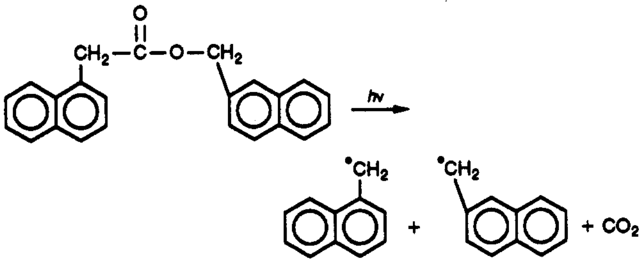
Communications to the Editor

Two-Photon Chemistry. 1. Fluorescence Labeling of Polystyrene and Poly(methyl methacrylate) by Laser Photolysis of 2-Naphthylmethyl 1-Naphthylacetate

Two-photon excitation yields electronically excited molecules, which possess a reactivity quite different from their ground-state and first electronically excited state counterparts. Studies of the fate of such species have been facilitated by the advent of high-intensity lasers and dual-laser techniques.[1] However, two-photon chemistry has yet to be employed for synthetic purposes.

This paper describes studies of fluorescence labeling of polystyrene (PS) and poly(methyl methacrylate) (PMMA) with naphthylmethyl chromophores using two-photon excitation of 2-naphthylmethyl 1-naphthylacetate (NMNA). The photochemistry of NMNA has recently been described.[2] When NMNA is irradiated with light of <320-nm wavelength, carbon dioxide is eliminated and the relatively stable naphthylmethyl radicals are produced.



The quantum yield for decarboxylation of NMNA in benzene is 0.2 ± 0.01. However, because of primary and secondary cage recombination, most radicals rapidly recombine. Only those that escape cage reactions are useful in the present study. The quantum yield for radicals that escape recombination processes has been determined, using free-radical traps, to be 0.06 ± 0.006 in benzene at 25 °C.[2]

Experimental Section. The synthesis of NMNA has been described previously.[2] The PS sample possessed a M_n of 1.61×10^5 and a polydispersity index of 2.9. The corresponding values for PMMA were 4×10^4 and 1.75. The concentrations of polymer and NMNA in benzene were 80 g/L and 6×10^{-3} M, respectively. The samples were degassed by freeze–pump–thaw cycles and sealed under vacuum. The solutions were irradiated either in melting point tubes (20–50 μL) or in quartz cells (4 mL). The optical path lengths were 0.1 and 1 cm, respectively. Samples were irradiated with a Lumonics HyperEX-400 excimer laser filled with XeCl. The intensity of the laser pulse was 20 mJ/cm^2/pulse at 308 nm and at a repetition rate of 10 Hz. The intensity of the laser decreased by ca. 25% over the frequency range of 1–100 Hz.

Following irradiation, the samples were injected into a Waters liquid chromatography system with a Du Pont bimodal column pair using tetrahydrofuran (1 mL/min) as eluant. A spectrofluorometer (Bio-Systems Model 900) was placed in tandem with a differential refractometer (Waters Model R410). An excitation wavelength of 300 nm was used for spectrofluorometric analysis and a 345-nm cutoff filter was used to discriminate between scattered light and fluorescence emission.

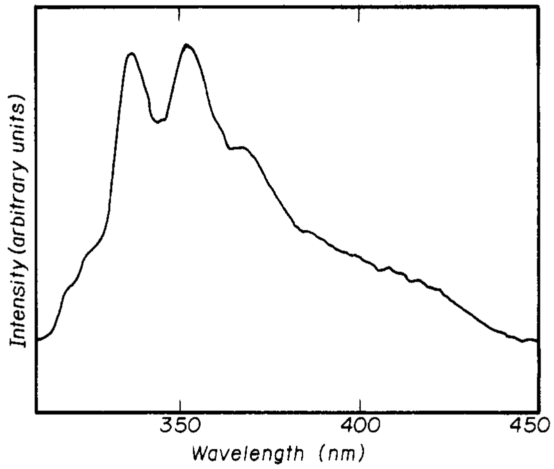


Figure 1. Fluorescence spectrum of polystyrene following laser irradiation in the presence of NMNA: $\lambda_{ex} = 280$ nm; concentration of polymer = 1 g/L; the sample contained 1.9×10^{-5} mol of fluorescent group/g of polymer.

