=}NOO^-$	oxoperoxonitrate (1-)	peroxynitrite
O=NOOH	hydrogen oxoperoxonitrate peroxynitrous acid	
${}^{\cdot}NO$	nitrogen monoxide	nitric oxide

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[5] Koppenol WH. (1990) What is in a name? Rules for radicals. *Free Radic. Biol. Med.* **9**:225-227.

E. More Free Radical Nomenclature You May Need to Know

Species/Abbreviation*	**Name**
Asc; $AscH^-$; $Asc^{\bullet -}$	ascorbate, general; ascorbate monoanion; ascorbate radical
CAT	catalase
Desferal®	trade name for deferrioxamine mesylate
DETAPAC or DTPA	diethylenetriaminepentaacetic acid
DMPO	5,5-dimethyl-pyrroline-1-oxide, a spin trap
EDRF	endothelium-derived relaxing factor
EDTA	ethylenediaminetetraacetic acid
EPR, ESR, identical	electron paramagnetic resonance, electron spin resonance
G	gauss
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	glutathione
GSSG	oxidized glutathione
GST	Glutathione-S-transferase
LDL	low density lipoprotein
MDA	malondialdehyde
NBT	nitroblue tetrazolium
NOS	nitric oxide synthase
PBN	α-phenyl-N-*tert*-butyl nitrone, a spin trap
PhGPx	phospholipid hydroperoxide glutathione peroxidase
POBN	α-[4-pyridyl 1-oxide]-N-*tert*-butyl nitrone, a spin trap
PUFA	polyunsaturated fatty acid
ROS	reactive oxygen species
SOD	superoxide dismutase
CuZnSOD	copper,zinc superoxide dismutase
MnSOD	manganese superoxide dismutase
FeSOD	iron superoxide dismutase
ECSOD	extracellular superoxide dismutase
TBARS	thiobarbituric acid reactive substances

*These are commonly used abbreviations. Others appear in the literature.

F. Even More Radicals and Related Species

Species	Name
$^1O_2$	singlet oxygen
$H^\bullet$	hydrogen atom
H^+	proton, hydron
$HO^\bullet$	hydroxyl radical
OH^-	hydroxide anion
H_2O_2	hydrogen peroxide
$RO^\bullet$	alkoxyl radical
$ROO^\bullet$	alkyldioxyl, alkylperoxyl radical
ROOH	alkyl hydroperoxide
$GS^\bullet$	glutathiyl radical
$^\bullet CH_3$	methyl radical
$^\bullet NO_2$	nitrogen dioxide
N_2O	nitrous oxide

G. Enzyme Reactions You Will Need to Know

(Absolutely, stone cold, verbatim)

$$O_2^{\cdot -} + O_2^{\cdot -} + 2\,H^+ \overset{\textbf{SOD}}{\text{¾ ¾ ®}} O_2 + H_2O_2$$

$$2\,H_2O_2 \overset{\textbf{CAT}}{\text{¾ ¾ ®}} O_2 + 2\,H_2O$$

$$ROOH + 2\,GSH \overset{\textbf{GPx}}{\text{¾ ¾ ®}} ROH + H_2O + GSSG$$

$$H_2O_2 + 2\,GSH \overset{\textbf{GPx}}{\text{¾ ¾ ®}} 2\,H_2O + GSSG$$

III. Types of radicals:

1. Sigma, s
2. pi - delocalized, p
3. Mixture of sigma and pi
4. carbon-centered $H_3C^{\cdot}$
5. O_2 - centered $H_3COO^{\cdot}$
6. Sulfur - centered $GS^{\cdot}$
7. Nitrogen - centered $R_2{\cdot}NO$
8. Metals: Cu^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Mo, Co^{2+}
9. Reducing radicals, $CO_2^{\cdot -}$, $PQ^{\cdot +}$
10. Oxidizing radicals, $HO^{\cdot}$, $LOO^{\cdot}$
11. ...

IV. Lipid Peroxidation

A. Lipid peroxidation is OXIDATIVE degradation of phospholipids, cholesterol, ... other

Results in:

1. membrane fluidity changes
2. increased ion permeability
3. covalent crosslinking - lipid and proteins
4. depletion of NADPH
5. inactivation of membrane enzymes and receptors
6. polypeptide strand scission
7. DNA damage and mutagenesis

B. Classic Lipid Peroxidation (The Basics)

1. Initiation

R_i

$LH + X^{\cdot}$ ¾ ® $L^{\cdot} + XH$

2. Propagation

k_o @ 3 x 10^{8} $M^{-1}s^{-1}$

$L^{\cdot} + O_2$ ¾ ¾ ¾ ¾ ¾ ¾ ¾ ¾ ® $LOO^{\cdot}$

k_p @ $10\text{-}50$ $M^{-1}s^{-1}$

$LOO^{\cdot} + LH$ ¾ ¾ ¾ ¾ ¾ ¾ ¾ ¾ ® $L^{\cdot} + LOOH$

3. Termination

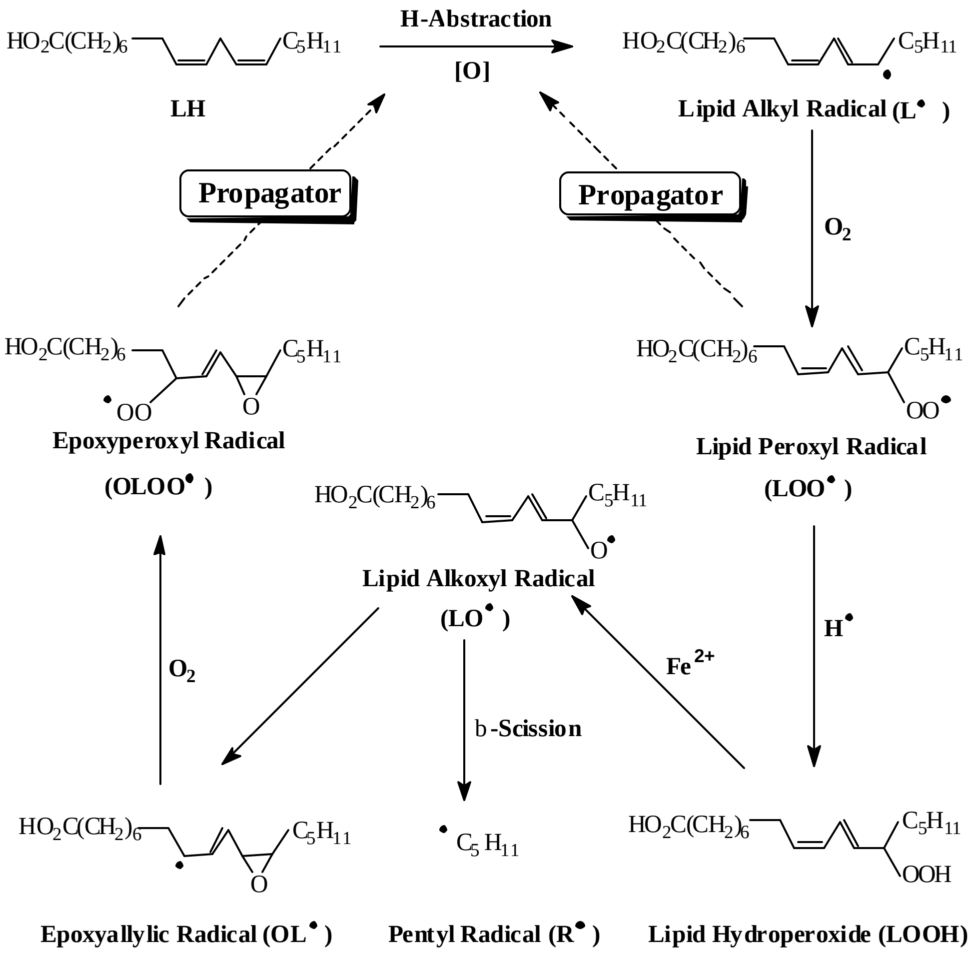
k_t @ $10^{6}\text{-}10^{7}$ $M^{-1}s^{-1}$

$2\ LOO^{\cdot}$ ¾ ¾ ¾ ¾ ¾ ¾ ¾ ¾ ® non-radical products

$L^{\cdot} + LOO^{\cdot}$ ¾ ® “

$L^{\cdot} + L^{\cdot}$ ¾ ® “

C. Some Details



Scheme. An overview of the chemistry of the formation of lipid-derived radicals ($L_d^\bullet$) produced during lipid peroxidation in the presence of ferrous iron. This scheme shows some of the radical species formed in the peroxidation of linoleic acid. Three different propagating species are shown. It is currently thought that $OLOO^\bullet$ may be the major propagating species in this type of system [6, 7]. The species $LO^\bullet$ is thought to be a minor propagating species because of its very short lifetime. It is estimated that the rate constant of cyclization of $LO^\bullet$ is approximately $2 \times 10^7\ s^{-1}$ while the rate constant for β-scission is $\sim 1 \times 10^6\ s^{-1}$ [8]. Scheme adapted from Qian *et al.* [9]

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[6] Marnett, LJ, Wilcox AL. (1996) The Chemistry of lipid alkoxyl radical and their role in metal-amplified lipid peroxidation. In: Rice-Evans, C.; Halliwell, B.; Lunt, G. G., eds. *Free Radicals and Oxidative Stress: Environment, Drugs and Food Additives*. Biochem. Soc. Symp. **61:** 65-72.

