

Preparation and Spectroscopic Properties of Phenanthrene-Labeled SEBS Triblock Copolymers

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ABSTRACT: Phenanthrene-labeled poly(styrene-*b*-poly(ethylene-*co*-butylene)-*b*-polystyrene) triblock copolymers (SEBS) have been prepared from a maleated derivative of the polymer. The dye was attached either through an imine or an ester linkage. The model compound **4**, *N*-phenanthrylmethyl succinimide, exhibits an exponential fluorescence decay in deaerated THF solution, with a lifetime of $\tau_0 = 44.5$ ns. However, the fluorescence decays of the labeled SEBS polymers, both in film and in solution, could only be fitted with a sum of three exponential functions, with lifetimes approximately equal to 2, 14, and 44 ns. The long lifetime coincides with that of unquenched phenanthrene. The shortest lifetime, 2 ns, was attributed to polystyrene (PS) emission and could be corrected using the decay of the PS component of unlabeled SEBS ($\tau_{PS} \approx 2$ ns). The 14 ns lifetime appears to be a result of phenanthrene self-quenching brought about by local aggregation, driven not by the dye itself but by the functional group used to attach the dye to the polymer, the succinimide group or the $-\text{COOH}$ groups of the succinate monoester derivative. Upon full methylation of the acid groups in the phenanthrylmethyl-ester-labeled SEBS copolymer, the interaction between the acid groups is removed and the extent of phenanthrene aggregation is strongly reduced. After correction for the PS emission, the phenanthrene decay profiles of the fully methylated SEBS copolymer, both in solution and in the film, could be fitted with only one exponential.

Introduction

SEBS is the term applied to the class of triblock copolymers obtained through the complete hydrogenation of the middle block of poly(styrene-*b*-butadiene-*b*-styrene). The EB refers to the ethylene-*co*-butylene structure of the middle block after hydrogenation. These polymers were first developed as thermoplastic elastomers.¹

They also have important applications as compatibilizers for immiscible polymer blends such as the blend of polyethylene with polystyrene.² Maleated derivatives of SEBS are available as reactive compatibilizers. These polymers are prepared commercially by the reaction of maleic anhydride with the unsaturated middle block (an ene reaction) prior to the hydrogenation step. The anhydride group serves as a useful functionality for reaction with appropriate nucleophiles such as amine or hydroxyl groups attached to other polymers.³

This anhydride group is also very useful as a site for attaching fluorescent dyes to the polymer. These dyes can be tracer dyes for fluorescence microscopy as a means of determining the location of SEBS in a mixture of polymers. Alternatively one can choose a dye suitable for nonradiative energy transfer (ET) measurements. ET measurements can provide useful information about mixing at the molecular level in polymer blends. Here we are interested in comparing features of the labeling process in which the dyes are attached by reaction of dye- NH_2 derivative to form an imine, as opposed to dye attachment via reaction with a dye- CH_2OH to form an ester linkage.

The dyes we examine are derivatives of phenanthrene (Phe), which is often the dye of choice as the donor in studies of polymer structure by energy transfer.^{4,5} As we indicate below, the spectroscopic properties of Phe attached to the polymer backbone are very sensitive to the nature of the attachment. This sensitivity arises because some functional groups promote aggregation of the dyes in solution as well as in polymer films. While Phe as a fluorophore tends to be relatively insensitive to self-quenching, particularly when compared to cyanine dyes or pyrene, self-quenching can become important when a significant fraction of the Phe substituents experience a high local concentration of other Phe groups. The possibility of Phe self-quenching in solution was established by Zachariasse et al.⁶ The authors used a series of symmetrically substituted diphenanthrylpropanes to create a large local concentration of Phe in the vicinity of the excited Phe*. They observed that, superimposed on the usual Phe* monomer fluorescence emission, a red-shifted structureless band appeared that could be assigned to a Phe excimer. The presence of the intramolecular excimer emission was also supported by single photon counting fluorescence decay curves of 1,3-bis(9,9-phenanthryl)propane in *n*-heptane, for which the authors found two decay times ($\tau_{\text{short}} = 14$ ns and $\tau_{\text{long}} = 44$ ns). In more general terms, the Phe group excited to form Phe* in the close proximity of other Phe groups can experience self-quenching. These Phe* will exhibit a reduced quantum efficiency of fluorescence and a faster fluorescence decay rate than unquenched Phe* elsewhere in the system.

Experimental Section

SEBS (Kraton G1652, with a block ratio of 1:5:1) and 2 wt % maleated PS-EB-PS (Kraton FG 1901X), both with $M_w = 97\,700$ and $M_n = 84\,400$, were obtained from Shell. In Kraton FG 1901X (KFG), maleic anhydride was grafted randomly to the middle polyethylenebutene (EB) block. Xylene and tetra-

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chloroethane were dried over 4A molecular sieves. Other solvents and reagents were used as received. The UV absorption spectra were measured with a Hewlett-Packard 8452A diode array spectrophotometer. IR transmission spectra were obtained with a Perkin-Elmer model FTIR spectrometer at a resolution of 4 cm^{-1} . Fluorescence spectra were measured with a SPEX Fluorolog 2.1.2 instrument equipped with a DMA 3000 data system. Fluorescence decay profiles were measured by the time-correlated single photon counting technique.⁷ The excitation wavelength was 300 nm; the emission was detected at 350 nm, and 20 000 counts were collected in the maximum channel. A $350 \pm 5\text{ nm}$ interference was placed in front of the emission monochromator to enhance the selectivity of the detection. Data were collected over three decades of decay and fitted by nonlinear least-squares methods using the δ function convolution method. All polymer solutions for the single photon counting measurements were degassed under a gentle flow of nitrogen for 20 min. Gel permeation chromatography (GPC) measurements in tetrahydrofuran (THF) solution were done using a Waters pump, two columns (HR3 and HR4, effective molecular weight range 500–500 000) and tandem fluorescence (Kratos FS 970) and refractive index (Waters R-400) detectors.

