

Maleimido-fluoroprobe: A Dual-Purpose Fluorogenic Probe of Polymerization Dynamics

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Maleimido-fluoroprobe (MFP) is a derivative of “fluoroprobe” (FP), the most sensitive known fluorescent polarity probe. MFP differs from FP in that it is completely nonfluorescent. The characteristic strong FP-type fluorescence is however switched on when the C=C bond of the maleimide group becomes saturated forming the succinimide derivative, SFP. This fluorogenic behavior is shown to be induced by copolymerization of MFP into growing polyacrylate chains. The increase in the intensity of the fluorescence, which is observable even in the early, low-viscosity stage of polymerization, provides therefore a measure of the degree of polymerization. The strong blue shift of the fluorescence of chain-incorporated SFP moieties fulfills the usual polarity probe function of monitoring changes in the microscopic dielectric environment of the polymer as vitrification takes place. This duality in the probe characteristics of MFP is illustrated by results on the radiation-induced bulk polymerization of methyl methacrylate.

Introduction

Fluorescent molecules have been used effectively in recent years to probe the micromorphological changes occurring during the course of bulk polymerization.^{1–16} The method depends on the sensitivity of the fluorescence spectrum and/or decay kinetics to the dielectric or viscoelastic properties of the surrounding medium. All probe molecules used to date fluoresce efficiently even prior to polymerization and monitor only morphological changes within their immediate environment which occur as a result of polymerization. On their own, they give no direct information on the degree of polymerization which can then be related to the morphological changes observed. In addition, when present as unincorporated, free molecules, they may be preferentially concentrated in monomer-rich regions even when polymerization is close to complete, thus resulting in a false impression of the changes occurring in the overall morphology of the medium.

To overcome these problems we have used the compound maleimido-fluoroprobe (MFP) whose molecular structure is shown in Figure 1 together with that of the parent compound fluoroprobe (FP). The maleimide group in MFP completely quenches the otherwise strong and highly polarity dependent fluorescence of the FP component of the molecule. However, when the C=C bond of the maleimide group becomes saturated, MFP switches to a highly fluorescent succinimide form, SFP,¹⁷ with photophysical properties very similar to those of FP.^{3,18} This fluorogenic effect of double-bond saturation has been shown to be induced by condensation reactions with, for example, thiols or amines.¹⁷ In the present work we show that the effect can also be induced by copolymerization of MFP into a growing polymer chain, as illustrated schematically in Figure 1. The resulting fluorescence comes therefore only from those probe molecules which have actually been incorporated into the

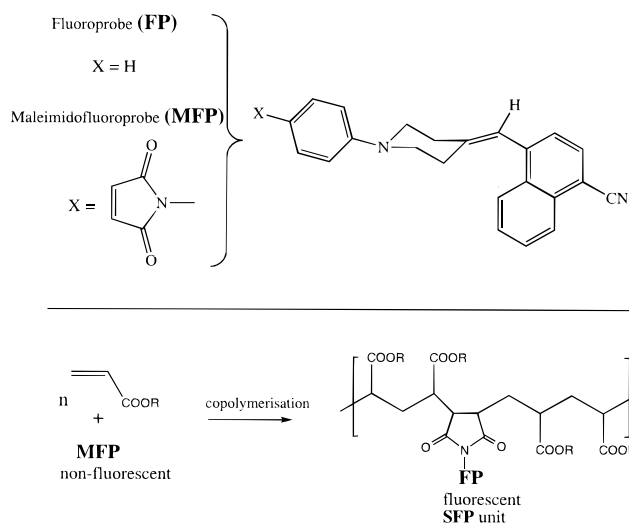


Figure 1. Top: Structures of fluoroprobe (FP) and maleimido-fluoroprobe (MFP). Bottom: Schematic representation of the fluorogenic activation of MFP by copolymerization into a polyacrylate chain.

polymer network. In this way the problem of the preferential concentration of the *fluorescent form* of the probe molecule in monomer-rich regions is avoided. In addition, the intensity of the fluorescence is potentially capable of providing a measure of the degree of polymerization. The application of MFP as a dual-purpose fluorogenic probe is illustrated here with results on the radiation-induced polymerization of methyl methacrylate.

Experimental Section

The molecular structures of fluoroprobe (FP) and maleimido-fluoroprobe (MFP) are shown in Figure 1. Their synthesis and physical characterization have been described elsewhere.^{17,18} Methyl methacrylate (MMA; Scientific Polymer Products, SPP)

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was passed over a DHR4 (SPP) column immediately prior to use to remove hydroquinone stabilizer. Optical absorption spectra of the solvent and solutions were measured using a Kontron Uvikon-940 UV/vis spectrophotometer. MMA solutions of MFP or FP of optical density *ca.* 0.2 per cm at 337 nm were placed in disposable 1 cm cuvettes, made from square Suprasil tubing as described previously.¹⁵ The optical densities used corresponded to solute concentrations of approximately 2×10^{-5} M. The solutions were deaerated by purging with argon prior to sealing the cuvette with a PTFE stopper and parafilm.

