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Fluorophore Exchange Kinetics in Block Copolymer Micelles with Varying Solvent-Fluorophore and Solvent-Polymer Interactions

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Abstract

Fluorescence spectroscopy was employed to characterize the kinetics of guest exchange in diblock copolymer micelles composed of poly(ethylene oxide-*b*- ϵ -caprolactone) (PEO-PCL) diblock copolymers in water/tetrahydrofuran (THF) mixtures which encapsulated fluorophores. The solvent composition (THF content) of the micelle solution was varied as a means of modulating the strength of interactions between the fluorophore and solvent as well as between the micelle core and solvent. A donor-acceptor fluorophore pair was employed consisting of 3,3'-di-octadecyloxacarbocyanine perchlorate (DiO, the donor) and 1,1'-di-octadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI, the acceptor). Through the process of Förster resonance energy transfer (FRET), energy was transferred from the donor to acceptor when the fluorophores were in close proximity. A micelle solution containing DiO was mixed with a micelle solution containing DiI at $t=0$, and the emission spectra of the mixed solution were monitored over time (at an excitation wavelength optimized for the donor). In micelle solutions containing 5 and 10 vol% THF in the bulk solvent, an increase in the acceptor peak intensity maximum occurred over time in the post-mixed solution, accompanied by a decrease in the donor peak intensity maximum, indicating the presence of energy transfer from the donor to the acceptor. At long times, the FRET ratios (acceptor peak intensity divided by the sum of the acceptor and donor peak intensities) were indistinguishable from that determined from pre-mixed micelle solutions of the same THF content (in pre-mixed solutions, DiO and DiI were encapsulated within the same micelle cores). In the micelle solution containing 20 vol% THF, the fluorophore exchange process occurred too quickly to be observed (the FRET ratios measured from the solutions mixed at $t=0$ were commensurate to that measured from the pre-mixed solution). A time constant describing the guest exchange process was extracted from the time-dependence of the FRET ratio through fit of

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ELECTRONIC SUPPLEMENTARY INFORMATION

The following are included in the Electronic Supplementary Information (ESI): dynamic light scattering data obtained on micelles in solutions of varying THF content (Figures S1 to S3), fluorescence spectroscopy data obtained on pre-mixed micelles in water with varying DiI:DiO ratios (Figure S4), calculations to estimate the micelle aggregation number, micelle concentration, and number of fluorophores per micelle (Tables S1 and S2), comparison of fluorescence spectra obtained from solutions in the absence and presence of micelles (Figure S5), normalized FRET ratio as a function of time (Figure S6), and parameters extracted from the fit of equation 3 to the FRET ratio (Table S3).

an exponential decay. An increase in the THF content in the micelle solution resulted in a decrease in the time constant, and the time constant varied over five orders of magnitude as the THF content was varied from 5–20 vol%.

Graphical abstract

Fluorescence spectroscopy probed the fluorophore exchange kinetics in a micelle system composed of poly(ethylene oxide-*b*- ϵ -caprolactone) diblock copolymers in water/tetrahydrofuran (THF) mixtures. The time constant for fluorophore exchange decreased dramatically as the THF content in the solution increased (decreasing the solvent-fluorophore and solvent-micelle core repulsion).

Keywords

block copolymer micelles; Förster resonance energy transfer (FRET) spectroscopy; guest exchange kinetics; donor and acceptor fluorophores

INTRODUCTION

Nanoscale assemblies in aqueous solutions which encapsulate hydrophobic molecules are highly relevant in applications as time-release drug delivery vehicles^{1–2} and nanoreactors.³ A variety of molecules spontaneously form such assemblies, including lipids, surfactants, and amphiphilic polymers.⁴ Diblock copolymers containing a hydrophobic block and a hydrophilic block are amphiphilic polymers which can self-assemble into spherical micelles, cylindrical micelles, and vesicles in selective solvents.^{1, 4–6} Though block copolymer micelle structures are similar to those formed by small-molecule surfactants, micelles formed from polymeric amphiphiles are often much more mechanically robust, and their molecular size and architecture can be more finely and widely tuned.^{7–8}