[7] Wilcox AL. Marnett LJ. (1993) Polyunsaturated fatty acid alkoxyl radicals exist as carbon-centered epoxyallylic radicals: a key step in hydroperoxide-amplified lipid peroxidation. *Chemical Research in Toxicology*. **6(4):**413-416.

[8] Grossi L. Strazzari S, Gilbert BC, Whitwood AC. (1998) Oxiranylcarbinyl radicals from allyloxyl radical cyclization: Characterization and kinetic information *via* ESR spectroscopy. *J. Org. Chem.* **63:** 8366-8372.

[9] Qian SY, Wang HP, Schafer FQ, Buettner GR. (2000) EPR detection of lipid-derived radicals from PUFA, LDL, and cell oxidations. *Free Radic Biol Med.* **29:**568-579.

V. ANTIOXIDANTS

A. ***A substance when present in trace (small) amounts inhibits oxidation of the bulk.***

There are two broad classes:

1. **Preventive**
2. **Chain-breaking**

B. Preventive Antioxidants reduce the rate of chain initiation

$L\text{-}H + X^{\cdot}$ ® $L^{\cdot} + XH$ **Initiation**

or $LOOH + Fe^{2+}$ ® $LO^{\cdot} + OH^{-} + Fe^{3+}$

or $H_2O_2 + Fe^{2+}$ ® $HO^{\cdot} + OH^{-} + Fe^{3+}$

or $Fe^{2+} + O_2$ ® $(FeO_2)^{2+}$**(oxidant)** $\overset{LH}{\text{¾ ®}}$ $L^{\cdot}$ [10]

or $^1O_2 + L\text{-}H$ ® $LOOH$ ® $LO^{\cdot}$

1. Targets for preventive antioxidants

a. **Metals - Fe, Cu**
- ***i.*** **Chelates-EDTA**
  - **DETAPAC**
  - **Desferal$^{\text{Ò}}$ º deferrioximine**
  - **Phytate ?**

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[10] Qian SY, Buettner GR. (1999) Iron and dioxygen chemistry is an important route to initiation of biological free radical oxidations: An electron paramagnetic resonance spin trapping study. *Free Radic Biol Med*, **26**: 1447-1456.

ii. **Proteins & metals**
Transferrin Fe
Ferritin Fe
Hemes, hemoglobin, myoglobin
:
Caeruloplasmin Cu

iii. **Key aspect is:**

Fe(III)chelate + $O_2^{\cdot -}$ ® Fe(II)chelate + O_2
(or $AscH^-$)

Fe(II)chelate + H_2O_2 ® $HO^\cdot$ + Fe(III)chelate
(or LOOH $LO^\cdot$)

b. Peroxide - Removing Antioxidants
H_2O_2, LOOH

i. **Catalase heme-enzyme**

$2\ H_2O_2$ ® $2\ H_2O + O_2$

ii. **GPx - Glutathione Peroxidases**
a. mitochondria & cytoplasm
b. $H_2O_2 + 2\ GSH$ ® $2\ H_2O + GSSG$

C. Chain-breaking Antioxidants

$3 \times 10^8\ M^{-1}s^{-1}$
$L^\cdot + O_2$ ¾ ¾ ¾ ¾ ¾ ¾ ® $LOO^\cdot$ Propagation

$10\text{-}50\ M^{-1}s^{-1}$
$LOO^\cdot$ + L-H ¾ ¾ ¾ ¾ ® $L^\cdot$ + LOOH Cycle

i. **Intercept $L^\cdot$? NO!**
ii. **Repair $LOO^\cdot$? Yes!**

The Donor Antioxidant Reaction:

k = 10^4 - 10^8 $M^{-1}s^{-1}$

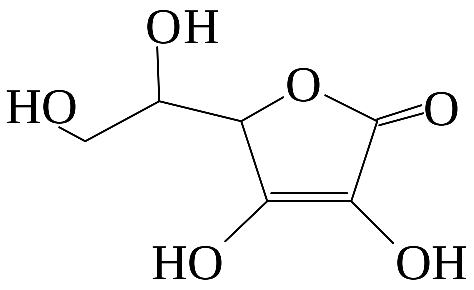
$LOO^{\cdot}$ + Antiox-H ¾ ¾ ® LOOH + $Antiox^{\cdot}$

iii. **Good antioxidant (chain-breaking)**

- **a. relatively Unreactive, both the Antioxidant & <u>Antiox</u>$^{\cdot}$**
- **b. $Antiox^{\cdot}$ - decay to harmless products**
- **c. Does not <u>add</u> O_2, *i.e.,* there is no $ROO^{\cdot}$ formed**
- **d. Renewed - somehow.**

VI. Vitamins C & E. (Donor Antioxidants)

a. Vitamin C (ascorbate)



Ascorbic Acid

$pK_1 = 4.2$; $pK_2 = 11.6$

Principal, water-soluble antioxidant, *i.e.* small molecule - chain breaking.

$AscH^{-}$ + $R^{\cdot}$ ® $Asc^{\cdot -}$ + R-H

b. Vitamin E – Tocopherols (a , b, g, d)

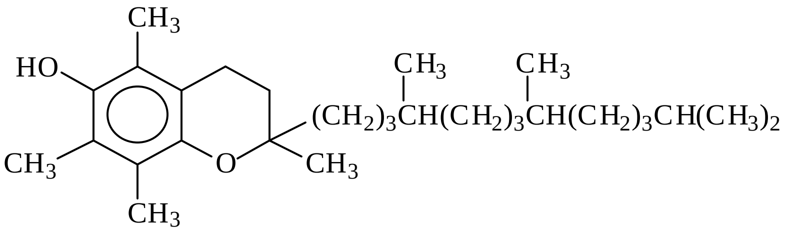


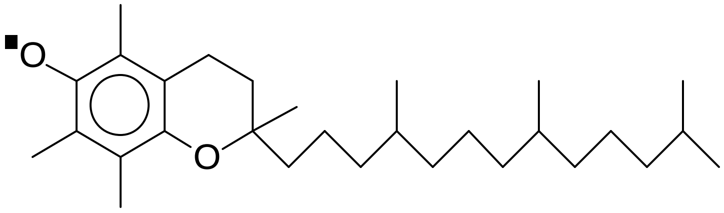
Table 1 Forms of Tocopherol

	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

c. The Antioxidant Reaction

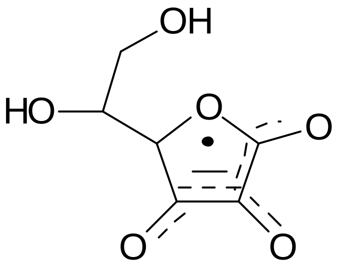
$R^{\cdot}$ + TOH ® RH + $TO^{\cdot}$

$R^{\cdot}$ + $AscH^{-}$ ® RH + $Asc^{\cdot -}$

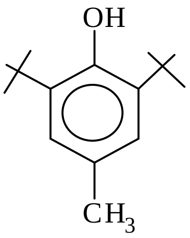


$TO^{\bullet}$

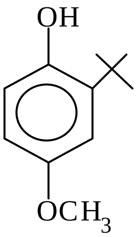
$Asc^{\cdot -}$



Note the structure of other common donor antioxidants below.