The samples were irradiated in a ^{60}Co γ -ray source at a dose rate of 1.05 kGy/h. After a given exposure the fluorescence was measured in a PTI Quantamaster spectrofluorimeter using an excitation wavelength of 337 nm. All fluorescence spectra could be described very well by a single Gaussian curve:

$$I(E) = I_{\text{max}} \exp\{ -[(E - E_{\text{max}})/0.60\Delta E]^2 \} \quad (1)$$

with E being the photon energy. The energies corresponding to the maximum intensity, E_{max} , and the full width at half-height, ΔE , were determined using the curve-fitting program TableCurve (Jandel Scientific).

Size exclusion chromatography (SEC) was carried out using a Polymer Mixed C column (7.5 mm i.d. \times 600 mm) and a PL-gel guard precolumn (7.5 mm i.d. \times 50 mm). A 50 μL aliquot of a 0.5 mg/4 mL solution of the irradiated sample in THF was injected. The elution profile was monitored using a Millipore Waters 410 differential refractometer with THF + 0.1% HAc as eluant. Molecular weight calibration was carried out using PMMA polymer standards. The fluorescence profile was monitored using a Perkin Elmer LS40 detector with excitation at 320 nm. Detection was restricted to wavelengths above 530 nm using a cutoff filter.

Results and Discussion

While fluoroprobe is highly fluorescent in all but very polar solvents,¹⁸ maleimido-fluoroprobe is universally nonfluorescent.¹⁷ This contrast in emissive properties is illustrated by the fluorescence measurements carried out on the unirradiated methyl methacrylate solutions which are shown for exposure time = 0 in Figure 2. For the MFP solution only a very low intensity fluorescence in the 400 nm region is observed. This was also present in the methyl methacrylate solvent and is attributed to a spurious, low-level impurity. This contrasts with the intense fluorescence from the FP solution with a maximum at *ca.* 560 nm.

When the MFP solution is ^{60}Co γ -ray irradiated, an emission with a maximum at *ca.* 540 nm does however appear, as shown in Figure 2. Up to a total radiation exposure of 4 h (4.2 kGy), the intensity gradually increases while the wavelength maximum and spectral shape remain almost unchanged. For the FP solution neither the intensity nor the spectral characteristics change appreciably over the same dose range. The results clearly indicate that the C=C double bond of the maleimide unit in MFP is becoming saturated on irradiation resulting in formation of an emissive SFP derivative. The question then arises as to whether this activation of the fluorogenic effect is caused by the formation of monomeric radiolysis products of MFP or is due to the copolymerization of MFP into poly-(methylmethacrylate) chains.

To answer this question we have carried out size exclusion chromatography (SEC) on 4 h irradiated samples of MMA containing FP and MFP using both refractive index (RI) and fluorescence (FL) detection. The RI elution profiles are similar