The stability of such assemblies in aqueous solutions governs their utility in applications such as drug delivery.^{9–10} Due to the large size of the molecules, dynamic processes in block copolymer micelles occur on significantly longer timescales than for conventional surfactants. For a narrow size distribution of micelles near equilibrium, the relaxation process is dominated by the exchange of individual polymer chains between the micelles, through insertion and expulsion processes.^{11–13} Numerous reports in the literature have established the prevalence of the single-chain exchange process employing neutron scattering experiments.^{14–19} However, recent experiments have indicated that, even near

equilibrium, fusion and fission processes may still occur.^{20–21} Fusion of micelles is also observed under flow conditions.²² Far from equilibrium, theoretical work by Semenov and coworkers suggest that insertion and expulsion are prevalent;²³ however, Dormidontova et al. have proposed that fusion and fission processes dominate.^{24–25} These dynamic processes in polymeric micelle assemblies can be arrested through crosslinking of the chains forming the micelle corona or core.²⁶ Controlled release of guest molecules can be mediated through a variety of processes, such as degradation of the polymer forming the assembly.²⁷

A rich body of literature exists for guest exchange mechanisms in micelle systems. One mode of guest exchange involves diffusive processes,²⁸ in which the guest molecules are expelled from a host, diffuse through the solvent, and reinsert into a guest, and the exchange dynamics are dominated by the rate at which guest molecules exit the host.²⁹ In addition, there are collision-based processes which involve fission or fusion.^{30–32} Many factors affect the prevalence of these exchange mechanisms as well as the partition coefficient for the guest molecule, including strength of interactions between the guest and micelle core (modulated by the block length in the hydrophobic block of block copolymer systems³³), and strength of interactions between the guest molecule and the bulk solvent. Indeed, prior work in polymer nanogels has shown that a highly hydrophobic core leads to collision-based pathways, whereas in a system containing a more hydrophilic core it is expected that diffusive-based processes are dominant.³⁴ Collision-based processes are also particularly relevant under flow conditions.²²

Fluorescence resonance energy transfer (FRET) is a powerful technique to probe the exchange (and therefore release) of encapsulated guest molecules (fluorophores) in micelle systems by monitoring the time-dependent emission spectra of the donor and acceptor fluorophores.³⁵ When the two fluorophores are encapsulated in separate micelle cores and are not in close proximity to one another, the recorded fluorescence emission spectra (at an excitation wavelength optimum for the donor, but not appropriate for the acceptor) are primarily associated with the donor. If the fluorophores co-exist in the same micelle core and thus are in close proximity, the emission from the excited donor is transferred to and excites the acceptor, resulting in the observation of the emission spectra of the acceptor.

Herein, we have characterized fluorescence spectra obtained from diblock copolymer micelles composed of poly(ethylene oxide-*b*- ϵ -caprolactone) (PEO-PCL) diblock copolymers in water/tetrahydrofuran (THF) mixtures which encapsulate fluorophores. A donor-acceptor fluorophore pair was employed consisting of 3,3'-diocadecyloxacarbocyanine perchlorate (DiO, the donor) and 1,1'-diocadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI, the acceptor). Emission spectra (at an excitation wavelength optimized for the donor) of the following three types of micelle samples were monitored over time: 1) micelles containing only one type of fluorophore (either DiI or DiO), 2) “pre-mixed” micelles which contain both fluorophores that were added at the very beginning of the sample preparation process, and 3) “post-mixed” samples in which a micelle solution containing DiO was added to a micelle solution containing DiI at $t=0$. Manipulation of the solvent composition in the micelle system is a facile mode of modulating the strength of interactions between the fluorophore and solvent, as well as

between the micelle core and solvent. The effect of the solvent composition (varied from 5–20 vol% THF) on the fluorophore exchange process was evaluated.

EXPERIMENTAL METHODS

Materials

All chemicals were purchased from Sigma-Aldrich unless otherwise specified.

Diblock Copolymer Synthesis and Characterization

ϵ -Caprolactone (ϵ -CL, 97%) was purified by distillation over calcium hydride (CaH_2 , ACS reagent, 95%). The catalyst 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD, 98%), was used as received and stored in the glove box to prevent deactivation. Poly(ethylene oxide-*b*- ϵ -caprolactone) (PEO-PCL) diblock copolymers were synthesized using monomethoxy-PEO (purchased from Polymer Source) as a macroinitiator for the ring-opening polymerization of ϵ -CL (Scheme 1). Benzene (ACS reagent, >99%) was distilled twice over CaH_2 to remove any contaminants prior to use. A stock solution of TBD in benzene was prepared with a concentration of 10 mg TBD/mL benzene prior to use. ϵ -CL was added to a solution of pre-dissolved PEO in benzene. The reaction started right after the TBD solution was added to the above solution (final ratio of TBD to ϵ -CL monomer was 1:125 by weight). The reaction proceeded at room temperature for 105 min and was quenched by the addition of benzoic acid (99.5%). Tetrahydrofuran (THF, chromatography grade, 99.5%, inhibitor free) was added and the polymer was purified by precipitation in hexanes (ACS grade, >99%) 5 times. The polymer was dried under vacuum at room temperature overnight, and then dried under vacuum at 60 °C for 8 h.