BHT



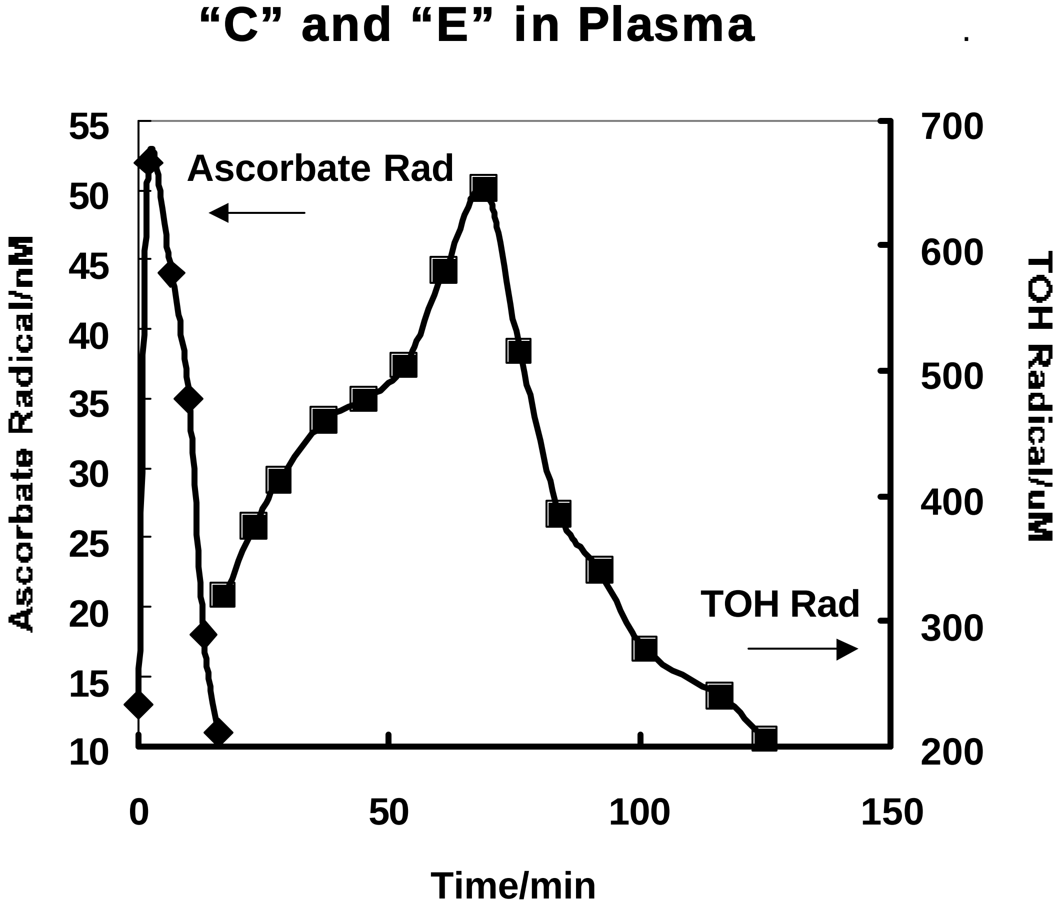
BHA

VII. "E", the principal nutritional chain-breaking antioxidant in membranes

A. Energetics

$LOO^{\cdot}$, H^{+}/LOOH	**E°¢ @ + 1000 mV**
$TO^{\cdot}$, H^{+}/TOH	**E°¢ @ + 480 mV**
$Asc^{\cdot -}$, H^{+}/$AscH^{-}$	**E°¢ = + 280 mV**

B. An Example:



Ascorbate and tocopheroxyl free radical concentrations over time in blood plasma subjected to oxidative insult. Time zero corresponds to the intensity observed at baseline in the plasma, *i.e.* prior to the introduction of hypoxanthine and xanthine oxidase to the sample. This result is representative of experiments with six different plasma samples. Each sample had different initial levels of vitamins C and E. The endogenous levels of vitamins C and E in this sample were 63 and 48 *m*M, respectively. Introduction of additional hypoxanthine and xanthine oxidase at a time point beyond 125 min produced no change in the TO$^{\bullet}$ ESR signal intensity, whereas introduction at some time point where significant TO$^{\bullet}$ is observed (*e.g.*, ≈ 45 minutes) produced a significant increase in [TO$^{\bullet}$]$_{ss}$. The poor signal-to-noise ratio in the TO$^{\bullet}$ signal intensity data will result in errors on the order of ≈ 10-25% in [TO$^{\bullet}$]$_{ss}$; the weaker the signal the more uncertainty. (It should be noted that under our experimental conditions, approximately 10 times more TO$^{\bullet}$ than Asc$^{\bullet-}$ is required to produce an ESR signal above the noise level. This is quite reasonable considering that TO$^{\bullet}$ has a broad 7-line spectrum whereas Asc$^{\bullet-}$ is a narrow doublet.) Adapted from [11].

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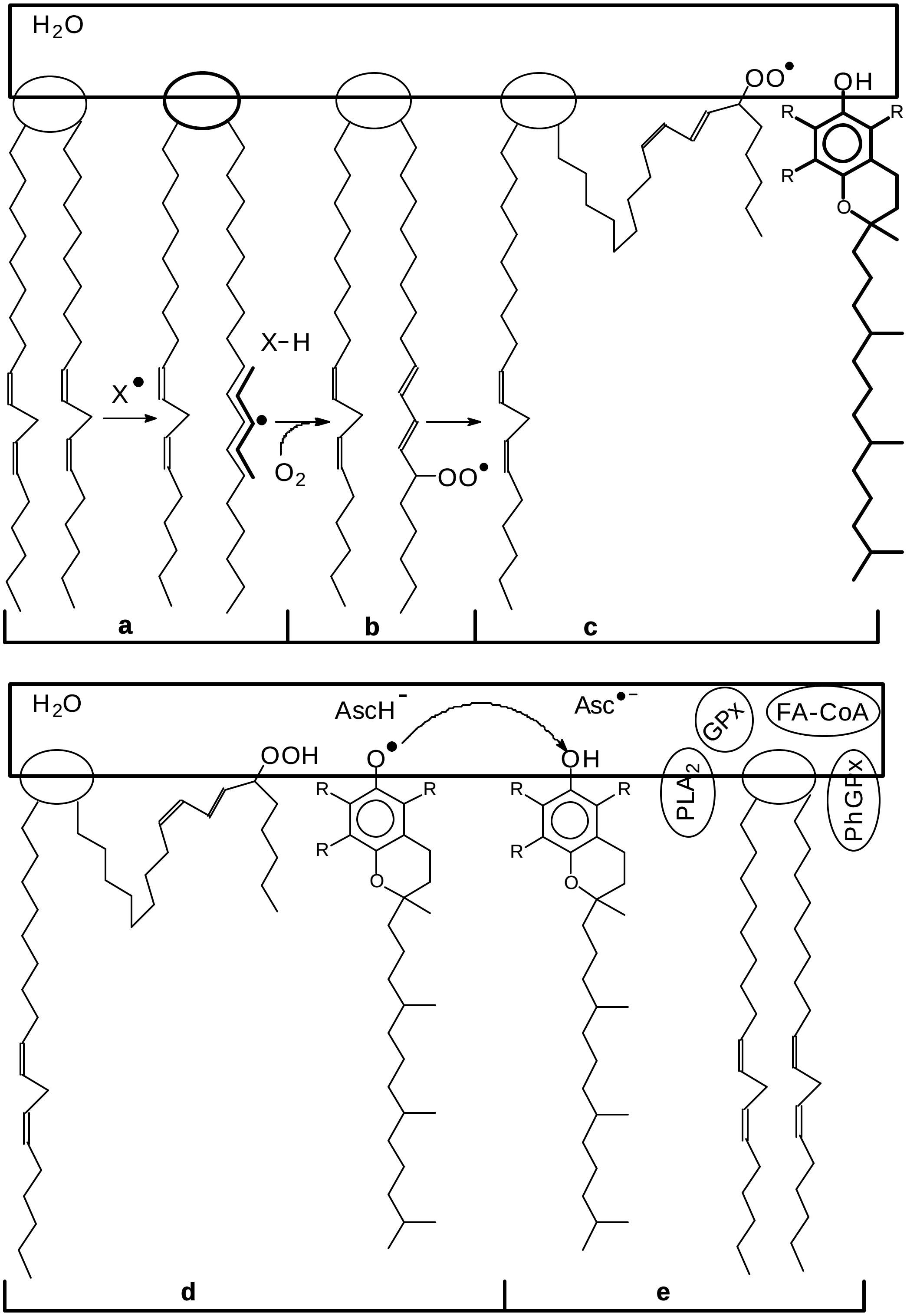
[11] Buettner GR, Jurkiewicz BA. (1993) The ascorbate free radical as a marker of oxidative stress: An EPR study. *Free Rad Biol Med* **14:**49-55.

