Fluorescence Study of the Coil−Globule Transition of a Poly(ϵ-caprolactone) Chain Labeled with Pyrenes at Both Ends

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Introduction

The conformation of a polymer chain in solution depends on both polymer (composition and structure) and solvent.[1,2] In a good solvent polymer adopts an extended coil conformation owing to the repulsive polymer segment−segment interactions (excluded-volume effect). When the quality of the solvent becomes worse, the coil is compressed, leading to the dominance of attractive segment−segment over segment−solvent interactions.[3] A compressed chain in poor solvents can evolve to a globular state once the attraction between segments is strong enough to force "condensation" of the polymer chain on itself.

The interest in the study of coil−globule transitions arises not only from their importance in biology (enzymatic activity and protein folding,[4,5] DNA packing[6]) but also from their fundamental role in the understanding of polymer segment−segment and segment−solvent interactions.[3]

The coil−globule transition theory was developed following the initial ideas of Flory[7] and Lifshitz[8] and has been recently reviewed by Grosberg and Kuznetsov.[3,9] It has been shown that the experimental observables generally used to follow the transition (gyration radius, intrinsic viscosity, hydrodynamic radius) depend on chain stiffness, ideal Gaussian coil state size, and reduced temperature $\tau_r = (T - \theta)/\theta$.[9,11] Long stiff chains go through a sharp transition just below the Flory θ temperature, while in the case of flexible chains the transition is smooth and includes the θ point in its temperature interval.[3] Following de Gennes,[10] the collapse kinetics of a flexible chain begins with the formation of successive crumples along the polymer backbone, resulting in a "new chain" that seems to be shorter and thicker. The final globule resembles a tight Gaussian random coil that attains equilibrium after the penetration of the chain ends through the crumples. For stiff chains, the globules are not spherical and show, instead, a well-defined ordered structure imposed by chain rigidity.[12]

The coil−globule transition has been studied using mainly light scattering[14,16,18,22] and intrinsic viscosity measurements.[17] Aggregation is the main concern in these studies, given that it can occur in the same temperature range as globule formation.[13,14]

Many experimental results on coil−globule transition have been performed in polystyrene solutions, either below the upper critical solution temperature (UCST)[2,14,16−18] or above the lower critical solution temperature (LCST).[19] Unfortunately, since polystyrene is stiff and light scattering and viscosimetry can only be performed for high molecular weight chains and relatively concentrated solutions, the transition could not be detected without interference of aggregation.[14]

Another interesting and well-studied system is poly(*N*-isopropylacrylamide) (PNIPAM) in water,[20−23] where a coil−globule transition occurs near the LCST at 32 °C. Wu et al.,[22] using light scattering measurements, were able to detect thermodynamically stable globules without formation of aggregates, but only in a very narrow temperature range. The peculiar behavior of PNIPAM in water is due to the variation of the partition of water hydrogen bonds between water molecules and polymer segments with temperature.

In the present study, fluorescence is used to prove that temperature quenching induces a coil−globule transition in a poly(ϵ-caprolactone) chain ($M_n = 19\,200$; $M_w/M_n = 1.07$) in THF. A stable globule was detected around 0 °C, prior to aggregation which was only observed at temperatures lower than −30 °C. This was only made possible due to the high sensitivity of fluorescence that allowed the use of very diluted solutions. These results confirm the theoretical prediction that, for diluted solutions of flexible polymer chains, the coexistence temperature should occur below their coil−globule transition.[15]

As far as we know, this is the first time that thermodynamically stable globules were observed in such a large (∼30 °C) temperature interval.