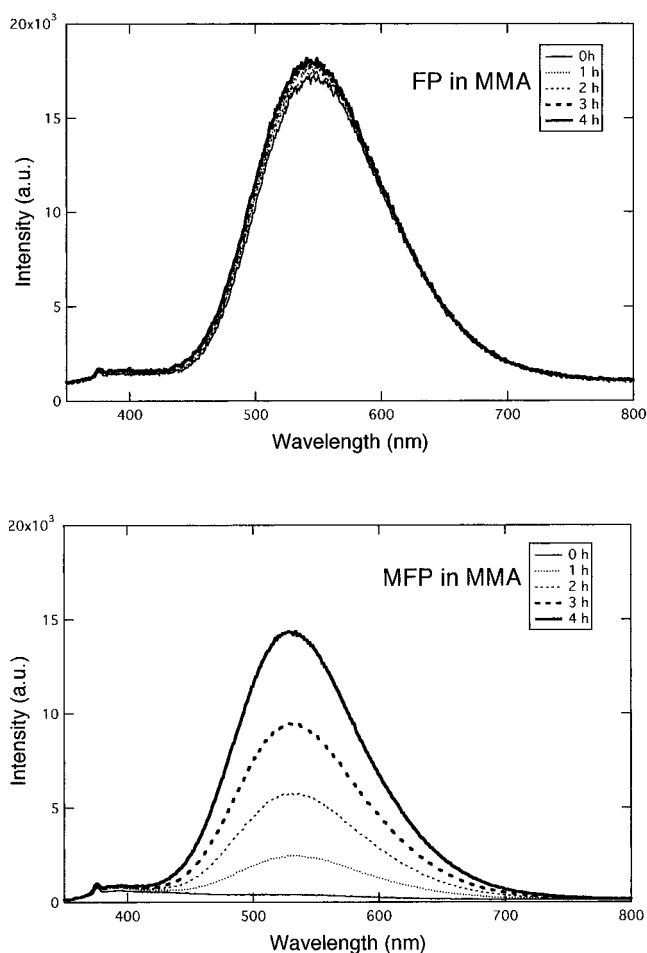


Figure 2. Fluorescence spectra taken after exposure of *ca.* 2×10^{-5} M MMA solutions of FP (top) and MFP (bottom) to ^{60}Co γ -ray irradiation with exposures from 0 to 4 h.

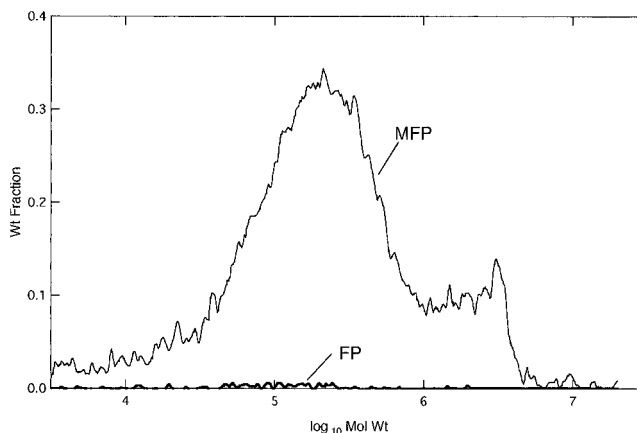


Figure 3. Elution profiles using fluorescence detection of MMA samples containing *ca.* 2×10^{-5} M FP or MFP after a γ -ray exposure of 4 h (4.2 kGy) plotted against the logarithm of the eluate molecular weight.

for the two samples and show the formation of polymeric products of similar molecular weight distribution and yield. The fluorescence profiles, presented in Figure 3, are however seen to differ dramatically and clearly show that only in the case of MFP is the emissive chromophore actually incorporated into the high molecular weight fraction of the eluate. The fluorescence of the FP sample is found to be concentrated entirely in the low molecular weight, solvent fraction, as expected. For MFP, some fluorescence was also observed in the low molecular weight, $<10^4$ Da, fraction. However, this was a minor

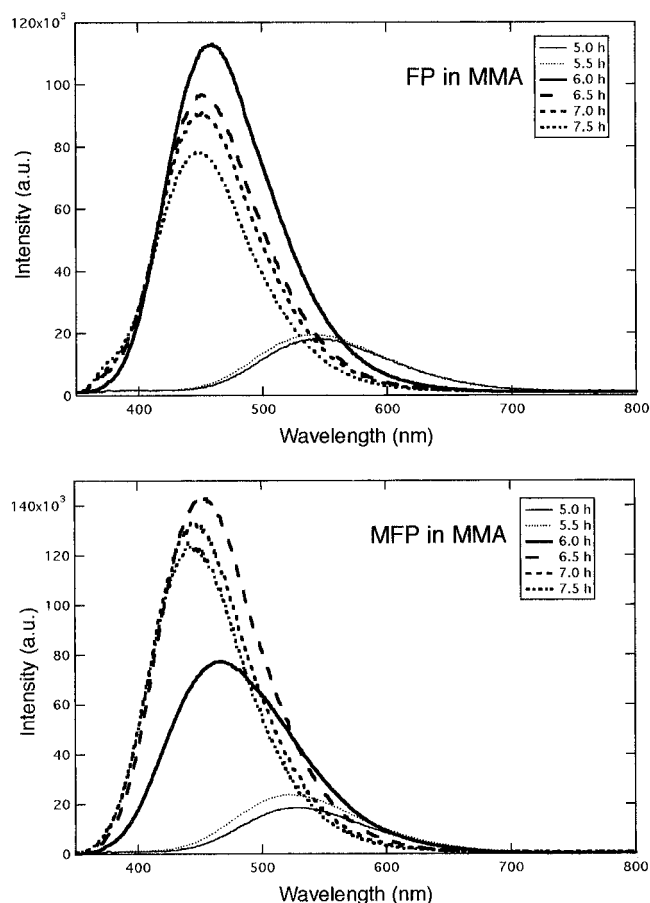


Figure 4. Fluorescence spectra taken after exposure of *ca.* 2×10^{-5} M MMA solutions of FP (top) and MFP (bottom) to ^{60}Co γ -ray irradiation with exposures from 5 to 7.5 h.

component of the total fluorescence observed. In addition, by comparison with the FP fluorescence, even the “low molecular weight” fraction of the emission of the MFP sample appeared to be associated with oligomeric $(\text{MMA})_n\text{SFP}$ species rather than with monomeric SFP compounds.

