LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

BNWL-SA-199

~~BNSA 199~~

CONF-650628-1

BNSA-

MASTER

FREE RADICALS FORMED BY
GAMMA IRRADIATION OF ORGANIC SOLUTES IN WATER¹

by

D. R. Kalkwarf²

and

R. N. Diebel²

Radiological Chemistry, Chemical Research
Chemistry Department
Pacific Northwest Laboratories
Battelle Memorial Institute
Richland, Washington

May 18, 1965

¹ Paper for oral presentation at the Northwest Regional Meeting of the American Chemical Society to be held at Richland, Washington, June 14, 1965.

² Work performed under Contract No. AT(45-1)-1830 between the Atomic Energy Commission and Battelle Memorial Institute.

PATENT CLEARANCE OBTAINED. RELEASE TO
THE PUBLIC IS APPROVED. PROCEDURES
ARE ON FILE IN THE RECEIVING SECTION.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

FREE RADICALS FORMED BY
GAMMA IRRADIATION OF ORGANIC SOLUTES IN WATER*

D. R. Kalkwarf and R. N. Diebel

Chemistry Department
Pacific Northwest Laboratory
Battelle Memorial Institute
Richland, Washington

The effects of ionizing radiation on even simple organic solutes in aqueous solution have been found to be complex primarily because of the intense and conflicting action of the radiolysis products of water. Hydrated electrons with a measured standard oxidation potential, $E^\circ = 2.7$ volts (1) and hydroxyl radicals with an estimated $E^\circ = -2.8$ volts (2) are the major products in neutral and basic solution; and this mixture of a potent reducing agent and a potent oxidizing agent exposes organic material to a variety of reactions, with the fastest ones determining the course of further events. In most cases, the products of these secondary reactions will themselves be free radicals and as such are amenable to detection and quantitative determination by electron spin resonance spectrometry. Such measurements offer the possibility of not only determining the structures of the organic radicals formed but also evaluating their rates of formation and disappearance so that the sequence of reactions initiated by irradiation can be better understood.

The lifetimes of radicals in aqueous solution can be quite short and their detection sensitivity by ESR measurements is lowered by the dielectric absorption of water. These conditions require that the solutions absorb relatively high

*Work performed under Contract No. AT(45-1)-1830 between the Atomic Energy Commission and Battelle Memorial Institute.

radiation dosages and then are transported rapidly to the detection equipment. In the present work, the flow system shown schematically in Figure 1 was used. A variable-speed rotary pump made of stainless steel propelled the solutions through a coil of 3/8-inch stainless steel tubing surrounding a circular array of cobalt-60 rods in an A.E.C.L. Gammacell 200. Polypropylene tubing then carried the solution to an aqueous sample cell of a Varian V-4500 spectrometer with 100 kc. modulation and Fieldial control of a magnet with 6-inch pole faces. From the sample cell, the solutions flowed through a glass flow-meter into a polypropylene reservoir which served as a place for adding reagents, removing entrained gas bubbles and sparging the solutions with gas, e.g., helium, to remove dissolved oxygen. Connective tubing was made of polyethylene and conducted the solution from the reservoir to the pump for recycling through the irradiation zone.

The dose rate to the solution in the irradiation zone was measured by chemical dosimetry and found to be $67.6 \text{ rads sec}^{-1}$, while the volume of solution present in the zone at any instant was measured to be 312 ml. Letting F represent the flow rate in ml sec^{-1} , the dose absorbed by a solution in each pass through this zone was $(67.6)(312)/F = 21100/F \text{ rads}$. Another key parameter of the flow system was the volume of solution between the exit of the irradiation zone and the sample cell. This was measured to be 11.5 ml. so that a time delay of $11.5/F$ took place before the ESR spectrum of the solutions could be determined.

Figure 2 shows the first-derivative ESR absorption spectrum of the product from the irradiation of $2.00 \times 10^{-3} \text{ M}$ sodium p-nitrobenzoate in deoxygenated water at pH 13. This spectrum is indicative of configuration I in which the unpaired electron interacts with the nitro-nitrogen atom to produce the spectral triplet of bands with 13.0 gauss splitting upon which is superimposed a triplet of lines with 3.3 gauss splitting due to the two ortho protons and a triplet of lines with 1.0 gauss splitting due to the meta protons. A theoretical spectrum

for configuration I is shown in Figure 2 below the observed spectrum and the relative line intensities are seen to be in agreement with the observed values.