Determination of Anhydride Contents of KFG1901. After dissolution of 0.9024 g of KFG in 100 mL of toluene, 200 μL of water was added to hydrolyze all anhydride functions into carboxylic acid functions. The solution was heated at reflux for 1 h.

(a) IR Measurement. A calibration curve of absorbance A measured by peak height at 1737 cm^{-1} vs concentration was constructed using succinic acid in THF. When hydrolyzed KFG (0.1981 g) was dissolved in THF (10.0 mL), the absorbance of the carbonyl acid group in the polymer solution was 0.2036 at 1737 cm^{-1} . Thus the succinic acid content in Kraton FG 1901X was calculated to be 0.17 mmol/g of polymer (1.7 wt %).

(b) Titration. Carboxylic acid concentration was determined directly by alkali titration using 0.021 N potassium hydroxide in methanol/benzyl alcohol 1/9 (v/v). The indicator used was a solution of phenolphthalein in ethanol. The KFG did not precipitate during titration. By titration, 0.16 mmol of succinic acid/g of KFG1901 was obtained.

Labeling KFG1901 with 9-Phenanthrylmethanol. KFG (2.0 g, 0.34 mmol anhydride) was dissolved in xylenes (10 mL), and a portion of molecule sieves (5A, 1.0 g) was added to the solution. The mixture was heated at $140\text{ }^{\circ}\text{C}$ for 6 h to convert the succinic acid in the polymer to its anhydride form (it was found later that addition of molecule sieves is not necessary for the dehydration of the succinic acid in the polymer). Then 9-phenanthrylmethanol (0.083 g, 0.4 mmol) was added to the polymer solution, and the mixture was heated at $140\text{ }^{\circ}\text{C}$ for another 14 h. The molecule sieves were filtered and the polymer **6-Phe** was precipitated from xylene by pouring the solution into excess methanol (300 mL), and washed first with methanol and then with acetone. Further purification was carried out by precipitation of the polymer from chloroform into methanol several times until no fluorescent molecules were detected at low molar mass by the GPC fluorescent detector.

Methylation of Acid Groups of PheCH₂OH-Labeled SEBS. The polymer **6-Phe** described above (0.3373 g) was dissolved in toluene (30 mL), and methanol (3 mL) was added to the solution. No precipitation occurred. The mixture was heated at reflux for 1 h. After the mixture was cooled to room temperature, trimethylsilyl diazomethane (0.2 mmol, 2.0 M in hexanes) was added. The reaction mixture was stirred overnight under nitrogen at room temperature. The polymer was purified by precipitation from toluene into excess MeOH (150 mL), washed successively with acetone and MeOH, and then dried under vacuum.

Labeling KFG with 9-Phenanthrylmethylamine. The solution of KFG1901 (3.0 g, 0.51 mmol of anhydride) in tetrachloroethylene (15 mL) was heated at reflux for 15 h to convert all the succinic acid in the polymer to its anhydride form. Then 9-phenanthrylmethylamine (0.12 g, 0.52 mmol) was added to the polymer solution. The mixture was refluxed

for another 14 h; the polymer was precipitated from tetrachloroethylene into methanol (300 mL) and washed with methanol and then with acetone. Further purification was carried out by precipitation of the polymer from chloroform into methanol several times until no fluorescence at low molar mass was detected by the GPC fluorescence detector. The polymer obtained was suspended in acetic anhydride (100 mL) and heated at reflux for 2 h. The mixture was cooled to room temperature; the polymer was filtered, washed with acetone, and then dried.

Preparation of Model Compounds. (a) Phenanthrylmethyl Succinate. Succinic acid anhydride (0.20 g, 0.002 mol) and 9-phenanthrylmethanol (0.42 g, 0.002 mol) were dissolved in pyridine (10 mL). The solution was refluxed for 3 h, and then was cooled to room temperature. After water (15 mL) was added, the organic layer was extracted with diethyl ether ($2 \times 10\text{ mL}$). The combined diethyl ether solution was washed with HCl ($2 \times 10\text{ mL}$) and water ($3 \times 10\text{ mL}$) separately and dried with MgSO_4 . Evaporation of the solvent yielded **4** as a yellow solid. Recrystallization of the yellow solid from toluene gave **4** as a yellow crystal, mp $140.0\text{--}141.0\text{ }^{\circ}\text{C}$. $^1\text{H NMR}$ (CDCl_3): δ 2.69 (m, 4H, CH_2CH_2), 5.62 (s, 2H, CH_2O), 7.46–8.52 (m, 9H, Phe–H). EIMS: m/z 308 (M^+ , 32.5), 208 (PheCH₂–OH, 9.7), 191 (PheCH₂⁺, 100). HRMS: m/z calculated for $\text{C}_{19}\text{H}_{16}\text{O}_4$, 308.1048; found, 308.1061. IR (CHCl_3): 1731 cm^{-1} . UV (THF): λ_{max} ($\epsilon = 11\,400$) = 298 nm.