C. Ascorbate and Tocopherol as Co-antioxidants [12].



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[12] Buettner GR (1993) The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol, and ascorbate. *Arch Biochem Biophys.* **300**: 535-543.

D. Getting Rid of Antioxidant Radicals

1. Chemistry

3.8 x 10^{4} M^{-1}s^{-1} estimate in membranes

TO$^{\cdot}$ + AscH^{-} ¾ ¾ ¾ ¾ ¾ ® **TOH + Asc$^{\cdot -}$**

TO$^{\cdot}$ Not good, but much better than LOO$^{\cdot}$

H^{+}

Dismutation: 2 Asc$^{\cdot -}$ ¾ ¾ ¾ ¾ ¾ ® **AscH^{-} + DHA**

2 x 10^{5} M^{-1}s^{-1}

2. Also, Enzyme Systems

Ascorbate - at the bottom of the Pecking Order

(Note: Ascorbate and tocopherol can also be pro-oxidants. But that is another complete lecture.)

VIII. The Pecking Order of Free Radicals [13]

Couple (one-electron)	**E°'/mV (pH)**
$HO^{\bullet},H^{+}/H_2O$	2,310
$H_3CH_2C^{\bullet},H^{+}/CH_3CH_3$	1,900
$RO^{\bullet},H^{+}/ROH$ (aliphatic alkoxyl radical)	1,600
$^{\bullet}CH_2OH,H^{+}/CH_3OH$	1,200
$HOO^{\bullet},H^{+}/H_2O_2$	1,060
$ROO^{\cdot},H^{+}/ROOH$ (alkylperoxyl radical)	**1,000**
$O_2^{\bullet-},2H^{+}/H_2O_2$	940
$RS^{\bullet}/RS^{-}$ (cysteine)	920
$C_6H_5O^{\bullet},H+/C_6H_5OH$	900
$PUFA^{\cdot},H^{+}/PUFA\text{-}H$	**600**
$HU^{\bullet-},H^{+}/UH_2^{-}$ (Urate)	590
α-tocopheroxyl$^{\cdot}$,$H^{\cdot}$/α-tocopherol ($TO^{\cdot},H^{+}/TOH$)	≈**500**
Trolox C ($TO^{\bullet},H^{+}/TOH$)	480
$H_2O_2,H^{+}/H_2O,\ {}^{\bullet}OH$	320
ascorbate$^{\cdot-}$,H^{+}/ascorbate monoanion	**282**
$CoQ^{\bullet-},\ 2H^{+}/CoQH_2$	200
Fe(III)EDTA/Fe(II)EDTA	120
Fe(III) Citrate/Fe(II) Citrate	100
Fe(III)DETAPAC/Fe(II)DETAPAC	30
$CoQ/CoQ^{\bullet-}$	- 36
riboflavin/riboflavin$^{\bullet-}$	- 317
$O_2/O_2^{\bullet-}$	- 330
adriamycin/adriamycin$^{\bullet-}$	- 341
Fe(III)Transferrin/Fe(II)Transferrin	- 400 (7.3)
paraquat/paraquat$^{\bullet+}$	- 448
Fe(III)Desferal/Fe(II)Desferal	- 450
$O_2,H^{+}/HO_2^{\bullet}$	- 460
$RSSR/RSSR^{\bullet-}$ (cystine or GSSG)	-1,500
$CO_2/CO_2^{\bullet-}$	-1,800
H_2O/e_{aq}^{-}	-2,870

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[13] Buettner GR (1993) The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol, and ascorbate. *Arch Biochem Biophys.* **300**: 535-543.

IX. Kinetics, Designing Free Radical Experiments

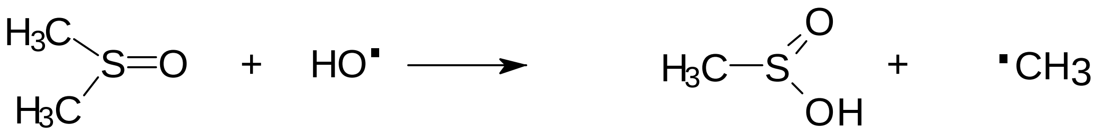
A. EPR Spin Trapping, An Example

The Fenton Rxn and DMSO

Fenton Rxn

Fe^{2+} + H_2O_2 ¾ ¾ ¾ ¾ ® **Fe^{3+} + $HO^{\cdot}$ + OH^{-}**

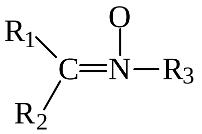
DMSO + $HO^{\cdot}$ ¾ ¾ ¾ ¾ ® **$(CH_3)S(=O)OH$ + $^{\cdot}CH_3$**



Spin Trapping, the Reaction

$R^{\bullet}$ + Spin Trap → Spin Adduct

There are two general classes of spin traps:

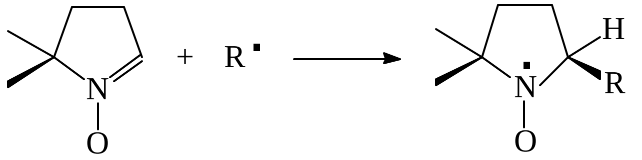


Nitrone

R—N=O

Nitroso

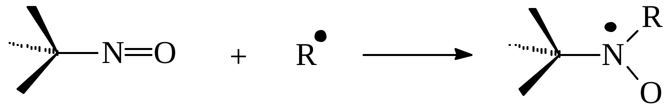
Example of nitrone trapping reaction:



DMPO
(Nitrone Spin Trap)

Nitroxide Radical
(Aminoxyl Spin Adduct)

Example of nitroso trapping reaction:



B. Why is spin trapping so popular?

1. **Can be done with room or physiological temperature aqueous solutions;**
2. **Can detect:**
   $HO^{\cdot}$, $O_2^{\cdot -}/HO_2^{\cdot}$, $GS^{\cdot}$
   $ROO^{\cdot}$, $RO^{\cdot}$, $L^{\cdot}$ (lipids);
3. **Integrative**[14]
   $[\text{Spin Adduct}]_{ss} >>> [R^{\cdot}]$
   Because
   $t_{1/2}$ (Spin Adduct) $>>> t_{1/2}[R^{\cdot}]$;
4. **Hyperfine splittings (a's) provide information**

Spin Adduct Hyperfine Splittings

Spin Adduct	a^N/G	a^H/G
$DMPO/^{\bullet}OH$	14.9	14.9
$DMPO/^{\cdot}OOH$	14.3	11.7 1.3
$DMPO/GS^{\cdot}$	15.3	16.2
$DMPO/CO_2^{\cdot -}$	15.6	18.8
$DMPO/CH_3{}^{\cdot}CHOH$	15.8	22.8
$DMPO/^{\cdot}CH_3$	16.4	23.4
$DMPO/H^{\cdot}$ (e^{-}_{aq})	16.7	22.4*(2)

*** Two hydrogens.**

STDBII: http://epr.niehs.nih.gov/stdb1.html

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[14] Vocabulary: A spin trap, such as DMPO, reacts with a radical to form a spin adduct.