Experimental Section

Instrumentation. Fluorescence spectra were recorded on a SPEX Fluorolog F112A fluorimeter at several temperatures using a cryostat from Oxford Instruments (DN 1704) that allows the temperature control within ±0.5 °C. The fluorescence spectra were recorded between 370 and 600 nm using 330 and 360 nm as excitation wavelengths. The NMR spectra of the polymer were recorded in a 300 MHz Varian spectrometer in $CDCl_2CDCl_2$. The absolute values of M_n and M_w of the polymer were obtained in a Waters 150 CV GPC operating at a flow rate of 1.0 mL/min and 35 °C. Three Waters Styragel HR1, HR3, and HR4 columns in series, coupled to a refractive index and a viscosimetry detector, were used. Time-resolved picosecond fluorescence intensity decays were obtained by the single-photon timing technique by laser excitation at the wavelength of 330 nm. The system consists of a mode-locked Coherent Inova 440-10 argon ion laser that synchronously pumped a cavity dumped Coherent 701-2 DCM dye laser, delivering 5−6 ps pulses at a repetition rate of 460 kHz. The fluorescence was observed using a polarizer at the magic angle, and the scattered light was eliminated by a cutoff filter. Detection was done by passing the light through a depolarizer and then through a Jobin-Yvon HR320 monochromator with a grating of 100 lines/mm. The fluorescence was detected by a Hamamatsu 2809U-01 microchannel plate photomultiplier. The instrument response function had an effective fwhm of 35 ps. To eliminate any interference of monomer emission, the excimer decay was recorded at 520 nm and a cutoff filter (λ_{cutoff} = 500 nm) was used at the entrance of the monochromator. Decay curve analysis was performed by a nonlinear least-squares method based on the Marquard algorithm.[26]

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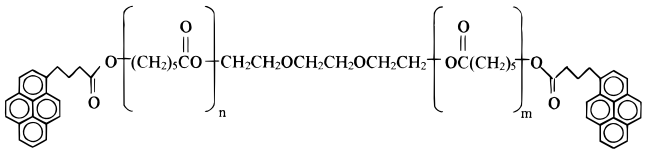
Polymer Characterization. The synthesis of the poly(ϵ-caprolactone) terminated at both ends with OH groups and its pyrene labeling was described by Sosnowski et al.,[27] originating the molecular structure shown in Scheme 1.

Scheme 1



The degree of polymerization determined by proton NMR spectra is 166, which corresponds to a molecular weight of 19 600. Using the ratio of the areas of the ^{1}H NMR bands of the terminal group and the repeating unit, a degree of pyrene labeling of 1.96 was found. This indicates that, within the experimental error, all polymer chains are both ends pyrene labeled. The GPC absolute value molecular weight of M_n = 19 200 agrees very well with the NMR data. The polymer chain has a very narrow molecular weight distribution (M_w/M_n = 1.07) obtained by GPC.

Sample Preparation. A 1.0×10^{-6} M polymer solution was prepared in tetrahydrofuran (THF). THF was freshly distilled under argon and its purity verified by fluorescence at the excitation wavelengths. The solution was degassed by the freeze−pump−thaw technique (six cycles), the final pressure being lower than 10^{-5} bar. The solution was kept in the dark between measurements. The 1-pyrenebutyric acid from Aldrich (97% pure) was used as received. A 1.0×10^{-6} M solution in THF was prepared and degassed as described for the polymer.

Results and Discussion

Figure 1A shows fluorescence spectra of both the poly(ϵ-caprolactone) chain (excitation at 330 and 360 nm) and a model compound, 4-(1-pyrenebutyric acid) (excitation at 330 nm) in THF, at 50 °C.

The polymer fluorescence spectrum shows, besides the pyrene monomer fluorescence, a weak broad band centered at 480 nm that corresponds to the emission of the pyrene excimer. The fluorescence spectrum by excitation at 360 nm shows, superposed on the monomer emission, three bands at λ_{max} ~382, 399, and 418 nm attributed to the emission of a pyrene dimer, which has already been observed for pyrene in silica glasses,[28] Langmuir−Blodgett films,[29] and sol−gel systems.[30] Figure 1B shows that the excitation spectrum recorded at the emission wavelength of 520 nm does not superpose the one at 376 nm, thus confirming the presence of pyrene dimers. Pyrene dimerization was also observed in a 10^{-3} M solution of pyrene in THF and probably results from the poor solvation of THF to pyrene due to the large differences in polarizability between both molecules.

An excimer can be formed either by a dynamic diffusion process involving the encounter of an excited pyrene molecule at one chain end with the other located at the other end or by a "static" process from the rotation of an excited pyrene dimer to form the excimer. As the polymer chains are very long, the dynamic contribution must be negligible.

Figure 2 shows the plot of the excimer (520 nm) to monomer (376 nm) fluorescence intensity ratio versus temperature, by excitation at 360 nm where pyrene absorption is very low. At this wavelength, the fluorescence intensity ratio is very sensitive to the processes of excimer formation from pyrene dimers.