(b) N-9-Phenanthrene Methylsuccinimide. 9-Phenanthrylmethanol (3.0 g, 0.014 mol) was suspended in concentrated HCl (50 mL). The mixture was stirred at room temperature for 72 h and then extracted with ethyl acetate ($3 \times 20\text{ mL}$). The organic layer was washed with water until neutral and then dried over anhydrous MgSO_4 . Evaporation of the solvents gave 9-phenanthrylmethyl chloride (2.5 g, 77%). $^1\text{H NMR}$ (CDCl_3): δ 5.10 (s, 2H, CH_2Cl), 7.57–8.79 (m, 9H, Phe–H). 9-Phenanthrylmethyl chloride (2.5 g, 0.011 mol) was dissolved in toluene at $80\text{ }^{\circ}\text{C}$, and sodium azide (1.8 g, 0.025 mol) and a small amount of tetrabutylammonium iodide were added to the solution. The mixture was stirred at $80\text{ }^{\circ}\text{C}$ overnight. The inorganic salts were removed by filtration; the filtrate was washed with water and dried over MgSO_4 . 9-Phenanthrylmethyl azide was obtained as a yellowish solid after evaporation of the solvent (1.5 g, 57%). $^1\text{H NMR}$ (CDCl_3): δ 4.80 (s, 2H, CH_2N_3), 7.60–8.78 (m, 9H, Phe–H). IR (CDCl_3): 2097 cm^{-1} ($-\text{N}_3$). The azide obtained (0.35 g, 1.5 mmol) was dissolved in dry diethyl ether (15 mL) and added dropwise to a suspension of lithium aluminum hydride (0.12 g, 3.0 mmol) in dry ether (40 mL). Following the addition of the azide, the mixture was kept at reflux temperature for 2 h. Moist ether was then added to destroy the excess lithium aluminum hydride, followed by the addition of water. The inorganic salts were removed by filtration and PheCH₂NH₂ was isolated from the ethereal layer of the filtrate. Recrystallization of the crude product from hexanes and toluene resulted in the formation of needlelike crystals of PheCH₂NH₂ (0.25 g, 82%), mp $106.0\text{--}107.0\text{ }^{\circ}\text{C}$. $^1\text{H NMR}$ (CDCl_3): δ 4.40 (s, 2H, CH_2), 7.60–8.78 (m, 9H, Phe–H). EIMS: m/z 207 (M^+ , 100.0), 191 ($\text{M}^+ - \text{NH}_2$, 31.9). HRMS: m/z calculated for $\text{C}_{15}\text{H}_{13}\text{N}$, 207.1048; found, 207.1047. Succinic acid anhydride (0.10 g, 1.0 mmol) was dissolved in toluene (10 mL) at $80\text{ }^{\circ}\text{C}$, and 9-phenanthrylmethylamine (0.19 g, 1.0 mmol) was added. The succinamic acid rapidly formed crystals. These were dehydrated in boiling acetic anhydride for 2 h to form imide **5**. The imide **5** was purified by recrystallization from dilute ethanol (yield 85%). $^1\text{H NMR}$ ($\text{DMSO}-d_6$): δ 2.52 (s, 4H, CH_2CH_2), 4.40 (s, 2H, $\text{CH}_2\text{--N}$), 7.65–8.80 (m, 9H, Phe–H). UV (THF): λ_{max} = 298 nm, $\epsilon = 12\,800\text{ M}^{-1}\text{ cm}^{-1}$. IR (KBr): 1700 cm^{-1} imide band.

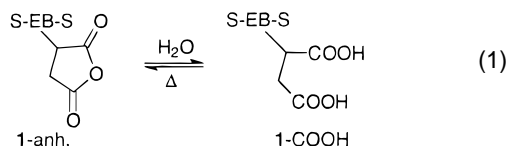
Labeling Content Determination by UV Absorbance Measurements. Standard solutions for UV absorbance measurements were prepared by dissolving different amounts of the model compounds in THF (50.0 mL). Calibration curves of UV absorbance at $\lambda_{\text{max}} = 298\text{ nm}$ vs concentration of each model compound in THF were plotted, and extinction coefficients were calculated from the slopes of the plots ($\epsilon = 11\,400\text{ M}^{-1}\text{ cm}^{-1}$ for the acid ester and $\epsilon = 12\,800\text{ M}^{-1}\text{ cm}^{-1}$ for the

imide). Samples of labeled polymers (13.5 mg each) were dissolved in THF (50.0 mL) and the absorbances at $\lambda_{\text{max}} = 298$ nm were measured. The label content for each was calculated from the corresponding ϵ value of the appropriate model compound.