Data acquisition and analysis were performed by using an IBM PC and custom-written software. Nine near-monodisperse PS and PMMA standards (Polymer Laboratories) of molecular weight 2000–1.8×10^6 were used to obtain the calibration curve of molecular weight versus elution volume. Both the refractive index and spectrofluorescence detectors were calibrated for quantitative evaluation of the polymers: aliquots of known mass of polymer were injected into the GPC, and the resultant area under the refractive index–GPC curves was integrated. The standard deviation of calibration plots of polymer mass versus integrated area was ca. 5%. The fluorescence detector was similarly calibrated by injecting known concentrations of 2-methylnaphthalene and integrating the area under the fluorescence–GPC peaks. The error in the calibration was ca. 1%. Calibration plots were corrected for the different quantum yields of fluorescence of 2-methylnaphthalene and 1-methylnaphthalene (0.32 and 0.25, respectively, in nonpolar solvents).[3]

Steady-state fluorescence excitation and emission spectra were recorded by using a Spex Fluorolog-2 spectrophotometer. Polymers for fluorescence spectroscopic measurements were purified by repeated precipitation until low molecular weight fluorescent material was removed.

Results and Discussion. Figure 1 shows the fluorescence emission spectra of PS following irradiation in the presence of NMNA and isolation of the polymer, as described above. The spectrum is characteristic of naphthalene. Since the polymer solution contained no low molecular weight fluorescent products, as determined by GPC, the naphthalene groups must be bound to the polymer chain.

Figure 2 shows the molecular weight distribution curves of PS before and after laser irradiation in the presence of NMNA. The fluorescence signal for the irradiated sample confirms that PS has been labeled with fluorescent groups.

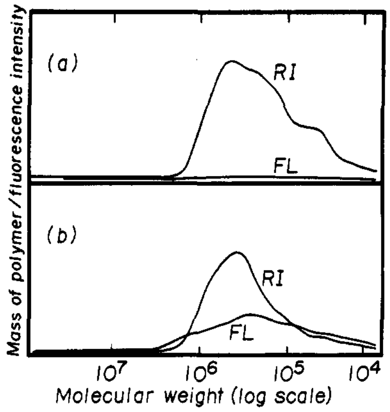


Figure 2. Molecular weight distribution and fluorescence intensity curves for polystyrene before (a) and after (b) laser irradiation in the presence of NMNA. RI = refractive index; FL = fluorescence; laser repetition rate, 100 Hz; number of pulses, 1440.

Table I
Degree of Photolabeling as a Function of Laser Repetition Rate

repetition rate,[a] Hz	moles of fluorescent groups[b] × 10^5	no. of fluorescent groups per chain[c]
100	10.93	17.60
50	2.37	3.82
20	1.08	1.74
10	1.00	1.61
0.5	0.64	0.10

[a] Number of laser pulses = 1440. [b] Moles of naphthylmethyl groups per gram of polystyrene. Determined by GPC. [c] M_n = 161 000.

Table II
Degree of Photolabeling as a Function of Number of Laser Pulses

no. of laser pulses,[a] ×10^3	moles of fluorescent groups,[b] ×10^5	no. of fluorescent groups per polymer chain[c]
11.52	27.7	44.61
5.76	7.9	12.72
1.44	2.4	3.86
0	0	0

[a] Laser repetition rate = 50 Hz. [b] Moles of naphthylmethyl groups per gram of PS. Determined by GPC. [c] M_n = 161 000.

No fluorescence of the polymer sample was observed prior to irradiation or when the polymer was irradiated in the absence of NMNA.

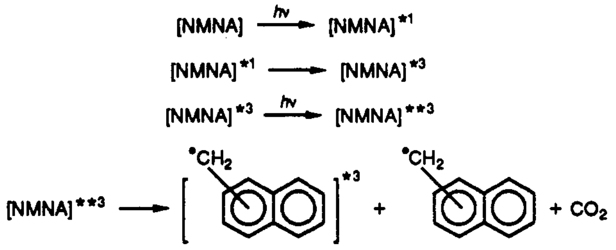
Important information was obtained from calculations of the number of chromophores per gram of polymer and per polymer chain. These data are shown in Table I. The number of fluorescent groups attached increases dramatically as the repetition rate is increased from 0.02 to 100 Hz, even though the total number of pulses was constant. This trend is indicative of a multiphotonic process.