The fluorescence intensity ratio remains practically constant with temperature from 50 to ≈0 °C and

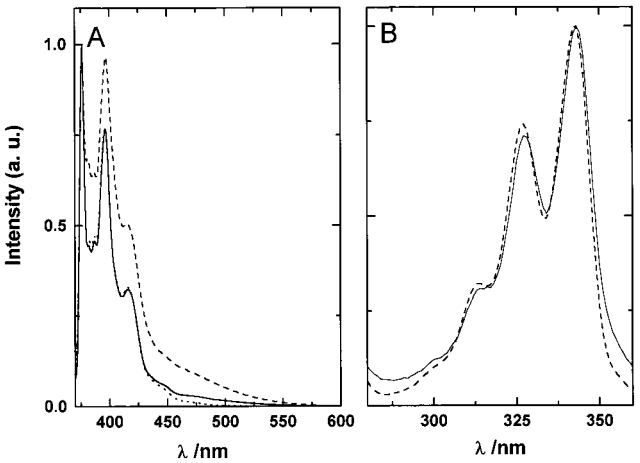


Figure 1. (A) Fluorescence spectra of a 1.0×10^{-6} M solution of poly(ϵ-caprolactone) in THF at t = 50 °C, using two excitation wavelengths: (—) λ_{ex} = 330 nm; (– – –) λ_{ex} = 360 nm. (• • •) Fluorescence spectrum of a 1.0×10^{-6} M solution of 4-(1-pyrenebutyric acid) in THF by excitation at λ_{ex} = 330 nm. (B) Excitation spectra of a 1.0×10^{-6} M solution of poly(ϵ-caprolactone) in THF, at t = 50 °C: (- - -) λ_{em} = 376 nm; (—) λ_{em} = 520 nm.

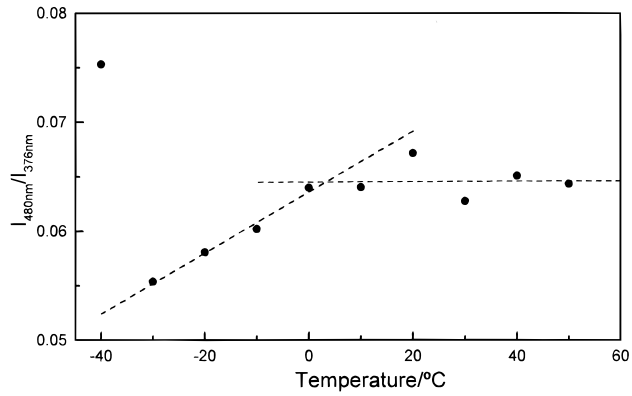


Figure 2. Plot of the excimer (λ_{em} = 520 nm) to monomer (λ_{em} = 376 nm) fluorescence intensities ratio with temperature, of a 1.0×10^{-6} M solution of poly(ϵ-caprolactone) in THF by excitation at λ_{ex} = 360 nm.

decreases afterward between 0 and −20 °C. This small variation cannot be due to experimental errors and points to a change in polymer conformation around 0 °C, attributed to the formation of polymer globules that entrap pyrene dimers. With the entrapment, the dimer to excimer conversion becomes slower since the polymer chain imposes restrictions to the rotation of the pyrene moieties, impeding the formation of the sandwich excimer configuration. For temperatures lower than −30 °C, the aggregation of globules leads to the overlap of different chains with the consequent increase on the fluorescence intensity ratio due to the formation of intermolecular excimers.

Figure 3 shows the monomer (A) and excimer (B) fluorescence decay curves of the poly(ϵ-caprolactone) polymer solution at 50 and −10 °C (above and below coil−globule transition temperature, respectively).

The excimer decay curve at +50 °C can be fitted by the sum of three exponentials (τ_1 = 2.0 ns, τ_2 = 53.9 ns, τ_3 = 186 ns), without any rise time component in this time range. This confirms that excimers are formed very efficiently by a static process from the excited dimers. The monomer decay can also be fitted by a sum of three exponentials (τ_1 = 5.0 ns, τ_2 = 64.3 ns, τ_2 = 197 ns),