Results and Discussion

Synthesis and Characterization of the Labeled Polymers. (a) Characterization of Maleated SEBS (Krayton FG 1901X, KFG). The Krayton block copolymers sold by Shell are relatively well-characterized materials with a narrow size distribution. In the experiments described here, we carry out reactions on Krayton FG 1901X. This polymer has $M_w = 97\,700$, $M_n = 84\,400$, and a block ratio of 1:5:1 as described by the manufacturer, with a reported degree of maleation of 2 wt %. We begin by characterizing the anhydride group content of the polymer as the basis for judging the extent of conversion of the anhydride to another dye-containing functionality.

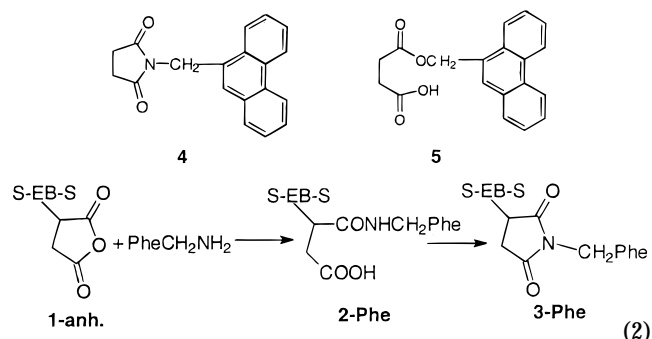
The IR spectrum of SEBS itself (e.g., Krayton G 1652) shows no absorbance in the carbonyl region. However, the strong absorbance at 1712 cm^{-1} in the IR spectrum of maleated SEBS (KFG, film cast from THF) shows that prolonged storage led to hydrolysis of about 95% of the anhydride groups in SEBS to the succinic acid form (eq 1). It is well-known that heating the polymer in a high boiling solvent (xylene, tetrachloroethylene) at $120\text{ }^\circ\text{C}$ for several hours results in the complete conversion of the succinic acid functionality back to the anhydride in the polymer. Under these circumstances, we find bands in the IR spectrum characteristic of the anhydride (strong absorbance at 1786 cm^{-1} and weak absorption at 1864 cm^{-1}).



We determined the functional group content of the triblock polymer in two ways, IR analysis and titration. For both approaches, the anhydride groups on the polymer were hydrolyzed completely to the diacid form. For the IR measurement, a calibration curve $A = 61.02C$ (mol/L) was constructed using succinic acid in tetrahydrofuran (THF) as a standard. The polymer in toluene solution was titrated with KOH in a mixture of benzyl alcohol and methanol, as described by Sclavons et al.⁸ By IR, we found 0.17 mmol of succinic acid/g of KFG, corresponding to 1.7 wt % anhydride groups. By titration, we found 0.16 mmol of succinic acid/g of KFG. On the basis of M_n , each polymer contains on average 14 anhydride groups, all localized in the EB block.

(b) Reaction of the Polymer with PheCH₂NH₂. All reactions with maleated SEBS followed a protocol in which the polymer was first heated in a high boiling solvent to convert all of the diacid groups to the anhydride. Addition of PheCH₂NH₂ to a solution of this polymer (**1**) in dry xylene solution results in rapid formation of the succinamic acid derivative **2-Phe**, which can be further dehydrated to the succinimide **3-Phe** by addition of a small amount of acetic anhydride to the reaction mixture (eq 2). Alternatively, the polymer **2-Phe** can be precipitated and heated as a suspension in acetic anhydride. Both approaches yield the dye-labeled polymer **3-Phe**. The level of dye incorporation

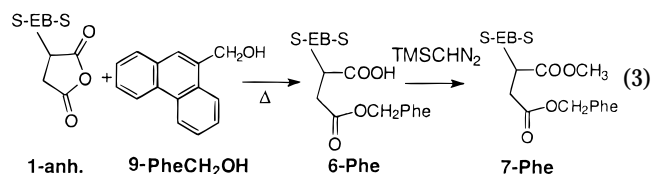
was determined by comparison of the UV absorption spectra of the dye-labeled polymer with that of *N*-phenanthrylmethyl succinimide **4**, prepared as a model compound. The label content of **3-Phe** was calculated to be 0.15 mmol PheCH₂ groups/gram of polymer, corresponding to 90% conversion of the anhydride groups to the imine derivative.



In many fluorescence experiments, one would like a polymer containing a level of dye incorporation less than that of the anhydride content of the polymer. One can achieve any level of dye incorporation by using limiting amounts of dye-CH₂NH₂.

(c) Reaction of the Polymer with PheCH₂OH. The reaction of anhydride groups with alcohols to yield the monoester is very sensitive to the concentration of alcohol derivative in the solution and to the presence of adventitious moisture. Addition of a small excess of PheCH₂OH to a solution of the polymer **1** in dry xylene led to the slow conversion to the monoester at $140\text{ }^\circ\text{C}$ (eq 3). Under these conditions, the reaction did not go to completion over 14 h. Unreacted anhydride groups were detected in the IR and some unreacted PheCH₂OH was detected in the gel permeation analysis of the precipitated polymer. The extent of reaction appears to depend on the time of reaction and the concentration of dye-CH₂OH derivative added.

