

## FLUORESCENCE DETECTION OF FREE RADICALS BY NITROXIDE SCAVENGING

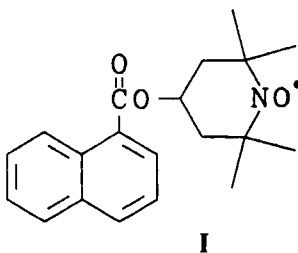
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The fluorescence quantum yield of 4-(1-naphthoxy)-2,2,6,6-tetramethylpiperidine-1-oxyl (*I*) in acetonitrile and hexane is 55 and 30-fold lower, respectively, than those of diamagnetic analogs. Experiments described herein demonstrate that this property makes possible the fluorescence detection of radical scavenging reactions in which the paramagnetic nitroxide-substituted naphthalene is converted to a diamagnetic N-alkoxy derivative. 2-Cyanopropyl free radicals were generated by the thermal decomposition of azobisisobutyronitrile (AIBN) in cyclohexane or in acetonitrile containing *I*. The fluorescence intensity of the sample increased proportionally to the decrease in its ESR signal intensity, indicating the conversion of the paramagnetic nitroxide to the diamagnetic product. The linear relationship between the increase in fluorescence intensity and decrease in ESR signal intensity shows that the changes in the fluorescence intensity can serve as a sensitive means for optically detecting radicals.

**KEY WORDS:** Radical scavenging, fluorescence, ESR, nitroxide

The fluorescence quantum yield of *I* in methanol was recently reported to be ~ 55-fold lower than that of its diamagnetic O-acetyl derivative.<sup>1</sup> This result suggests that the



fluorescence intensity changes associated with the conversion of nitroxides such as *I* to diamagnetic products could be used to monitor, with very high sensitivity, free radical formation in systems not amenable to standard ESR techniques. In particular, it suggests the possibility of optically imaging domains of free-radical reactions in materials and in biological systems. In this report, the feasibility of this approach is demonstrated.

The production of 2-cyanopropyl radicals by thermolysis of azobisisobutyronitrile (AIBN) in oxygen-free cyclohexane and acetonitrile was selected as a model system.<sup>2</sup> The reaction of *I* with free radicals to form diamagnetic N-alkoxy derivatives was followed by both fluorescence and electron spin resonance (ESR) spectrometry. A weighed amount of AIBN was sealed under vacuum in a breakseal connected to a Pyrex sample cell equipped with a 1 cm quartz cuvette (fluorescence and fluorescence

life-time measurements), and a 3 mm quartz ESR cell (flattened to minimize dielectric losses in experiments with acetonitrile). Solutions of **I** were deaerated by three or more freeze-pump-thaw cycles in the cell and then sealed under vacuum. Concentrations of **I** were determined from the ratio of the area under its ESR signal to the area under the ESR signal from known amounts of recrystallized 4-hydroxy-2,2,6,6-tetramethylpiperidiny-N-oxyl (TEMPOL) recorded in the same sample cell under identical conditions.<sup>3</sup> Fluorescence and ESR spectra were recorded prior to the addition of AIBN. No change was seen upon addition of AIBN, prior to heating.

Thermolysis was carried out stepwise by heating the sample cell in a 60 °C oil bath for 10–20 minutes followed by rapid cooling in an ice-water bath. The fluorescence intensity, measured by the signal amplitude at the 348 nm emission peak, increased with heating time in direct proportion to the decrease in the concentration of **I** (Figure 1). The experiment was concluded when **I** could not be detected by ESR; the corresponding fluorescence intensity increased by about 20 times, but the shape of the emission band was unchanged. This increase was reproducible, though less than half that reported between **I** and its O-acetyl derivative.

The fluorescence lifetimes of the diamagnetic species resulting from reaction of **I** with AIBN thermolysis products were measured using a photon-counting hydrogen flash-lamp spectrometer (Photochemical Research Associates, London, Ontario). The lifetimes in both acetonitrile ( $1.75 \pm 0.1$  nsec) and in cyclohexane ( $\sim 1$  nsec) agreed well with values reported for the O-acetyl derivative of **I** in these solvents.<sup>4</sup> The lifetime of pure **I** is  $< 0.1$  nsec,<sup>4</sup> shorter than the response time of the instrument used here. Prior to thermolysis, the lifetime measurement of solutions of the reaction mixture showed a component with a lifetime equal to that measured for the final products. Thus, we attribute the discrepancy between the 20-fold increase in fluorescence intensity measured in these experiments and the 55-fold increase cited above to the presence, in the starting reaction mixture, of a small amount (1%) of diamagnetic impurity, possibly generated during sample preparation. As a control experiment, heating of **I** in cyclohexane in the absence of AIBN caused about a 10% increase in

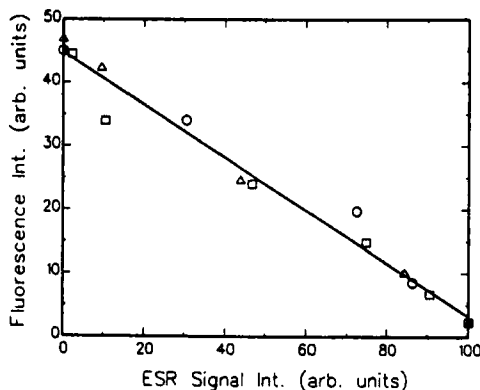


FIGURE 1 Relationship between the fluorescence intensity increase and ESR signal decrease produced by reaction of Nitroxide **I** with 2-cyanopropyl radical generated by thermal decomposition of AIBN in cyclohexane and in acetonitrile. Data shown for 133  $\mu\text{m}$  of **I** in acetonitrile with 10 mM AIBN (○); 97.2  $\mu\text{m}$  of **I** in cyclohexane with 10 mM AIBN (□); and 27.5  $\mu\text{m}$  of **I** in cyclohexane with 2 mM AIBN (△). Spectra intensities were normalized to the same amplitude for the starting material. Fluorescence spectra were recorded with an SLM 8000 system. The excitation wavelength was 300 nm; the spectra bandwidth was 2 nm; the photon counting period 1 sec. The ESR spectrometer was a Bruker-IBM ER 200 D system.

fluorescence intensity, corresponding to less than 0.2% thermal conversion of I to a diamagnetic species. AIBN alone in cyclohexane showed no fluorescence. The quantum yields of I and of the diamagnetic products were insensitive to the fluorophore concentrations over the range used in these experiments, and they were also insensitive to oxygen.

The decay rate of I during AIBN thermolysis in oxygen-free acetonitrile and cyclohexane was in good agreement with free radical initiation rates previously reported.<sup>5</sup> An identical decay rate was obtained for TEMPOL, indicating that the naphthylate group did not interfere with scavenging. The linear relationship observed between fluorescence intensity increase and nitroxide concentration decrease demonstrates that fluorescence spectrometry can be used to quantify free radical formation rates. The present experiments were carried out with a relatively high starting concentration of I (28–133  $\mu\text{M}$ ) to facilitate accurate concentration measurements by ESR. In principle, fluorescence spectrometry could be used to follow free radical scavenging by nitroxides, such as I, at much lower starting concentrations, or more importantly, reactions wherein free radical formation is much slower. Our results indicate that free radical formation at concentrations less than 100 nM are measurable by fluorescence detection of I. Even lower concentrations may be detectable with other fluorophores.<sup>6</sup>

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