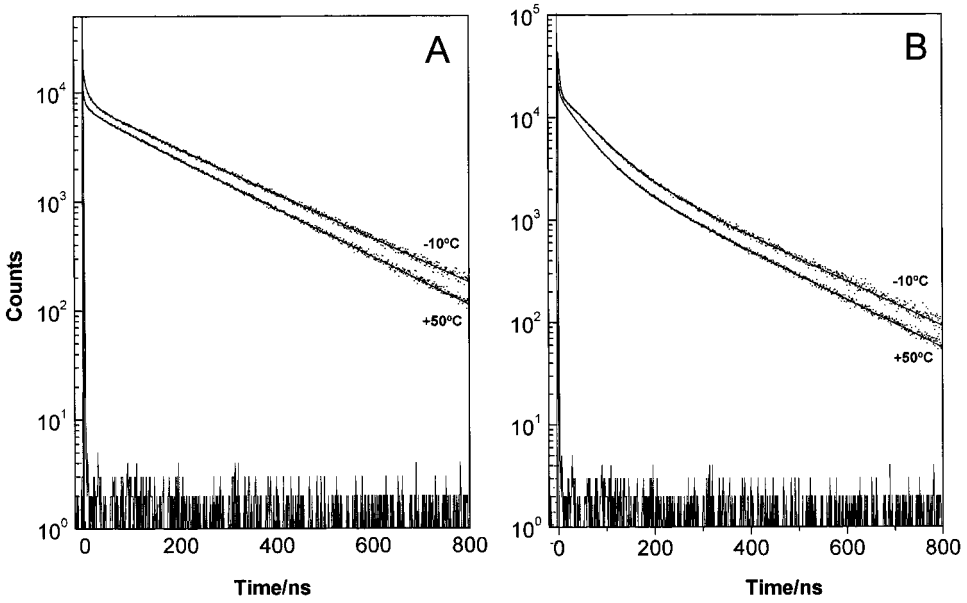


Figure 3. Monomer (A) and excimer (B) fluorescence decay curves of a 1.0×10^{-6} M solution of poly(ϵ-caprolactone) in THF at +50 and −10 °C, by excitation at 330 nm. (A) $\lambda_{em} = 376$ nm; (B); $\lambda_{em} = 520$ nm.

Table 1. Monomer and Excimer Decay Curves Parameters of a 10^{-6} M Solution of Poly(ϵ-caprolactone) in THF at 50 and −10 °C, by Excitation at $\lambda_{exc} = 330$ nm[a]

	a_1	τ_1/ns	a_2	τ_2/ns	a_3	τ_3/ns	a_4	τ_4/ns	χ^2
				$t = 50$ °C					
monomer	0.0 4	5.0	0.03	64.3	0.46	197			1.0
excimer	0.14	2.0	0.29	53.9	0.12	186			1.1
				$t = -10$ °C					
monomer	0.10	3.1	0.09	16.6			0.16	210	1.0
excimer	0.63	3.7	−0.55	29.0	0.20	58.3	0.07	200	1.1

[a] a_i = preexponential factor; τ_i = decay time.

with a contribution of the long component to the steady-state fluorescence spectrum of 95%. Table 1 summarizes the results of decay curves analysis. The 197 ns component coincides exactly with the 1-(4-pyrenebutyric acid) lifetime in the same conditions and shows that most of the excited pyrenes decay with their intrinsic lifetime. This demonstrates that most of the polymer chains adopt an extended coil configuration that prevents the encounter of pyrenes located in opposite chain ends, during the pyrene lifetime. Note that this component cannot be attributed to one end pyrene labeled chains, as NMR results show that both chain ends were pyrene terminated. The other components are associated with those cyclized chains forming ground-state pyrene dimers. These components are different from the ones recovered from the excimer decay, indicating that the kinetics are complex and/or that the small contribution to the monomer decay curve impeaches its correct evaluation.

A very short ~200 ps rise-time component was also detected in both monomer and excimer decay curves, using a smaller time range, which shows a very fast dimer to excimer/monomer conversion. Both excimer