The level of dye incorporation in the polymer was determined by comparison of the UV absorption spectra of the dye-labeled polymer with that of the model compound phenanthrylmethyl succinate **5**. The label content of the polymer acid ester **6-Phe** obtained in this way was calculated to be 0.053 mmol of PheCH₂ groups/g of polymer. Thus, under these conditions, only about 30% anhydride group in the original polymer reacted with 9-phenanthrene methanol to form the acid ester. The lower reactivity of alcohols compared to amines with SEBS-anhydride was previously reported by Taha and Frerejean.³ In order for us to compare the fluorescence properties of SEBS labeled through reaction with alcohol and amine, a polymer containing a low label content of PheCH₂-imine (0.046 mmol of dye/g of polymer) was also synthesized by reducing the amount of PheCH₂NH₂ in the labeling reaction.



Polymers containing free -COOH groups tend to associate in nonpolar media. As we show below, this association process complicates the fluorescence behav-

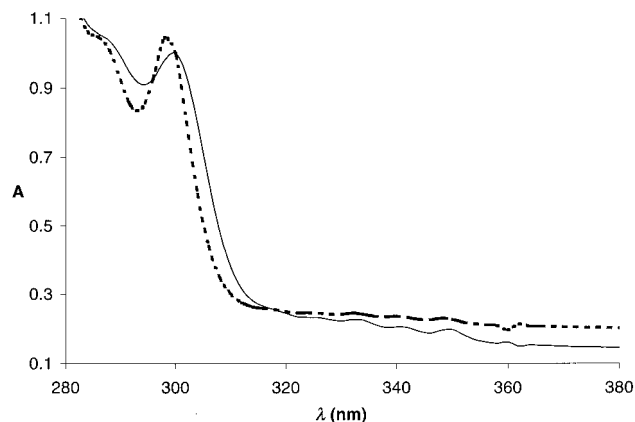


Figure 1. UV spectra of **3-Phe** (—) and **6-Phe** (---) polymer films cast from cyclohexane.

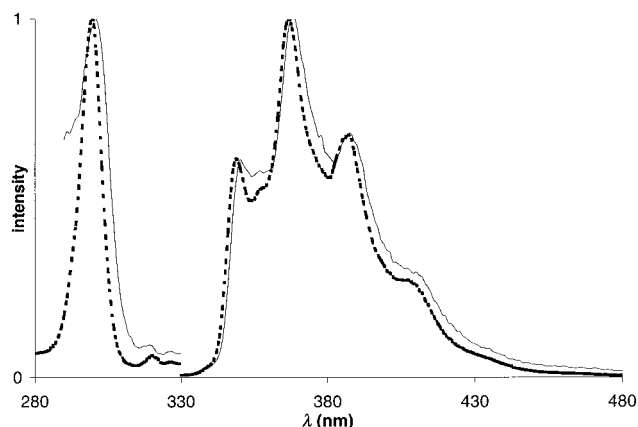


Figure 2. Normalized fluorescence excitation and emission spectra of model compound **4** in THF (---) and polymer **3-Phe** in toluene (—).

ior of the dye. To overcome this problem, we sought a strategy for converting the free $-\text{COOH}$ groups to the corresponding methyl ester. We first exposed the polymer to excess methanol to convert the unreacted anhydride to the monomethyl ester and then treated the polymer in situ with trimethylsilyldiazomethane ($\text{TMS}-\text{CHN}_2$). $\text{TMS}-\text{CHN}_2$ is a stable and safe substitute for the hazardous diazomethane. In the presence of methanol, $\text{TMS}-\text{CHN}_2$ will effectively carry out the complete methylation of carboxylic acids (eq 3).⁹ The IR spectrum of the product **7-Phe** showed that all the acid groups were methylated.

Spectroscopic Properties of the Labeled Polymers. The UV spectra of the phenanthrylmethyl-imide- and phenanthrylmethyl-ester-labeled polymers (**3-Phe** and **6-Phe**), in films cast from cyclohexane, are similar but not very distinctive, as shown in Figure 1. One sees the characteristic (0,0) band of the $S_2 \leftarrow S_0$ (L_a) transition of phenanthrene at around 300 nm superimposed on the tail of adsorption due to the PS component of the polymer. The tiny peaks in the UV spectrum in the region 330–350 nm are due to the weaker L_b transition of the phenanthrene chromophore. The normalized fluorescence excitation and emission spectra of the model compound **4** and polymer **3-Phe** are shown in Figure 2. They are very similar and have features typical of phenanthrene fluorescence. Careful inspection indicates an underlying background fluorescence at shorter wavelengths, which we imagine is due to the PS portion of the polymer. We will later argue that the difference between the emission of model

Table 1. Decay Analysis of **3-Phe** in Solution

solvent	τ_{short}	τ_{long}	$w_{\text{short}},^a \%$
THF	13.9	43.9	5
toluene	13.9	44.1	5
cyclohexane	13.5	45.7	7

^a Weight of the shorter component calculated according to eq 4.

compound **4** and polymer **3-Phe** at longer wavelengths results from Phe self-quenching, caused by local aggregation of the imide groups.