Now for a real-world example of the influence of kinetics on the results seen in a free radical experiment:

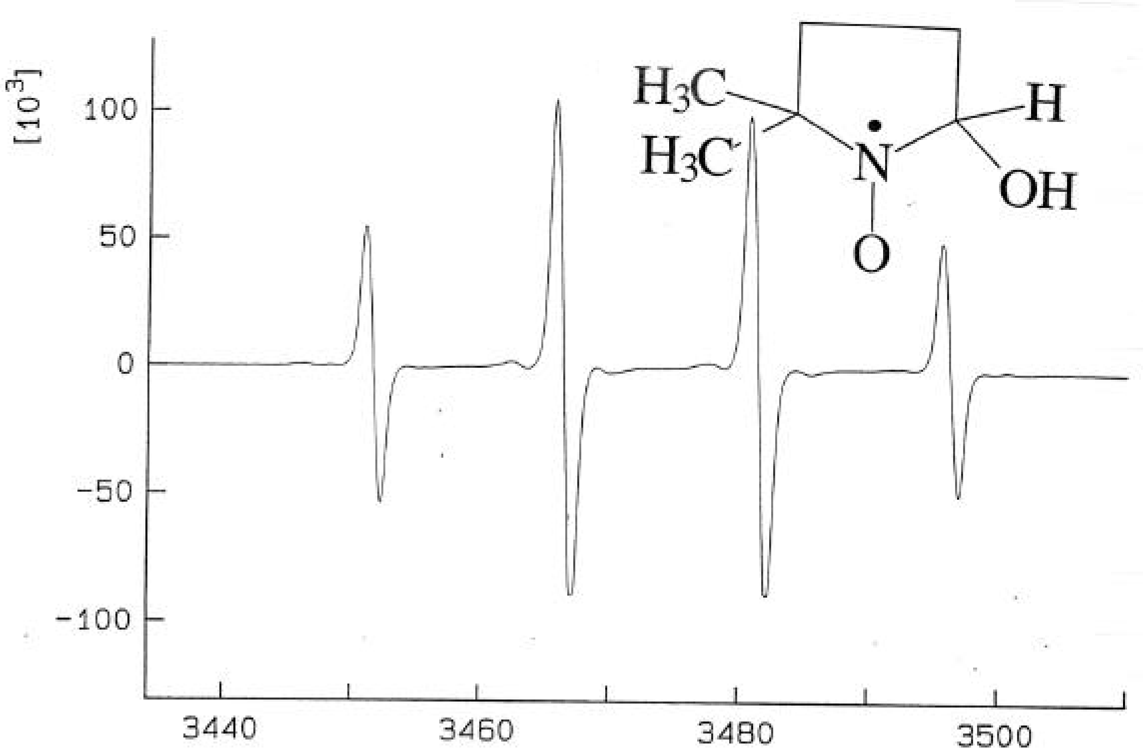
Experiment #1. Spin trapping and the Fenton reaction:

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\bullet} + OH^{-}$

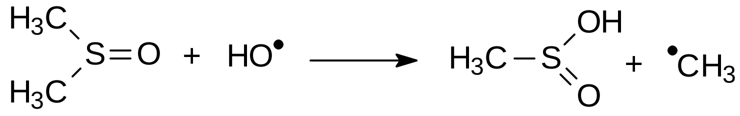
Then

$DMPO + HO^{\bullet} \longrightarrow DMPO/HO^{\bullet}$

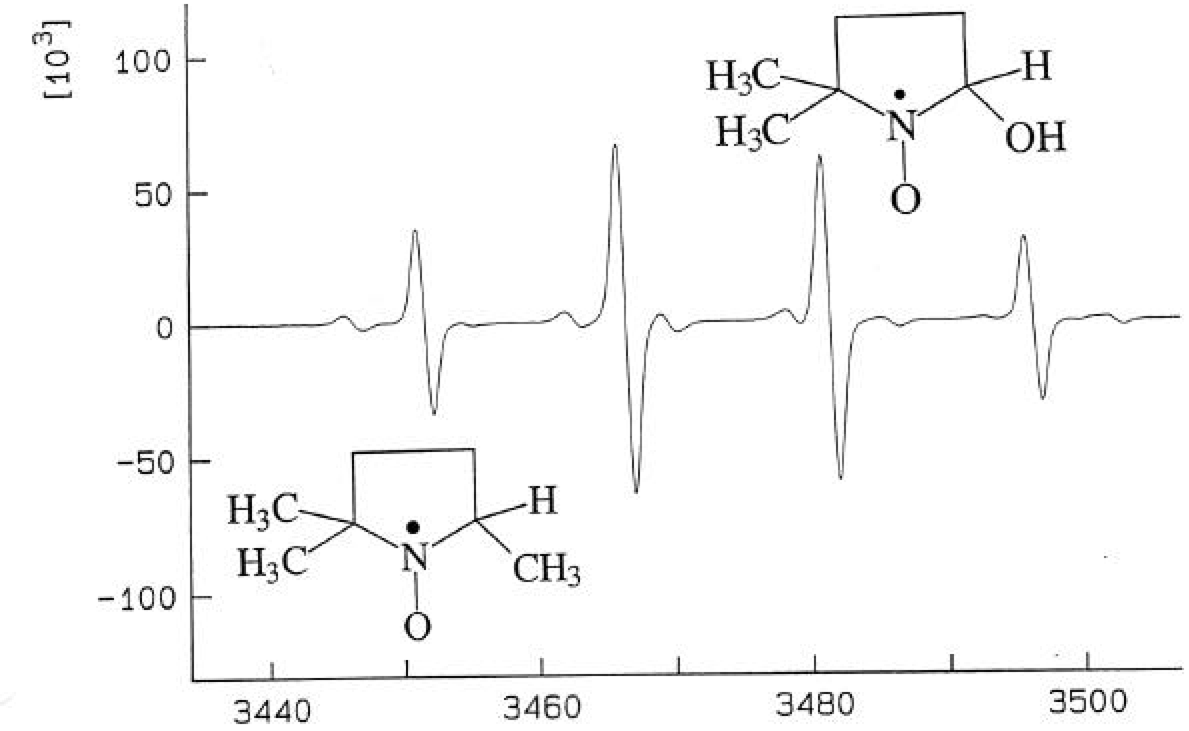
$[DMPO] = 100$ mM
$[H_2O_2] = 200\ \mu M$
$[Fe^{2+}] = 100\ \mu M$



Experiment # 2; Spin trapping of the methyl radical



Fenton system plus
[DMPO] = 100 mM;
[DMSO] = 10 mM.



Why do we see DMPO/HO$^\bullet$? KINETICS

$$\frac{\text{rate (HO}^\bullet + \text{DMPO)} = 4.3 \times 10^9\ \text{M}^{-1}\text{s}^{-1}\ [\text{DMPO; 100 mM}]\ [\text{HO}^\bullet]}{\text{rate (HO}^\bullet + \text{DMSO)} = 6.5 \times 10^9\ \text{M}^{-1}\text{s}^{-1}\ [\text{DMSO; 10 mM}]\ [\text{HO}^\bullet]}$$

or $\frac{\text{rate(DMPO)}}{\text{rate(DMSO)}} = \frac{7}{1}$

i.e. DMPO wins!

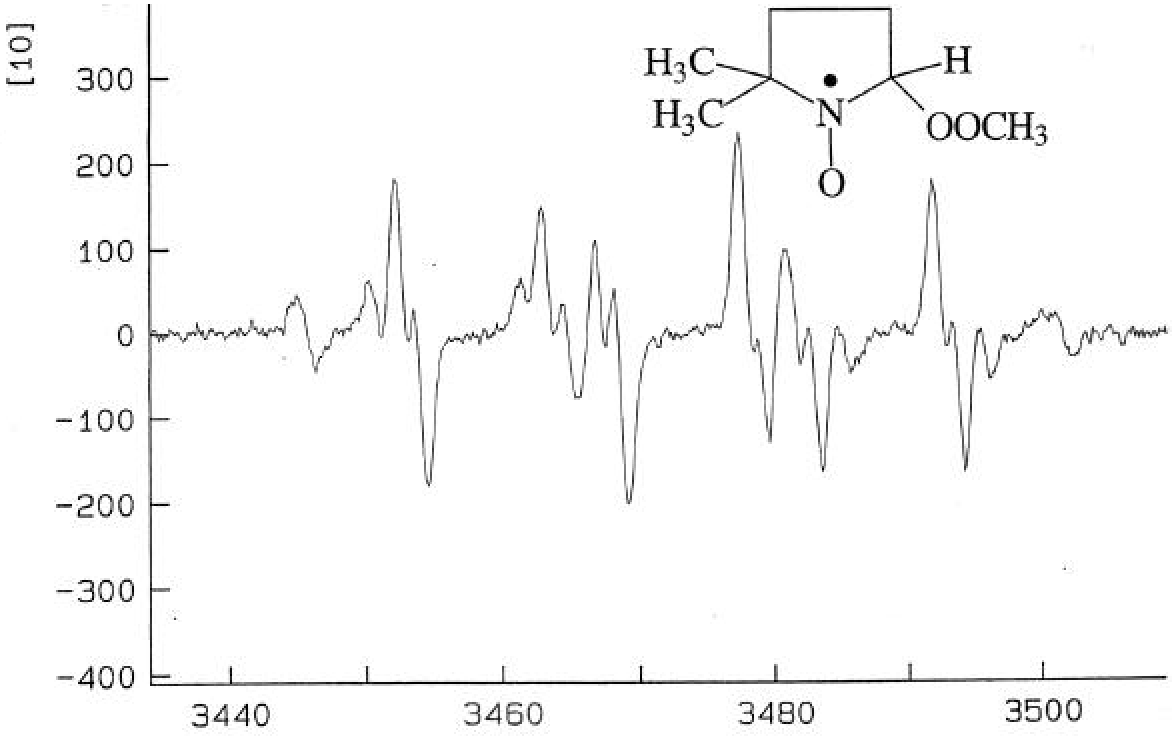
(Assumption: Other routes of HO$^\bullet$ decay are minor)

Experiment # 3 Rethink the kinetics

Fenton system plus

[DMPO] = 25 mM;
[DMSO] = 100 mM

What do we see?