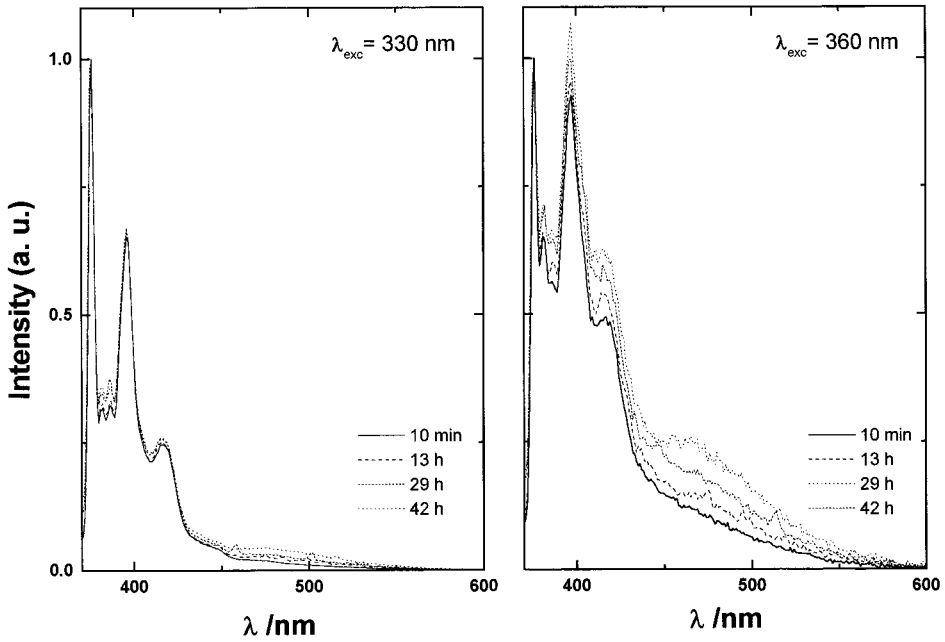


Figure 4. Normalized fluorescence spectra of a 1.0×10^{-6} M solution of poly(ϵ-caprolactone) in THF, by excitation at 330 and 360 nm at several times after temperature quenching to −40 °C.

and monomer decays remained almost constant when the temperature was lowered until 0 °C. Below this temperature a rise-time component appears in the excimer decay curve. This is shown in Figure 3B for the decays obtained at −10 °C. The decay is very complex and can only be fitted with a sum of four exponentials. The monomer decay can be fitted by a sum of three exponentials with a dominant long component of 210 ns, still attributed to the emission of isolated pyrenes (see Table 1). The global analysis of the monomer and excimer decay curves is not possible, suggesting very complex excited-state kinetics, involving a monomer, one or two excimers, and dimers. Similar kinetics has already been observed, although in different media.[28−30] The shape of the decay curves remained constant in time for more than 50 h, showing that at this temperature polymer aggregation does not occur.

The formation of dynamic excimers detected by the rise-time component on the excimer decay is only possible when the pyrenes at both chain ends are at short distances from one another. This becomes possible when the polymers form globules where pyrenes are entrapped (in the form of dimers or separated from each other). Those pyrenes that are separated by small distances can then form intramolecular excimers. This confirms our suggestion from steady-state measurements that a globular state was reached around 0 °C.

The dynamic excimer formation should increase the I_E/I_M ratio. However, this was not observed (see Figure 2) since the slowing down of the dimer to excimer conversion compensates for the dynamic contribution.

The time needed to obtain the equilibrium globule must be smaller than the value of 10 min reported by Chu et al.[14] for a much higher molecular weight and stiff polystyrene chain. This shows that, within our experimental conditions, we cannot observe the kinetics of equilibrium globule formation. As fluorescence spectra and decay curves were invariant in time between 0 and −30 °C, we conclude that stable globules were observed, without aggregation interference. This interval is much larger than the one detected for PNIPAM thermodynamically stable globules in water.[20]

For temperature quenching below −30 °C, the global fluorescence intensity decreases and the excimer to monomer fluorescence intensity ratio substantially increases with time. Figure 4 shows the normalized fluorescence spectra in time for both excitations at 330 and 360 nm, after temperature quenching to −40 °C.

This all shows that globules begin to aggregate, forming clusters that grow in time and finally precipitate.

Conclusions

The coil−globule transition induced by temperature lowering of a pyrene labeled poly(ϵ-caprolactone) chain in THF was followed by fluorescence. Globules were detected at 0 °C both by a break in the plot of excimer to monomer fluorescence intensity ratio with temperature and by the appearance of a rise-time component in the excimer decay. From 0 to −30 °C, the fluorescence spectra and decays were invariant in time for more than 50 h. This proves that the coil−globule transition was detected without aggregation interference. The transition temperature can be slightly influenced by the pyrene labeling. Below −30 °C, the excimer to monomer fluorescence intensity increases with time, owing to globule aggregation. This was the first time that polymer stable globules were observed in such a large interval (∼30 °C), with no polymer aggregation interference. This behavior is a consequence of the flexibility of the polymer and the use of very diluted polymer solutions.

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