(a) The Phe- CH_2 -Imide Derivative in Solution. The model compound **4**, *N*-(phenanthrylmethyl)succinimide, exhibits an exponential decay in deaerated solution in THF, with a lifetime of $\tau_0 = 44.5$ ns and $\chi^2 = 1.05$. The corresponding labeled polymer **3-Phe** in THF solution has a nonexponential decay. For comparison with the polymer labeled via an ester group, we examine the **3-Phe** polymer sample with a similar label content. When this decay was fitted to a sum of exponential terms, there appeared to be three components to the decay: a long-lived term with a lifetime comparable to unquenched phenanthrene, a short component with a lifetime of ca. 2 ns, and an intermediate component with a lifetime on the order of 14 ns. Normally one does not place much confidence in attributing the parameters of a three-exponential fit to individual components of the system. Here, a somewhat deeper analysis is possible.

The SEBS triblock copolymer contains 28 wt % polystyrene. We found that polystyrene itself (0.018 M in THF), when excited at 300 nm and monitored at 350 nm, has a weak fluorescence with a lifetime of 2.2 ns. In systems where the ratio of Phe to PS is small, the contribution of PS fluorescence will introduce a fast component with a lifetime of about 2 ns into the measured decay profile. Introduction of an aliquot of PS into a solution of **3-Phe** perturbs the fluorescence decay profile to amplify the magnitude of the 2 ns component, but the remainder of the profile remains parallel to that of the labeled polymer itself. The same behavior can be observed when we add nonlabeled SEBS polymer to a solution of **3-Phe** in toluene. A useful strategy for analyzing the decay profile of the Phe-labeled polymer is to subtract the signal due to emission of the same concentration of the unlabeled polymer, measured under conditions identical to that of the labeled polymer. Under these circumstances, the 2 ns component is removed, but the 14 ns component and the long-lived 44 ns “unquenched” component remain.

Experiments were carried out in solvents (THF, toluene, and cyclohexane) that differ in their polarity and polarizability. The results are shown in Table 1. In all three solvents $\tau_{\text{short}} \approx 14$ ns and the longer lifetime is also approximately constant ($\tau_{\text{long}} \approx 44$ ns), although slightly longer in cyclohexane. The weight w_i , with which a lifetime τ_i with preexponential factor A_i contributes to a n -exponential decay, can be defined as

$$w_i = \frac{A_i \tau_i}{\sum_{j=1}^n A_j \tau_j} \quad (4)$$

In cyclohexane, the weighting of the short component w_{short} is somewhat larger than for the other solvents. We will argue in a subsequent section of this paper that the short component is due to local aggregates that

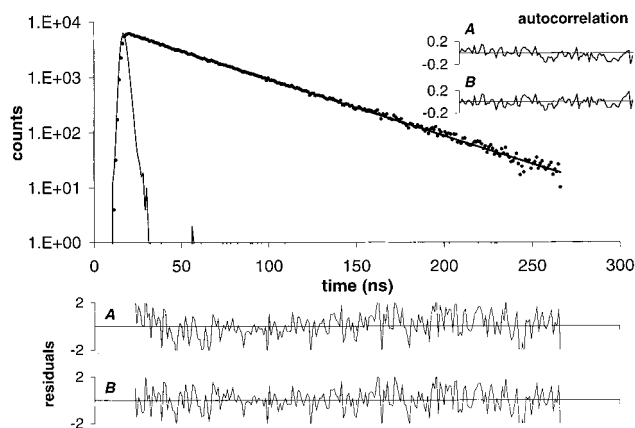


Figure 3. Fluorescence decay profile of polymer **6-Phe** in THF, corrected for the PS component using the fluorescence decay of a sample of unlabeled SEBS as described in the text. Weighted residuals and autocorrelation of the residuals of the fits with one (A) and two (B) exponential functions.

bring Phe groups into proximity and lead to self-quenching. The long component is attributed to isolated Phe groups whose emission is not quenched in this way.

(b) The Phe-CH₂-Ester Derivatives in Solution. Phe-CH₂-OH and its simple ester derivatives give exponential decays in solution with lifetimes close to 44 ns. We carefully compared the fluorescence decay of the polymer **6-Phe** (Phe-SEBS-COOH) in toluene (polymer = 1.2 g/L, Phe = 0.053 mM) before and after subtraction of the emission of unlabeled SEBS. The differences are impossible to see by eye, but before the correction, a weak 2 ns component in the decay is detected by the computer when we use a three-exponential fit. In Figures 3 and 4 we show the corrected decays for solutions of the polymer in THF and in toluene, respectively, with the residuals and autocorrelation of fitting the decays with one and two exponential terms. The fits to a single exponential are acceptable, although they show some problems in the autocorrelation of the residuals. The fitted lifetimes are 42.4 ns in THF and 42.1 ns in toluene. When fitted with two exponential terms, a short component of 15 ns appears and the quality of the fitting is improved. For the polymer in both THF and toluene, τ_{long} increases slightly to 43.2 ns. The weight of the short 15 ns component is $w_{\text{short}} = 2\%$ in THF and $w_{\text{short}} = 4\%$ in toluene.

In the absence of other evidence, the differences cited above might be very difficult to interpret. What is of striking significance to us is that the fluorescence decay of the fully methylated polymer **7-Phe**, in toluene solution (Figure 5), is well fitted by a single-exponential term with a lifetime of 45.5 ns ($\chi^2 = 1.16$). With two exponential terms, the computer still finds a short component with $\tau_{\text{short}} = 15$ ns, but the weight of this term is negligible ($w_{\text{short}} < 1\%$). All of these results are consistent with a small amount of phenanthrene fluorescence self-quenching, brought about primarily through dimerization of the free -COOH groups in the polymer. This interaction is removed by conversion of the carboxylic acid groups to the methyl ester.