(I)

(II)

(III)

Formation of configuration I during irradiation can be easily explained as the addition product of a hydrated electron to the p-nitrobenzoate ion, i.e.,

The rate constant for this reaction can be estimated to be $1.2 \times 10^{10} \text{ M}^{-1} \text{ Sec}^{-1}$ at room temperature based on data obtained for similar compounds during pulse radiolysis (3). At first, another radical product, configuration II, was also expected since aqueous solutions of benzenoid derivatives generally yield phenolic compounds during irradiation in the absence of air. The hydroxycyclohexadienyl radical, configuration III, has been shown to be an intermediate formed during the irradiation of aqueous solutions of benzene (4); but using the rate constant for its formation together with the relative rate constants for reactions of hydroxyl radical with benzene and with p-nitrobenzoate ion (5) a value of only $1.2 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ was obtained for the reaction:

Since the radiolysis yield of hydrated electrons is 2.8 per 100 e. v. absorbed compared with a value of 2.4 hydroxyl radicals per

100 e.v. absorbed (6), the lack of evidence for radicals with configuration II seems due to their relatively slower rate of formation.

The absolute concentration of radicals in the aqueous sample cell was determined with standard solutions of Wursters Blue perchlorate in deoxygenated water and diphenylpicrylhydrazyl in benzene. The modulation amplitude of the spectrometer was increased until the fine structure in the spectra of these solutions broadened into simple bands and the first moments of these band spectra were obtained on a moment balance in the same manner as described by Kohnlein and Muller (7). These moments are proportional to the concentration of radicals in the sample cell, and the proportionality constant was used to calculate the concentration of radicals in the irradiated solutions from the first moments of their overmodulated spectra. Table I lists the concentration of radicals obtained from irradiated p-nitrobenzoate solutions under various conditions.

TABLE I

Radical Concentrations Measured at the Aqueous Sample Cell

<u>Solution Composition</u>	<u>Flow Rate</u>	<u>Radical Concentration</u>
1.00×10^{-3} M sodium p-nitrobenzoate at pH 12.9	3.9 ml sec ⁻¹	4.9×10^{-6} M
2.00×10^{-3} M sodium p-nitrobenzoate at pH 12.9	3.9 ml sec ⁻¹	4.5×10^{-6} M
1.00×10^{-3} M sodium p-nitrobenzoate at pH 12.9	3.9 ml sec ⁻¹	8.5×10^{-6} M
2.00×10^{-3} M sodium p-nitrobenzoate, 2.88×10^{-3} M ethanol at pH 12.9	3.9 ml sec ⁻¹	10.9×10^{-6} M

The concentration of radicals is seen to increase with concentration of p-nitrobenzoate ion and with addition of ethanol to the solution. Ethanol is a fast hydroxyl radical scavenger but a slow reactant with hydrated electrons so that it appears that part of the action of hydroxyl radicals in the solution is to react with radicals of configuration I to form a non-radical species.

After stopping the pump, the decay of signal amplitude from the solution in the sample cell was measured and found to be surprisingly slow, e.g., see Figure 3. The intensities of the spectral lines decreased proportionally with time, and no new lines or changes in line splitting were observed. It was thus concluded that only one radical species was involved. The decay rates were not simply first or second order with respect to radical concentration, but they could be resolved into the sum of two first-order rate expressions with half-lives of around 14 minutes and of around 1 hour. Further experiments are required to unravel this complex behavior as it appears that the radicals are reacting simultaneously with several components in the solutions.

Irradiated solutions of p-nitroaniline in deoxygenated water at pH 13 were also found to contain relatively long-lived radicals with an ESR spectrum as shown in Figure 4. Casual examination of this spectrum indicates the presence of a radical with configuration IV in accord with that found by electrolytic reduction (8) and chemical reduction with dithionite (9). In the irradiation

zone, this radical can be formed by the reaction:

Higher resolution of the ESR spectrum for the radical produced is shown in Figure 5; and it can be seen that additional structure is present. No data are available for the evaluating k_3 or the rate constant for the competing reaction of p-nitroaniline with hydroxyl radicals; however, it has been shown that the presence of

an amino group in an aromatic compound decreases its reactivity with hydrated electrons (3) while enhancing its reactivity with hydroxyl radicals (5). It seems reasonable to conclude that the rates of these competing reactions will be more equal in the case of p-nitroaniline than they were in the case of p-nitrobenzoate ion and that the effect of hydroxyl-radical attack would be more apparent.

Table II lists the concentrations of radicals produced in irradiated solutions of p-nitroaniline under various conditions.

TABLE II

Radical Concentrations Measured at the Aqueous Sample Cell

<u>Solution Composition</u>	<u>Flow Rate</u>	<u>Radical Concentration</u>
2.00×10^{-3} M p-nitroaniline at pH 12.9	2.4 ml sec ⁻¹	4.3×10^{-6} M
2.00×10^{-3} M p-nitroaniline, 1.44×10^{-3} M ethanol at pH 12.9	2.8 ml sec ⁻¹	4.4×10^{-6} M

The concentration of radicals obtained from irradiated p-nitroaniline is seen to be about the same as from p-nitrobenzoate, but the effect of added ethanol was found to be less.