The number of fluorescent labels attached to the polymer chains is strongly dependent on the number of laser pulses. Table II shows the number of fluorophores attached per gram of polymer, and per polymer chain, as a function of the number of laser pulses.

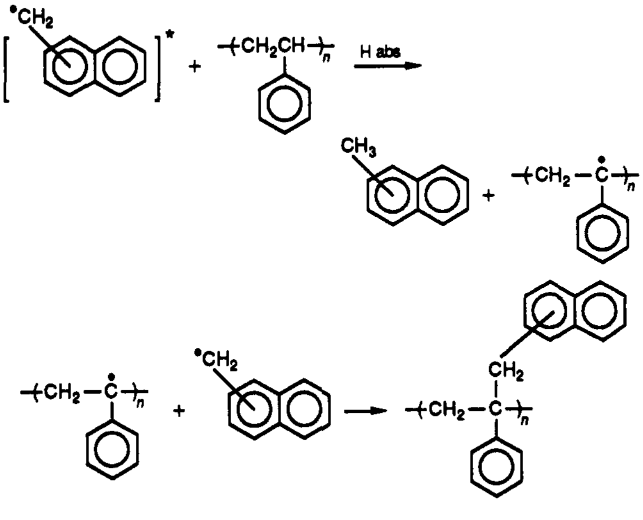
Similar experiments in which NMNA was replaced with 2-methylnaphthalene did not lead to fluorescent polymer products, proving that naphthylmethyl radicals are important intermediates. Ground-state naphthylmethyl radicals, however, can be disregarded as being the reactive species in the photolabeling process because of the observed dependence on laser repetition rate. Photolabeling was also achieved under steady-state irradiation using a 500-W Hg lamp. log–log plots of the degree of photolabeling versus intensity of irradiation gave straight lines of gradient 2.0 ± 0.1. This is convincing evidence for a two-photon process. Detailed studies using steady-state photolysis will be described in a separate paper.

The experimental evidence indicates that the reactive species are electronically excited naphthylmethyl radicals. It is postulated that these originate from a two-photonic event in which the initial photon yields naphthylmethyl radicals. The radicals, which are sufficiently long-lived due to resonance stabilization, then absorb a second photon to give the excited-state radical. However, in this mechanism the lifetime of the radical must exceed tens of milliseconds in order to absorb a photon from the subsequent laser pulse.

Alternatively, generation of excited-state naphthylmethyl radicals could involve triplet–triplet absorption by NMNA and subsequent elimination of carbon dioxide via the second excited triplet state, i.e., a biphotonic event. Further experiments to clarify the mechanism are underway.



Three modes of H atom abstraction are available with PS: fission of the α-C–H bond, the β-C–H bond, and the aromatic ring C–H bond. The α-C–H bond is the least stable of the three, and it is this bond that predominantly ruptures.[4] The following mechanism of photoaddition can be inferred.



Fluorescence labeling by two-photon chemistry is not restricted to aromatic polymers. Similar observations were made with PMMA. Figure 3 shows the molecular weight distribution curves and associated fluorescence of PMMA samples following irradiation in the presence of NMNA. No fluorescence from the polymer was observed prior to irradiation or following irradiation in the absence of NMNA.

In conclusion, labeling of polymer molecules with fluorescent functionality has been demonstrated. The number of fluorophores per chain can be controlled by

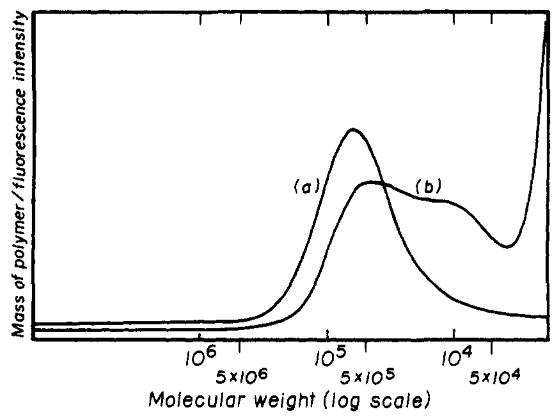


Figure 3. Molecular weight distribution and fluorescence intensity curves for PMMA after laser irradiation in the presence of NMNA: (a) refractive index; (b) fluorescence; laser repetition rate, 20 Hz; number of pulses, 710.

varying the repetition rate and number of laser pulses. These studies signify the usefulness of two-photon processes in synthetic chemistry.

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References and Notes

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