The acid groups in the polymer may play an important role because they can form hydrogen-bonded dimers which bring pairs of phenanthrene groups into proximity. The self-quenching which results contributes to the short lifetime species. In the more polar THF, the interaction among the acids groups of Phe-SEBS-

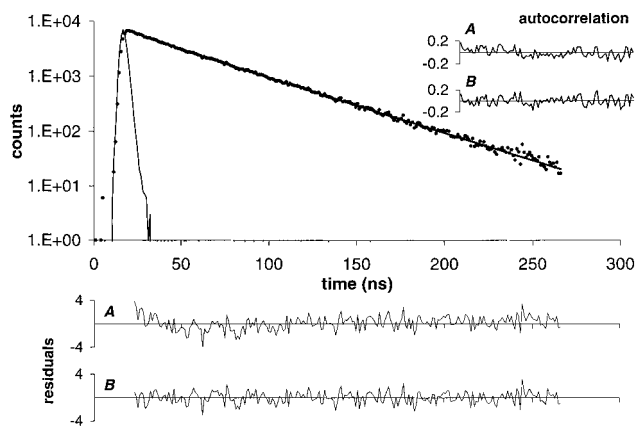


Figure 4. Fluorescence decay profile of polymer **6-Phe** in toluene, corrected for the PS component of the emission. Weighted residuals and autocorrelation of the residuals of the fits with one (A) and two (B) exponential functions.

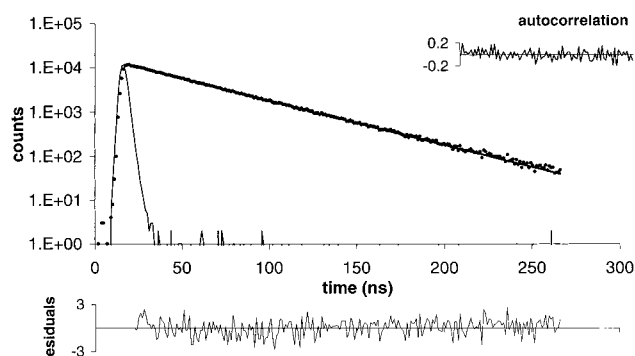


Figure 5. Fluorescence decay profile of polymer **7-Phe** in toluene, corrected for the PS component of the emission. Weighted residuals and autocorrelation of the residuals of the fit with one exponential function.

COOH is weaker and the derivation from monoexponentiality is smaller ($w_{\text{short}} = 2\%$) than that in toluene ($w_{\text{short}} = 4\%$).

The effect of phenanthrene self-quenching is apparent mainly in the dynamic fluorescence measurements, from the appearance of the 14 ns component, also observed by Zachariasse et al.⁸ for diphenanthryl propanes in solution. Phenanthrene self-quenching does not make a major contribution to the emission spectra of the labeled polymers (cf. Figure 2). However, a small but observable blue shift can be observed in the fluorescence spectra, again similarly to the one observed by Zachariasse et al.⁸

(c) Fluorescence Decay Profiles in the Solid State. Fluorescence decay profiles of the labeled polymer samples were measured in solid films prepared by solvent casting. It is difficult to correct the emission of a solid sample for the contribution of emission from the polystyrene component of the SEBS. One can, of course, use a film of unlabeled polymer as a reference, but the count rate in the detection is very sensitive to the orientation and optical quality of the films. On the other hand, it is possible to correct for the contamination of the decays by PS emission using a technique similar to the one proposed by Martinho et al.¹⁰ for correction of light scattering contamination. In here, we can simply add an extra exponential term to the decay equation, with the lifetime fixed and equal to the one obtained for the unlabeled polymer. The weight of this term gives