Proton nuclear magnetic resonance (^1H -NMR) experiments were performed on a JEOL ECA-500 instrument using deuterated chloroform (99.8 atom % D) as the solvent, for the determination of weight fractions of the PEO and PCL blocks, and the number-average molecular weight (M_n). The molecular weight distribution (including the dispersity, M_w/M_n) was characterized by a Viscotek gel permeation chromatography (GPC) instrument containing Agilent ResiPore columns using THF (OmniSolv, HPLC grade) as the mobile phase at 30°C. The flow rate was 1 mL/min and the injection volume was 100 μL . Universal analysis, using the refractometer and viscometer, was employed for the characterization of M_n (the low light scattering signal precluded light scattering analysis). The PEO precursor purchased from Polymer Source had the following characteristics: $M_n = 1.9 \text{ kg/mol}$ and $M_w/M_n = 1.05$. The PEO-PCL block copolymer characteristics are listed in Table 1.

Micelle Preparation for Fluorescence Measurements

Inhibitor-free THF (EMD Millipore, 99.5%) was used as purified from a solvent purification column (Pure Process Technology). Water (H_2O) was purified using a Millipore MilliQ Gradient water purification system (containing RO cartridge, UV lamp). The donor fluorophore, 3,3'-diocetadecyloxacarbocyanine perchlorate (DiO, 98.0%), and the acceptor fluorophore, 1,1'-diocetadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI, BioReagent, 98.0%), were used as received. Excitation / emission maxima are described

for membrane-bound fluorophores as 484 / 501 nm and 549 / 565 nm for DiO and DiI, respectively (reported by Molecular Probes).

All vials used in the preparation of fluorophore-encapsulated micelles were coated prior to use with SafetyCoat (Ultrapure bioreagent, J. T. Baker). The vials were filled with the SafetyCoat reagent, allowed to sit for a minimum of 2 hours, emptied and rinsed with DI water, and finally dried in an oven at 100 °C for 2–3 h.

PEO-PCL was dissolved in THF in a 4 mL safety-coated vial. Fluorophore stock solutions were prepared by dissolving DiO and DiI in THF, respectively. The desired amount of the fluorophore stock solution (either DiO, DiI, or both) was added to the PEO-PCL solution, with stirring. Water was subsequently injected into the solution at a speed of 8 mL/hr using a syringe pump (Fisher Scientific 78-01001) under vigorous stirring. After removing the stir bar, the vial was capped and sealed by parafilm. The micelle solutions were sonicated (VWR Symphony, 35 kHz) at room temperature for 1 h and then filtered through a 0.45 μ m Nylon syringe filter (VWR) prior to being transferred to a vial for the fluorescence measurements.

The targeted final polymer concentration in all micelle solutions was 1 wt %. For the pre-mixed micelles, which contained both fluorophores, the final concentrations of the fluorophores in the micelle solution were 19.3 and 3.9 μ mol/L for DiI and DiO, respectively (5:1 molar ratio of DiI:DiO). For the time-resolved experiments, one micelle solution was prepared containing only DiI (at a concentration of 38.6 μ mol/L), and a second micelle solution was prepared containing only DiO (at a concentration of 7.8 μ mol/L). In time-resolved experiments, each micelle solution (one containing DiI and one containing DiO) was separated into two parts. At $t = 0$, half of the DiI micelle solution was mixed with half of the DiO micelle solution, at which point the time-resolved fluorescence data was monitored. Concurrently, the remaining half of each micelle solution was diluted with DI water such that the final fluorophore concentration was 19.3 and 3.9 μ mol/L for DiI and DiO, respectively, so that the control micelle solutions containing only one fluorophore (DiI or DiO) could also be monitored with fluorescence spectroscopy. Therefore, all fluorescence measurements were conducted on samples which contained either one or both fluorophores at the following concentrations: DiI (19.3 μ mol/L) and DiO (3.9 μ mol/L). The final THF concentration in the micelles was fixed at 5, 10 or 20 vol%. This volume percentage encompassed additive volumes of THF and water. The amounts of polymer and fluorophores in each solution were assumed to be negligible percentages of total micelle solution volume.

