Journal of Chromatography, 206 (1981) 139–142
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,362

Note

Integral high-performance liquid chromatographic–electron spin resonance spectrometer for *in situ* studies of thermal and photochemical free radical reactions

Separation of phenoxy, hydrazyl, nitroxide and silyl-substituted semiquinone radicals

TERRY REICH, KUANG S. CHEN and JEFFREY K. S. WAN*
Department of Chemistry, Queen's University, Kingston, Ontario K7L 3N6 (Canada)
(Received September 22nd, 1980)

The potential and wide applications of high-performance liquid chromatography (HPLC) in chemical and biochemical studies are beyond debate. In the past ten years we have extensively used electron spin resonance (ESR) spectroscopy to characterize transient free radicals and paramagnetic intermediates involved in organic and organometallic reactions. Many of these radical intermediates are very persistent and it is often desirable to separate them for a full spectroscopic characterization, including electronic, vibrational and nuclear magnetic spectroscopy. Prompted by the recent reports of a Japanese group[1–4] on the separation of the stable nitroxide radicals formed in radiolysis by HPLC with ESR detection, we wish to present here a more extensive use of a simple integral HPLC–ESR system assembled in our laboratory for *in situ* studies of thermal and photochemical free radical reactions. Three separate chemical systems will be used to illustrate the general applications of our integral HPLC–ESR spectrometer. They include the thermal reactions of diphenylpicrylhydrazyl radical (DPPH) and di-*tert.*-butyl peroxide (BOOB) with substituted phenols[5], the photochemical reaction of 2-methyl-2-nitrosopropane (TBNO) and the photochemical reaction of 2,6-di-*tert.*-butyl-*p*-benzoquinone (DTBQ) and triphenylsilane in the presence of BOOB[6].

EXPERIMENTAL

The principal components of the integral HPLC–ESR system are shown in Fig. 1. It consists of a positive-displacement pump (Laboratory Data Control, Model 396) coupled to a home-made pulse damper, a Valco sample-injection 6-port valve (universal HPLC injection system) with a sample loop modified for *in situ* thermal and photochemical reactions. For thermal reactions, the sample coil was thermostatted in an oil bath at a pre-selected temperature for a pre-determined period of time, usually 20 sec. For photochemical studies, a short length of a small quartz tubing was included in the modified sample loop for irradiation by a mercury arc. All columns were packed in our laboratory with Whatman Partisil-10 (silica gel); the short column is 30 cm × 2 mm and the long one measures 60 cm × 3 mm.

0021-9673/81/0000–0000/$02.50

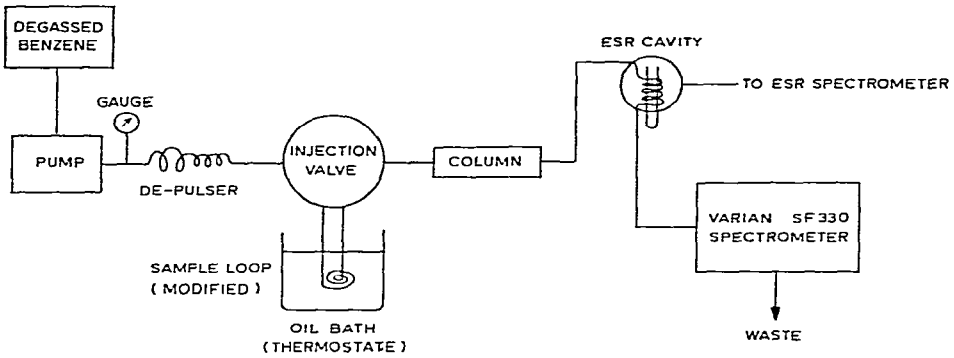


Fig. 1. Flow diagram of an integral HPLC–ESR system.