Why do we see a DMPO/methoxyl radical adduct? Kinetics

rate ($HO^\bullet$ + DMPO) = 4.3 x 10^9 $M^{-1}s^{-1}$ [DMPO; 25 mM] [$HO^\bullet$]
rate ($HO^\bullet$ + DMSO) = 6.5 x 10^9 $M^{-1}s^{-1}$ [DMSO;100 mM] [$HO^\bullet$]

rate((DMPO)/rate(DMSO) ~ 1/6

but O_2 and DMPO compete for $^\bullet CH_3$

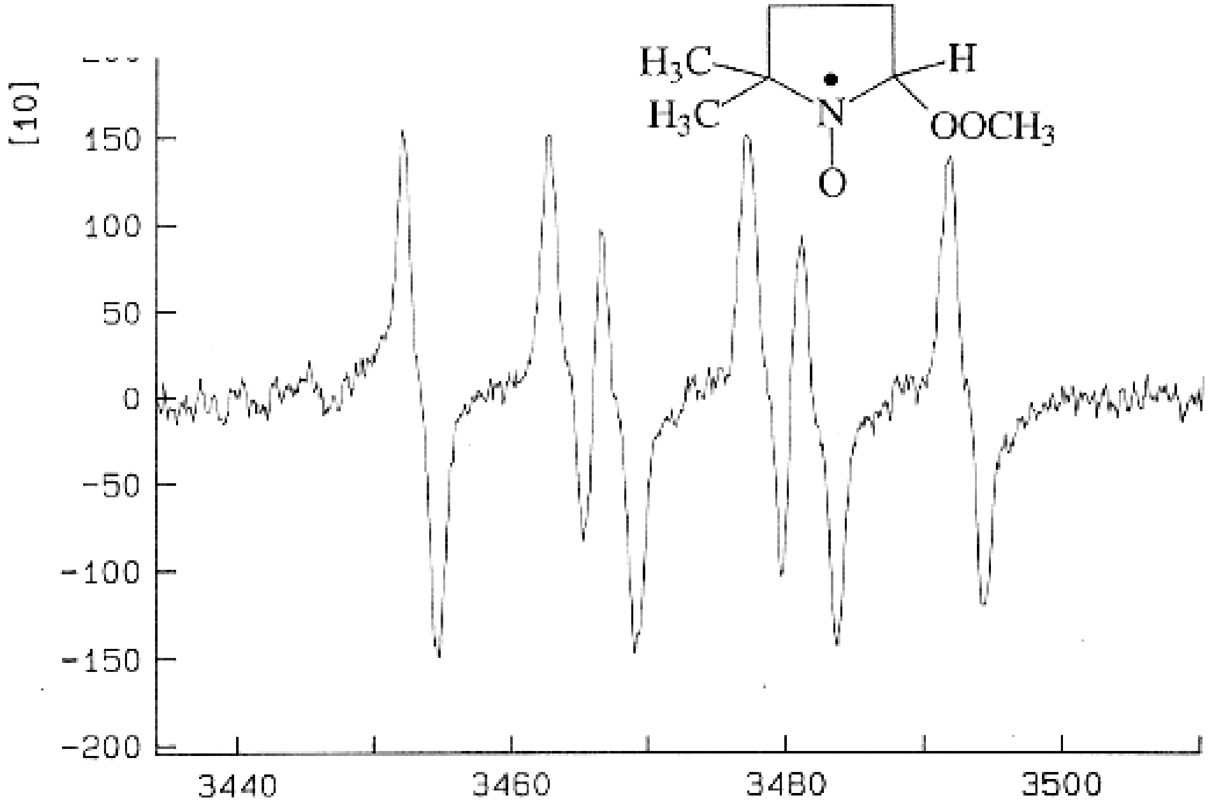
rate ($^\bullet CH_3$ + O_2) = 3.7 x 10^9 $M^{-1}s^{-1}$ [O_2; 250 μM] [$^\bullet CH_3$]
rate ($^\bullet CH_3$ + DMPO) = 10^6 $M^{-1}s^{-1}$ [DMPO; 25 mM] [$^\bullet CH_3$]

rate(O_2)/rate(DMPO) ~ 10/1

DMSO wins $HO^\bullet$, but oxygen wins the $^\bullet CH_3$ produced.

Experiment # 4 Increase [O_2]

[DMPO] = 25 mM;
[DMSO] = 100 mM
Bubble with O_2, [O_2] goes from 0.25 to 1.25 mM



Note the line broadening caused by oxygen.

Where could the methoxyl radical come from? Proposal,

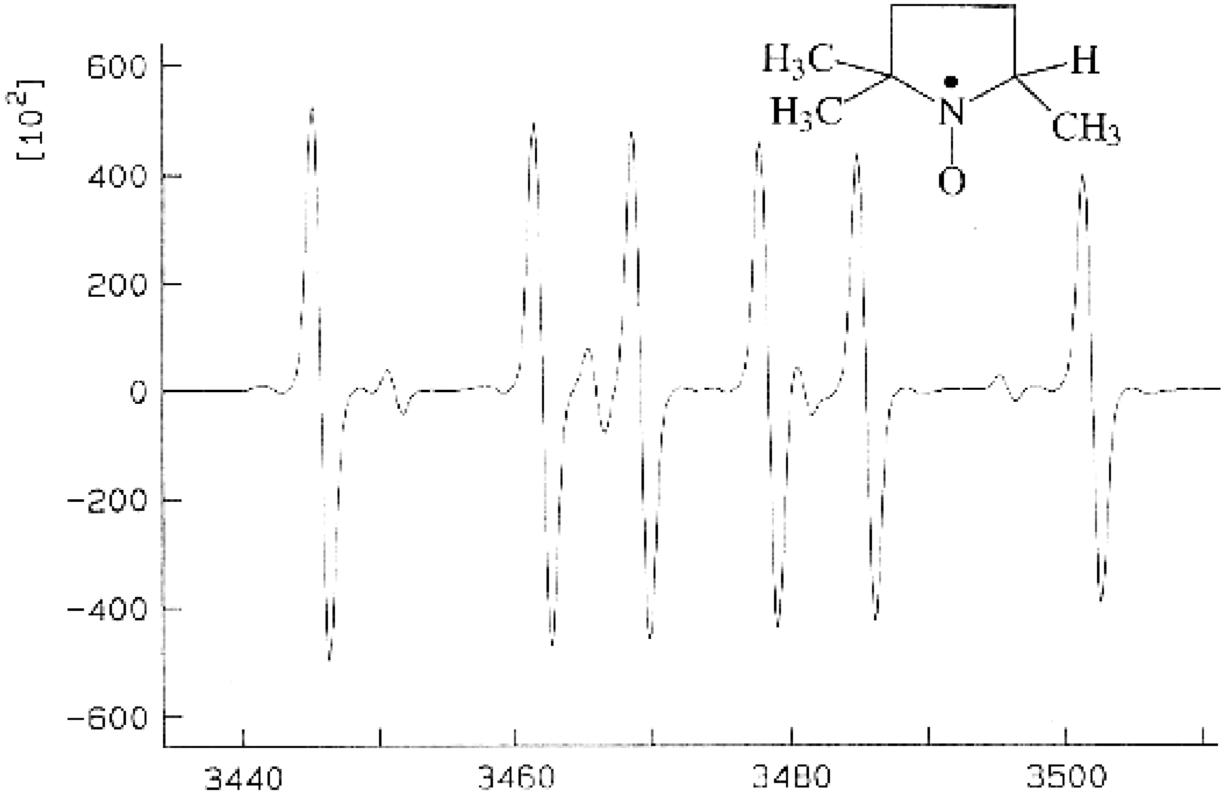
$$^{\bullet}CH_3 + O_2 \longrightarrow H_3COO^{\bullet}$$

$$2\ H_3COO^{\bullet} \longrightarrow 2\ H_3CO^{\bullet} + O_2$$

Experiment # 5 decrease [O_2]

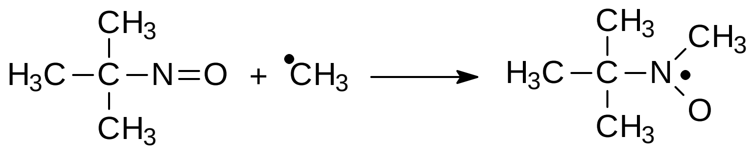
[DMPO] = 25 mM;
[DMSO] = 100 mM

Bubble with N_2



Experiment # 6 Have we really observed $^{\cdot}CH_3$?