Fluorescence Spectroscopy to Identify Spectral Overlap of Donor and Acceptor Fluorophores

Fluorescence spectroscopy experiments were conducted to identify the spectral overlap of the DiI and DiO molecules using an OLIS DM 45 fluorometer (Olis Inc., Bogart, GA, USA) equipped with an 150W Xenon lamp and single grating excitation and emission monochromators. A slit of 3.16 was used and the bandpass was 13 nm. In the measurement of the emission spectra: 1) DiO was excited at 470 nm (secondary excitation) and the emission spectrum was measured at 495–600 nm; 2) DiI was excited at 520 nm (secondary excitation) and the emission spectrum measured at 545–650 nm. In the measurement of the excitation spectra: 1) the DiO emission wavelength was fixed at the primary peak

wavelength (515 nm) and the absorption spectrum was collected at 395–495 nm; 2) the DiI emission wavelength was fixed at the primary peak wavelength (565 nm) and the absorption spectrum was collected at 455–555 nm. In this experiment only, the following procedure was used for the micelle preparation: PEO-PCL (1 wt%), DiI (20 µg/mL) and DiI (20 µg/mL) were dissolved in acetone, transferred to a dialysis bag, and allowed to dialyze for 24 h in Millipore water (water was replaced every 12 h). The PEO-PCL diblock copolymer had the following characteristics (used in this experiment only): $M_{n, \text{PEO}} = 2 \text{ kg/mol}$, $M_{n, \text{PCL}} = 3 \text{ kg/mol}$, 60 wt% PCL.

Time-Resolved Fluorescence Spectroscopy

Excitation and emission fluorescence spectra were obtained on micelle solutions using a Horiba Scientific NanoLog spectrofluorometer. The excitation source was a 450 Watt continuous xenon lamp and the detector was a UV/VIS R928 PMT (FL-1073) single channel detector (wavelength range 190 – 860 nm, lifetime resolution 200 picoseconds – 1 millisecond). The instrument and lamp were turned on and allowed to equilibrate for 15–20 min before use. The excitation spectrum of water was used as a standard to monitor the consistency of the instrument over time (the water peak maximum showed little variation, < 5%). Emission spectra were obtained using a slit width of 1 nm and excitation wavelengths of 450 and 505 nm (ideally suited for the donor and acceptor fluorophores, respectively). In time-resolved experiments, the emission spectra (at an excitation wavelength of 450 nm) of three types of micelle samples were monitored over time: 1) micelles containing only one type of fluorophore (either DiI or DiO), 2) pre-mixed micelles which contain both fluorophores that were added from the very beginning of the sample preparation, and 3) post-mixed samples in which micelles containing DiI were mixed with micelles containing DiO at $t = 0$.

Micelle Hydrodynamic Radius through Dynamic Light Scattering

Micelle solutions were prepared as described above for the time-resolved fluorescence experiments (though in this case, without the addition of fluorophores) and transferred to glass cuvettes for dynamic light scattering (DLS) measurements. DLS measurements were performed on a Brookhaven Instruments BI-200SM Research Goniometer System with a He-Ne laser of wavelength 637 nm (30 mW, 400 micron aperture). The detector angle was 90 degrees and the measurements were made at 25 °C. Six separate measurements of the correlation function were taken at each delay time, and the average was used in analysis of the data. At long decay times, the data fluctuated around zero intensity, at which point the data was no longer used in the analysis. The autocorrelation function was analyzed using the method of cumulants to determine the average diffusion coefficient. The hydrodynamic radius of the diffusing particles was calculated using the Stokes-Einstein relation:

$$R_h = \frac{k_B T}{6\pi\eta D} \quad (1)$$

where D , k_B , T and η are the diffusion coefficient of the micelles, Boltzman's constant, temperature and viscosity of the solution respectively. The viscosities of water/THF

mixtures at 25 °C were obtained from ref. ³⁶ and are summarized in Table 2. The correlation functions of all samples are included in Figures S1–S3 in the Electronic Supplementary Information.

Measurement of Fluorophore Solubility

Fluorophore solubilities in water/THF solutions were examined. Each fluorophore was dissolved in a water/THF solution of known composition, and then allowed to stir for 24 hours, after which the solution was visually inspected to ascertain the fluorophore solubility. Table 3 indicates the solution compositions which were examined.