All HPLC components were mounted on a platformed next to the 10-in. magnet of an old Varian V4500 X-band ESR spectrometer with 100 kHz field modulation. The connection between the HPLC column and the ESR cavity is made by PTFE tubings (0.03 in. I.D.). The PTFE tubings are coiled around a quartz tube inserted into the cavity and the coil is approximately 2 cm in length and in double layers. A standard radical sample placed in a capillary tube can be inserted into the quartz tube in the cavity without distrubing the PTFE sample coil. The magnetic field is manually set by the standard sample and locked in by a Varian F-8A fluxmeter. The standard is then removed before the HPLC operation begins. In order to enhance the sensitivity of the old ESR spectrometer, the state-of-the-art low noise GaAs microwave preamplifier (Narda, Model N6244S-43) was installed at the signal front ends. This provides an approximately 3-fold improvement in sensitivity and a detection limit of about 10^{-12} mole of free radicals.

In the dual-detector configuration, the sample solution was routed from the ESR cavity directly to a Varian SF330 double beam spectrofluorometer for either emission or absorption studies.

All chemicals used were supplied by Aldrich; quinones and phenols were vacuum sublimed before use. Benzene was used as the solvent in all chemical systems and degassed benzene was also used as eluting solvent. In a typical operation, a benzene solution of the chemical system was injected into the sample loop (modified as the *in situ* reactor, 30–50 μl) and after a pre-determined reaction time the system was eluted at a flow-rate of 0.6 ml/min.

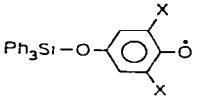
RESULTS AND DISCUSSION

Table I summarizes the retention times of various free radicals in benzene under identical operational conditions as detected by ESR. It is remarkable that the nitroxide radicals have a relatively very long retention time. This is not too surprising as the nitroxide is the most polar radical among those studiec here. The potential usefulness of the HPLC–ESR systems in studies of free radical reactions is illustrated in the following three chemical systems.

TABLE I

RETENTION TIMES OF SOME PHENOXY, HYDRAZYL, NITROXIDE AND SILYL-SUBSTITUTED SEMIQUINONE RADICALS IN BENZENE

Flow-rate: 0.6 ml/min.

Radical	*Retention time (min)*
2,4,6-tri-*tert.*-Butylphenoxy	0.70
1,1-Diphenylpicrylhydrazyl	1.06
$(t\text{-Bu})_2N^{\cdot}\text{–O}$	8.03
	0.72
For comparison, the parent quinone, 2,5-di-*tert.*-butyl-*p*-benzoquinone	2.22

Thermal reaction of DPPH and BOOB with phenol

The thermal reactions of BOOB and DPPH with substituted phenols are well established and accounted for by the following mechanisms:

$$\text{BOOB} \xrightarrow{\triangle} 2\,\text{B}\dot{\text{O}} \quad (1)$$

$$\text{B}\dot{\text{O}} + \text{PhOH} \longrightarrow \text{BOH} + \text{Ph}\dot{\text{O}} \quad (2)$$

$$\text{DPP}\dot{\text{H}} + \text{PhOH} \xrightarrow{\triangle} \text{DPPH}_2 + \text{Ph}\dot{\text{O}} \quad (3)$$

Here PhOH represents the 2,4,6-tri-*tert.*-butylphenol. In the first BOOB–PhOH system, the increase in $\text{Ph}\dot{\text{O}}$ radical concentration with temperature as seen by the HPLC–ESR spectrometeer reflects the increases of rates for both reactions 1 and 2.In the second system, both the $\text{DPP}\dot{\text{H}}$ and the $\text{Ph}\dot{\text{O}}$ were detected and separated by the HPLC–ESR spectrometer but the ratios of the concentrations of the two radicals varied with temperature. For example, $[\text{DPP}\dot{\text{H}}]/[\text{Ph}\dot{\text{O}}] = 0.82$ at 30°C and the ratio decreased to 0.72 at 49°C. This is consistent with the fact that although reaction 3 is "reversible"[5], the forward reaction proceeds much faster with increasing temperature.