The decay of signal amplitude from the p-nitroaniline solutions was faster than in the case of p-nitrobenzoate solutions. As before, this decay was not simply first or second order in radical concentration but could be resolved into the sum of two first order rate expressions with half-lives of 20 ± 5 seconds and 90 ± 15 seconds. Again, multiple reaction pathways are indicated.

Aqueous solutions of 9×10^{-3} M benzene at pH 7 and of 1.0×10^{-2} M sodium benzene sulfonate at pH 13 were irradiated in the flow system; however, no ESR signal was obtained from either of the product solutions. The specific rate constant for the reaction of benzene with hydrated electrons is 1.4×10^7 M⁻¹ sec⁻¹, (3) while that with hydroxyl radicals is 4.3×10^9 M⁻¹ sec⁻¹ (4). Both of these

are apparently too low to produce a sufficient concentration of radicals for detection 0.3 seconds after irradiation. Substitution of a sulfonate group on the benzene ring raises its specific rate constant for reaction with hydrated electrons to $4.0 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ (3) and its specific rate constant for reaction with hydroxyl radicals to $7.3 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ (10); however, these reactions still do not seem to be fast enough to produce observable concentrations of the radicals.

Another compound which forms easily detected free radicals during irradiation in aqueous solution is flavin mononucleotide. The spectrum obtained from a $1.00 \times 10^{-2} \text{ M}$ solution of this compound at pH 11 is shown in Figure 6, and its structure corresponds closely to that of the radical formed by dithionite reduction (11). It thus seems probable that this radical is formed in the irradiation zone as a result of reaction between flavin mononucleotide and the hydrated electron. The signal from this radical persists for hours if the solution does not contain molecular oxygen, but disappears as oxygen is dissolved in the system.

The existence of free radicals for seconds to hours after their formation in an irradiation zone has important implications for radiation chemistry and radiobiology. For many years, the "free-radical stage" in the irradiation of aqueous systems has been considered over within microseconds after irradiation, based on the mobilities and expected reactivities of the radicals formed. As can be seen from the examples described in the present paper, some of the radicals formed during irradiation can be quite selective in their reactions. They can, thus, confuse the interpretation of radiation effects on aqueous solutions *in vitro*, but they also offer new hope for the chemical therapy of living tissue after irradiation. Their observation and identification by ESR spectrometry should play a key role in solving both of these problems.

REFERENCES

1. J. H. Baxendale, Radiation Research, Supplement 4, 139-140 (1964).
2. W. M. Latimer, "Oxidation Potentials", Second Edition, Prentice-Hall Inc., New York, 1952; page 48.
3. M. Anbar and E. J. Hart, J. Amer. Chem. Soc. 86 5633-5637 (1964).
4. L. M. Dorfman, I. A. Taub and D. A. Harter, J. Chem. Phys. 41 2954-2958 (1964).
5. D. R. Kalkwarf, Proc. Second Int. Conf. Peaceful Uses Atomic Energy, United Nations, Geneva, 1958. Vol. 29, pp 379-384.
6. A. O. Allen, Radiation Research, Supplement 4, 54-73 (1964).
7. W. Kohnlein and A. Muller, Phys. Med. Biol. 6 599-604 (1962).
8. L. H. Piette, P. Ludwig and R. N. Adams, Anal. Chem., 34 916-921 (1962).
9. R. L. Kolker and W. A. Waters, J. Chem. Soc., 1136-1141 (1964).
10. D. R. Kalkwarf, Unpublished data.
11. L. E. G. Eriksson and A. Ehrenberg, Acta Chem. Scand., 18 1437-1453 (1964).

FIGURE 1

FLOW SYSTEM FOR IRRADIATION OF SOLUTIONS

FIGURE 2

ESR Spectrum for Irradiated 2.00×10^{-3} M Sodium p-Nitrobenzoate
in Water at pH 13, and the Theoretical Spectrum for Structure I

FIGURE 3

Decay of Signal Amplitude from Irradiated 1.00×10^{-3} M Sodium p-Nitrobenzoate
at pH 12.9 and 22°C.

FIGURE 4

ESR Spectrum of Irradiated 2.00×10^{-3} M p-Nitroaniline in Water at pH 13

FIGURE 5

The Low-Field Band of the ESR Spectrum for Irradiated
 2.00×10^{-3} M p-Nitroaniline in Water at pH 13.

FIGURE 6

ESR Spectrum of Irradiated 1.00×10^{-2} M
Flavin Mononucleotide in Water at pH 11