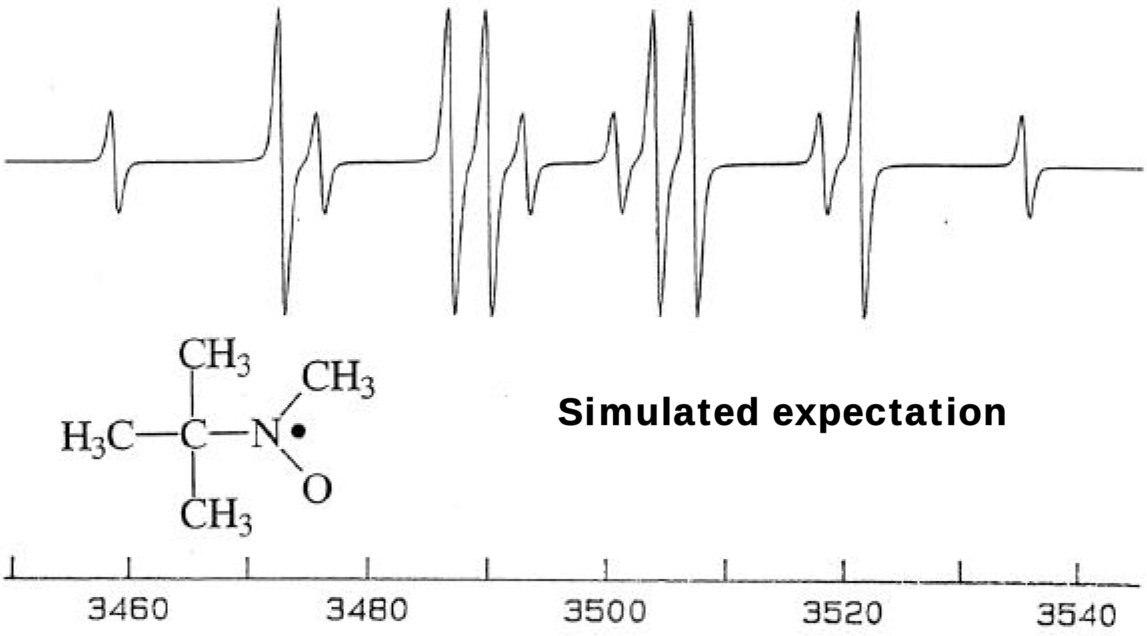
Use MNP, a nitroso spin trap



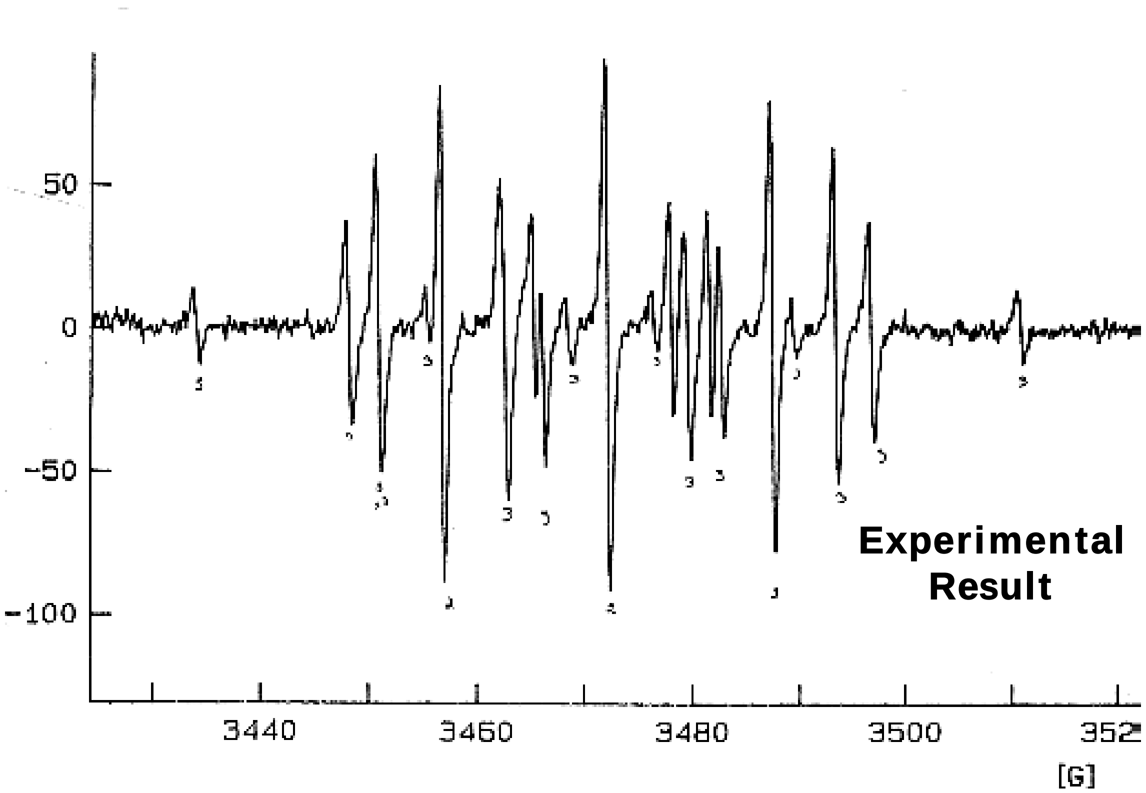
$(CH_3)_3C{-}N{=}O + {}^{\bullet}CH_3 \longrightarrow (CH_3)_3C{-}N^{\bullet}(O)CH_3$

Fenton system plus

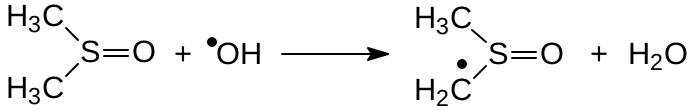
[DMSO] = 1 M
[MNP] = 25 mM;