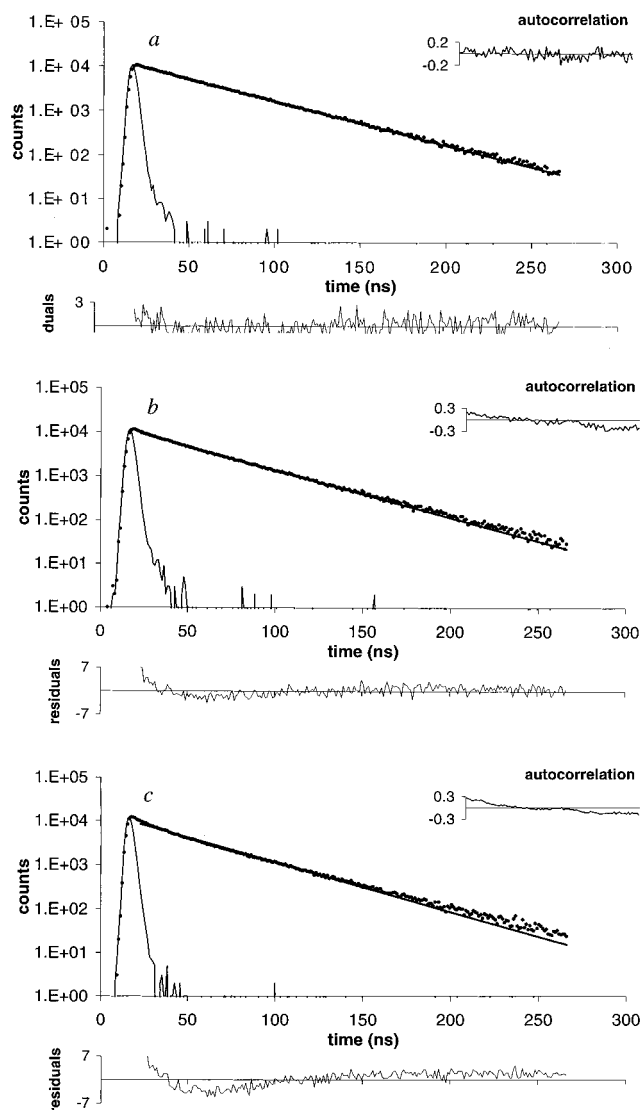


Figure 6. Exponential fits of the phenanthrene fluorescence decay profiles of three polymer film samples: (a) **7-Phe**; (b) **6-Phe**; (c) **3-Phe**.

us an indication of the importance of the PS emission relative to that of the Phe emission.

The fluorescence decay of film samples of the fully methylated Phe-SEBS polymer **7-Phe** gave reasonable fits to a single-exponential term (Figure 6a), with a lifetime of 44.0 ns. The value of $\chi^2 = 1.1$ indicates an almost perfect fit. The deviation from the single-exponential behavior occurs at short times, and we believe that the major contribution to the short component in the decay comes from emission of the PS in the block copolymer. Indeed, from a two exponential fit we determine that the total weight of the $\tau_{\text{short}} = 2.2$ ns component associated with PS emission is only about $w_{\text{short}} = 2\%$. Therefore, this decay profile fits well enough to a single-exponential term that one can use this polymer as the donor component in energy transfer experiments.

The decay curves of Phe-SEBS-COOH (**6-Phe**) and Phe-SEBS-imine (**3-Phe**) exhibit strong deviations from an exponential form, as seen in parts b and c of Figure 6. Good fits could only be obtained using three exponential terms. The weight of the short $\tau_{\text{short}} = 2.2$ ns component associated with PS emission is again about $w_{\text{short}} = 2\%$, but the component around 15 ns,

which we attribute to Phe aggregation increases its weight from about 7% in **6-Phe** to 15% in **3-Phe**. From these results, we establish that dye aggregation leading to significant self-quenching of the phenanthrene is more prominent in the solid state than in polymer solutions, but can still be avoided by methylation of the Phe-CH₂-ester derivatives.

Implications for Future Work

In carrying out experiments on labeled polymers, one often assumes that the presence of the label does not perturb the properties of the polymer. When the label is a fluorescent dye, one sometimes needs to assume that the attachment of the dye to the polymer does not perturb the fluorescence of the dye. The proper interpretation of energy transfer experiments often depends on this assumption. In many of these experiments, for mixtures of donor- and acceptor-labeled polymer, one interprets deviations from exponentiality in the donor decay profiles in terms of energy transfer to the acceptor. For this interpretation of the data to be correct, it is essential that the donor decay profile in the absence of acceptor be exponential.

It is also essential that the dyes in the system do not aggregate. The label is introduced as a tracer for one of the components of a polymer system. Data interpretation relies on the assumption that the distribution of dyes in the system measures the distribution of polymer segments. If the dyes have a proclivity to aggregate, this assumption breaks down.

We have taken great care in the experiments reported here to show that the tendency of simple aromatic dyes to aggregate when attached to a polymer can be seriously affected by the nature of the functional groups used to attach the dye to the polymer. We were stimulated in this investigation by the reports of Farinha et al.¹¹ and Duhamel et al.¹² that pyrene, attached both to PS as an amide derivative and to polyolefins as a succinimide derivative, tend to aggregate in solution. This aggregation is easier to detect for pyrene because associated pyrene often fluoresces as an excimer.

Phenanthrene does not form excimers except under unusual circumstances,⁸ and normally it is insensitive to self-quenching. Its tendency for exhibiting simple exponential decays in a variety of polymer media make it the dye of choice for energy transfer measurements. Our finding here that Phe fluorescence in the presence of polystyrene is contaminated by independent emission from the polystyrene introduces a note of caution in the quantitative interpretation of Phe decay profiles in systems containing PS. We have reported such studies for poly(styrene-*b*-methyl methacrylate) (PS-PMMA) diblock copolymers labeled at the junction with Phe and anthracene.¹³ More recent studies with poly(styrene-*b*-butyl methacrylate) (PS-PBMA), as yet unpublished,¹⁴ did not fit well to the models we employed previously. The ratio of styrene units to Phe is much higher in the PS-PBMA samples. We now suspect that fluorescence decay data from these polymers must be corrected for the PS emission before they can be interpreted. We are currently investigating the magnitude of this effect in the PS-PMMA polymers we have previously described. In those polymers, the PS emission is weak. We believe that in polymers where the ratio of PS/Phe is not very large the correction will be small and may even be negligible.

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