Photolysis of 2-methyl-2-nitrosopropane in benzene

TBNO has been used widely as a spintrap to convert short-lived intermediate radicals into the more stable nitroxides. However, TBNO itself is relatively photosensitive and in a benzene solution a self-trapping reaction will proceed as follows:

$$t\text{-BuNO} + h\nu \rightarrow (\text{CH}_3)_3\dot{\text{C}} + \text{N}\dot{\text{O}} \quad (4)$$

$$(\text{CH}_3)_3\dot{\text{C}} + t\text{-BuNO} \rightarrow [(\text{CH}_3)_3\text{C}]_2\text{N}^{\cdot}\text{–O} \quad (5)$$

The concentration of the nitroxide radicals increases with the irradiation time. Because of the extraordinarily long retention times, Makino and co-workers[1–4] have demonstrated that in the radiolysis of an aqueous solution of TBNO, at least five kinds of self-trapped nitroxids radicals were separated by HPLC and characterized by ESR. The photodecomposition of TBNO in a benzene solution appears to be much less extensive and only one major nitroxide radical was produced via reaction 5.

Photolysis of DTBQ–triphenylsilane–BOOB in a benzene solution

The photolysis of a typical benzene solution containing 10^{-3} *M* of DTBQ and triphenylsilane with an excess of BOOB led to a strong blue fluorescence and the observation of a persistent ESR spectrum. The ESR spectrum can be assigned to the triphenylsilyl radical adduct of DTBQ formed by the following mechanism[6]:

$$\text{BOOB} + h\nu \rightarrow 2\,\text{BO} \tag{6}$$

$$\text{BO} + \text{Ph}_3\text{SiH} \rightarrow \text{BOH} + \text{Ph}_3\dot{\text{S}}\text{i} \tag{7}$$

$$\text{Ph}_3\dot{\text{S}}\text{i} + \text{DTBQ} \rightarrow \text{Ph}_3\text{Si}\text{–O–}\langle\text{C}_6\text{X}_2\rangle\text{–}\dot{\text{O}} \tag{8}$$

It was not immediately obvious whether the strong blue fluorescence is due to the persistent triphenylsilyl radical adduct of DTBQ, since both the fluorescence and the ESR spectrum appear simultaneously. When this chemical system was studied in the HPLC–ESR spectrometer with the sample irradiation time of about 15 min, the persistent radical adduct formed in reaction 8 was separated and identified by ESR. However, the eluent containing this radical did not give any fluorescence. Subsequently, the fraction of eluent containing the fluorescent material was run through another commercial high-performance liquid chromatograph (Waters Assoc., refractive index detector) and the fluorescent material was tentatively identified as the di-silyl substituted hydroquinone, probably formed by the combination of the DTBQ radical adduct with another triphenylsilane radical:

$$\text{Ph}_3\text{Si}\text{–O–}\langle\text{C}_6\text{X}_2\rangle\text{–}\dot{\text{O}} + \text{Ph}_3\dot{\text{S}}\text{i} \longrightarrow \text{Ph}_3\text{Si}\text{–O–}\langle\text{C}_6\text{X}_2\rangle\text{–O–SiPh}_3$$

Finally, while the present note demonstrates adequately the potential and the wider general applications of an integral HPLC–ESR spectrometer in free radical studies, other novel metalorganic radicals involved in organometallic reactions are now being successfully separated and characterized by this technique.

ACKNOWLEDGEMENTS

This research is supported by the Natural Sciences and Engineering Research Council of Canada. The authors are grateful to the Department of Pharmacology at Queen's for providing the facility for column packing.

REFERENCES

1 K. Makino, N. Suzuki, F. Moriya, S. Rokushika and H. Hatano, *Anal. Lett.*, Part A, 13 (1980) 301.
2 K. Makino, N. Suzuki, F. Moriya, S. Rokushika and H. Hatano, *Anal. Lett.*, Part A, 13 (1980) 311.
3 K. Makino, *J. Phys. Chem.*, 84 (1980) 1012, 1016.
4 K. Makino and H. Hatano, *Chem. Lett.*, (1979) 119.
5 P. B. Ayscough and K. E. Russell, *Can. J. Chem.*, 43 (1965) 3039.
6 K. S. Chen, T. Foster and J. K. S. Wan, *J. Chem. Soc. Perkin II*, (1979) 1288.