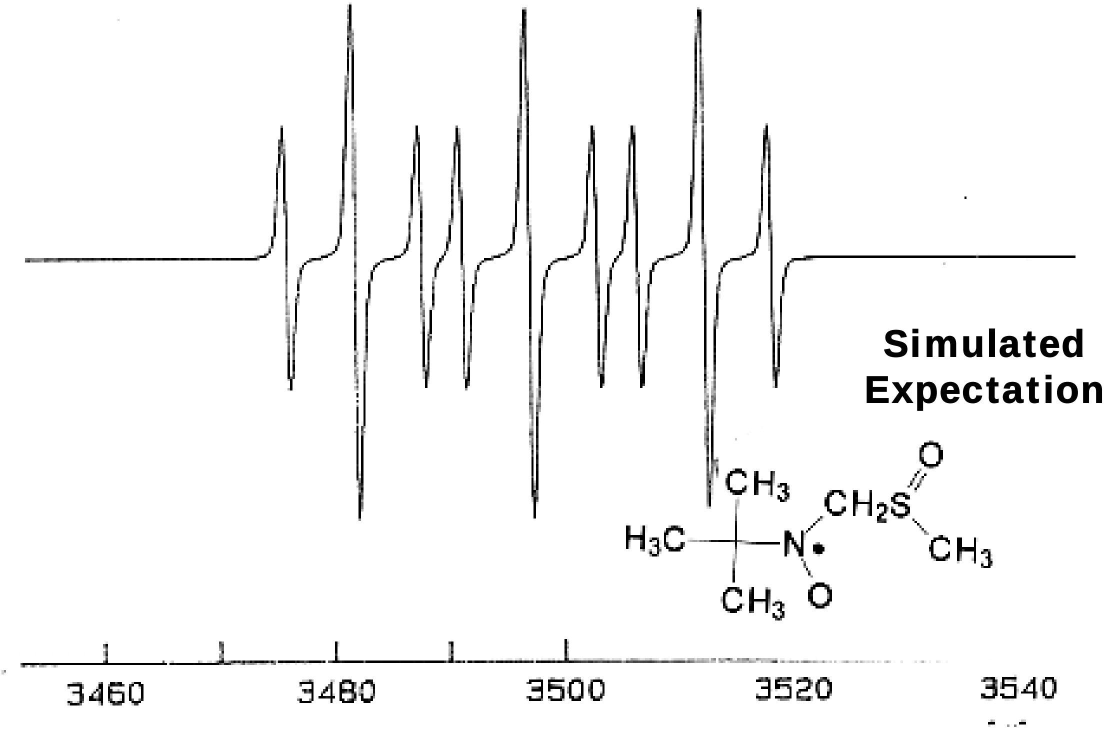


Simulated expectation

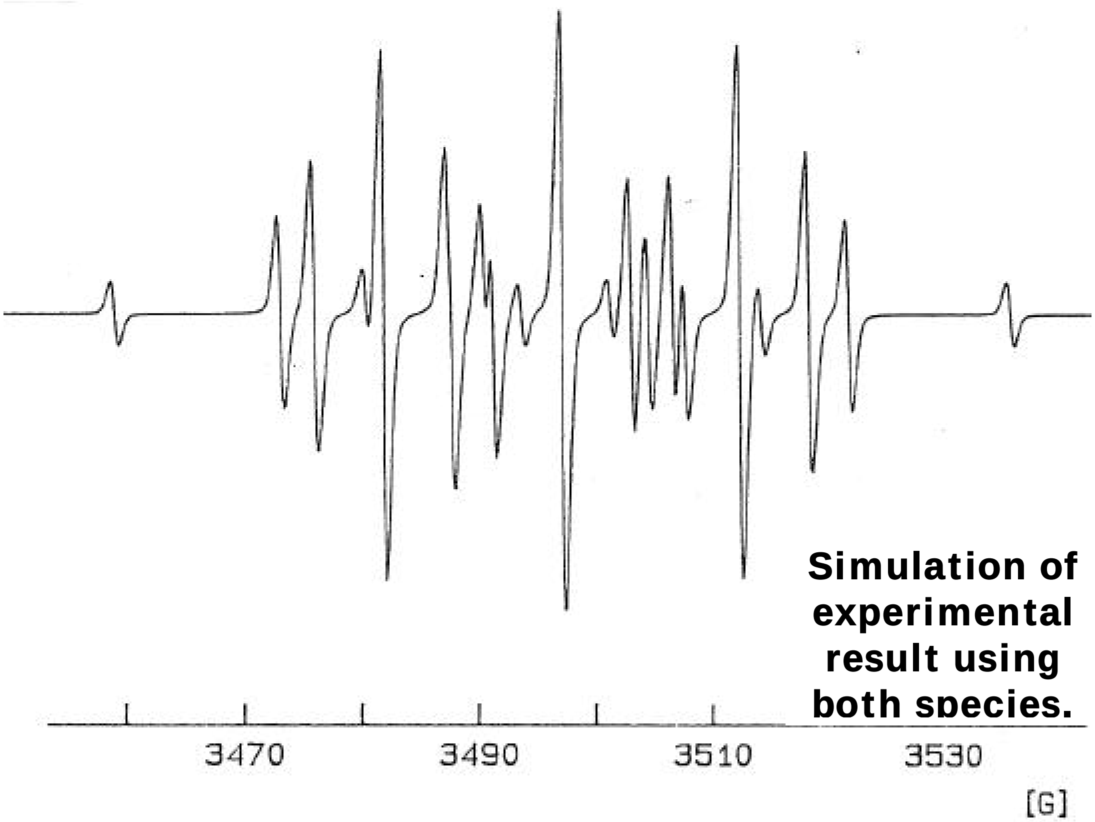


Experimental Result





Simulated Expectation



Simulation of experimental result using both species.

Conclusions:

THINK about

Thermodynamics and Kinetics

For Understanding and Success

APPENDIX

Basic Free Radical Chemistry Resources

NDRL/RCDC*[15] Reprints in *J. Phys. Chem. Ref. Data* published by the American Chemical Society. Parital list:

Reactivity of $HO_2^\bullet/O_2^{\bullet-}$ Radicals in Aqueous Solution, by Benon H.J. Bielski, Diane E. Cabelli, Ravindra L. Arudi, and Alberta B. Ross. *J. Phys. Chem. Ref. Data*, **14**(4):1041-1100, 1985. Reprint No 285, $16.00.

Triplet-Triplet Absorption Spectra of Organic Molecules in Condensed Phases, by Ian Carmichael and Gordon L. Hug. *J. Phys. Chem. Ref. Data*, 15(1):1-250, 1986. Reprint No. 288, $40.00.

Rate Constants for Reactions of Radiation-Produced Transients in Aqueous Solutions of Actinides, by S. Gordon, J.C. Sullivan, and Alberta B. Ross. *J. Phys. Chem. Ref. Data*, **15**(4):1357-1367, 1986. Reprint No. 308, $10.00.

Extinction Coefficients of Triplet-Triplet Absorption Spectra of Organic Molecules in Condensed Phases: A Least-Squares Analysis, by Ian Carmichael, W.P. Helman, and G.L. Hug. *J. Phys. Chem. Ref. Data*, **16**(2):239-260, 1987. Reprint No. 322, $12.00.

Critical Review of Rate Constants for Reactions of Hydrated Electrons. Hydrogen Atoms and Hydroxyl Radicals ($^\bullet OH/^\bullet O^-$) in Aqueous Solution, by George V. Buxton, Clive L. Greenstock, W. Phillip Helman, and Alberta B. Ross. *J. Phys. Chem. Ref. Data*, **17**(2):513-886, 1988. Reprint No 343, $56.00.

Rate Constants for Reactions of Inorganic Radicals in Aqueous Solution, by P. Neta, Robert E. Huie, and Alberta B. Ross. *J. Phys. Chem. Ref. Data*, **17**(3):1027-1284, 1988. Reprint No. 346, $42.00.

Rate Constants for the Quenching of Excited States of Metal Complexes in Fluid Solution, by Morton Z. Hoffman, Fabrizio Bolletta, Luca Moggi, and Gordon L. Hug. *J. Phys. Chem. Ref. Data*, **18**(1):219-543, 1989. Reprint No. 360, $50.00.

Reduction Potentials of One-Electron Couples Involving Free Radicals in Aqueous Solution, by Peter Wardman. *J. Phys. Chem. Ref. Data*, **18**(4):1637-1755, 1989. Reprint No. 372, $24.00.

Rate Constants for Reactions of Peroxyl Radicals in Fluid Solution, by P. Neta, Robert E. Huie, and Alberta B. Ross. *J. Phys. Chem. Ref. Data*, **19**(2):413-513, 1990. Reprint No. 384, $22.00.

Quantum Yields for the Photosensitized Formation of the Lowest Electronically Excited Singlet State of Molecular Oxygen in Solution, by Francis Wilkinson, W. Phillip Helman, and Alberta B. Ross. *J. Phys. Chem. Ref. Data*, **22**(1):113-262, 1993. Reprint No 449, $28.00.

Rate Constants for the Decay and Reactions of the Lowest Electronically Excited Singlet State of Molecular Oxygen in Solution. An Expanded and Revised Compilation, by Francis Wilkinson, W. Phillip Helman, and Alberta B. Ross. *J. Phys. Chem. Ref. Data*, **24**(2):663-1021, 1995. Reprint No. 489, $54.00.

Rate Constants for Reactions of Transient from Metal Ions and Metal Complexes in Aqueous Solution, by George V. Buxton, Q.V. Mulazzani, and Alberta B. Ross. *J. Phys. Chem. Ref. Data*, **24**(3):1055-1349, 1995. Reprint No. 491, $46.00.

Order directly from the American Chemical Society, Post Office Box 57136, Washington, D.C. 20037-0136. Orders must be PREPAID. Telephone: (800) 227-5558 or (202) 872-4363.

Many of the rate constants for the reactions of free radical and related species, e.g. $^1O_2$, can be found at the Web Site of the Notre Dame Radiation Laboratory at: **http://allen.rad.nd.edu** . On the NDRL Radiation Chemistry Data Center pages go to the section devoted to online resources.

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[15] NDRL, Notre Dame Rad. Lab.; NIST, National Institute of Science and Technologies; RCDC, Radiation Chemistry Data Center.