We conclude that the fluorescence which gradually develops on irradiation of a methyl methacrylate solution of MFP results mainly from the incorporation of MFP into the growing polymer chains which leads to the fluorogenically activated SFP chromophore as illustrated schematically in Figure 1. The intensity of the fluorescence is therefore a measure of the degree of polymerization. In future work we will attempt to quantify this relationship and compare the results with other methods of determining the extent of monomer conversion.

A dramatic change in the spectral characteristics of both the MFP and FP solutions suddenly occurs above an exposure of 5.5 h, as shown in Figure 4. This involves a very large, *ca.* 100 nm (0.5 eV), blue shift in the emission maxima and a 6-fold increase in intensity. These effects can be ascribed to a sudden autoacceleration in the polymerization process and a concomitant rapid vitrification of the sample; a phenomenon which is known as the Trommsdorff effect.¹⁹ This sudden spectral shift is similar to the abrupt 565–450 nm shift found on thermal curing of MMA containing FP¹ and somewhat larger than that found for ^{60}Co γ -irradiation using the conjugated donor–acceptor molecule (dimethylamino)nitrofluorene (DNF) as probe.¹⁵

Radiation exposures in excess of 6 h result in only a slight further shift to the blue in the emission spectra and a gradual decrease in intensity, which may be due to degradation of the chromophoric units.

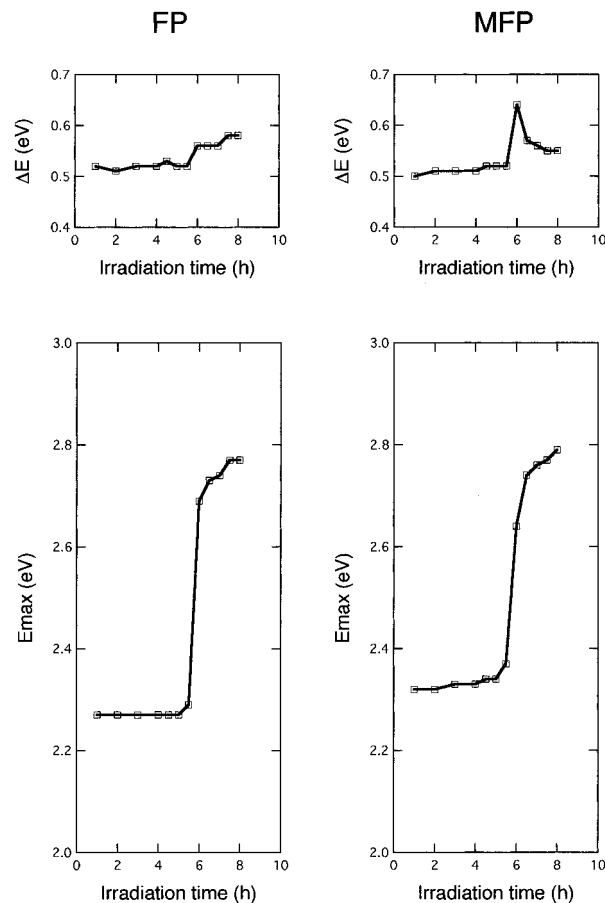


Figure 5. Change with irradiation exposure time of the fluorescence maxima (lower plots) and spectral half-widths (upper plots) of *ca.* 2×10^{-5} M MMA solutions of FP (left) and MFP (right).

In Figure 5 are shown the changes in the maximum and the half-width of the spectra for the FP and MFP samples as a function of the exposure time. The E_{max} plots illustrate even more clearly the extreme abruptness of the Trommsdorff effect. A tendency for the sudden blue shift to be somewhat less abrupt for the MFP sample may reflect the different “observation points” of the fluorescent probes within the matrix: FP in “monomer-rich” regions and SFP units incorporated within the polymer network. The behavior of the half-width in the Trommsdorff transition region indicates even more clearly that the two probes are probably sending out different messages with MFP indicating a larger heterogeneity in the matrix than in the neighborhood of FP molecules. In-situ irradiation experiments are being devised to study in greater detail the fascinating Trommsdorff transition region.

Conclusions

The fluorogenic molecule maleimido-fluoroprobe (MFP) fluoresces only when it is copolymerized into a polymer chain. It is therefore a sensitive probe for studying concurrently both the degrees of polymerization and the changes in dielectric properties associated with the development of the polymer matrix. By applying size exclusion chromatography with fluorescence detection, MFP can in addition provide information on the development of the molecular weight distribution during the course of polymerization. The application of such fluorogenic probes to study directly the kinetics of polymerization using time-resolved pulsed-initiation techniques can also be envisaged.

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