RESULTS AND DISCUSSION

PEO-PCL Diblock Copolymer Synthesis and Micelle Preparation

The PEO-PCL diblock copolymer was synthesized through the ring-opening polymerization of ϵ -caprolactone (Scheme 1) using a monomethoxy-PEO macroinitiator (monomer conversion was held at around 80% to avoid transesterification). A ^1H NMR spectrum obtained from the purified PEO-PCL (Figure 1) shows characteristic peaks associated with the respective PEO and PCL repeat units. Analysis of the spectrum (using peaks a/b and g determined that the diblock copolymer contained 67.6 wt% PCL and the PCL block molecular weight (M_n) was 4.0 kg/mol (the PEO precursor $M_n = 1.9$ kg/mol). GPC analysis (Figure 2) indicated the presence of a narrow molecular weight distribution with dispersity = 1.06.

Micelles containing the desired fluorophore concentration (donor, acceptor, or both) were prepared in THF/water mixtures (varying from 5–20 vol% THF) using the solvent switch method, followed by filtration and sonication. We note that the polymer concentration in the micelle solutions (1 wt%) was well above the previously reported critical micelle concentration of a PEO-PCL diblock copolymer (0.002 wt%).³⁷ The PEO-PCL diblock copolymer used in this study was chosen to contain a PCL content (67.6 wt% PCL) within the range previously reported to adopt a spherical micelle morphology (in ref. ³⁸, spherical micelles were observed within a range of 59–70 wt% PCL in the block copolymer). Dynamic light scattering analyses of the micelle solutions show correlation functions characteristic of a unimodal distribution of micelle sizes (Figures 3 and S1–S3). The micelle diffusion coefficients were measured to be in the range of $2.3 - 3.2 \times 10^{-11} \text{ m}^2/\text{s}$ and the calculated hydrodynamic radii were in the range of 6.1 – 6.8 nm (Table 2) using the Stokes-Einstein relation (equation 1). We estimate the aggregation number of these micelles to be between 110 – 226, using the volume of a PEO-PCL diblock copolymer (and PEO and PCL densities of 1.125 and 1.14 g/mL, respectively),^{39–41} and assuming the micelle forms a sphere with hydrodynamic radius reported in Table 2 (this simplified calculation ignores many effects such as swelling of the micelle core with the co-solvent THF, swelling of the corona with water, the diffuse nature of the core-corona interface, and diffuse boundary between the corona and bulk solvent). Using this estimate of the aggregation number, we calculate the micelle concentration to be in the range of $5 - 9 \times 10^{18}$ micelles per L solvent, accounting for a critical micelle concentration of 0.002 wt%, reported previously for a PEO-

PCL block copolymer in water.³⁷ Further details of calculations used to estimate the aggregation number and micelle concentration are provided in Tables S1 and S2.

Though PCL is a semi-crystalline polymer, we do not anticipate that the PEO-PCL micelle solutions in water/THF mixtures studied here contain semi-crystalline cores for the following reasons: 1) a co-solvent additive like THF generally swells the micelle core,⁴² which is anticipated to disrupt crystallization, 2) even when a co-solvent is not added, there is often sufficient water swelling of the PEO-PCL micelle core to disrupt crystallization (in a prior study, crystallization was only observed at low temperatures, or with higher molecular weight PCL blocks than used in our study⁴³), and 3) crystallization promotes formation of morphologies other than spherical micelles.^{43–44}

Time-Resolved FRET Spectroscopy on Diblock Copolymer Micelles with Varying Solvent-Core Interactions

The DiO-DiI donor-acceptor fluorophore pair was chosen for this study due to its established use in literature reports of FRET studies.^{22, 45–48} The spectral overlap of the donor and acceptor fluorophores was confirmed through measurement of the excitation and emission spectra for each individual fluorophore (encapsulated within the micelle solution), shown in Figure 4 (and consistent with ref. ^{22, 48}). Based on the data in Figure 4, an excitation wavelength of 450 nm was chosen for FRET experiments. At 450 nm, there is sufficient excitation of the donor (DiO), and minimal excitation of the acceptor (DiI). Once excited, the donor fluorophore emission spectrum indicates a peak maximum at 505 nm. During the FRET process, the emission of the donor subsequently excites the acceptor; 505 nm is a sufficient excitation wavelength for DiI (Figure 4).

Literature studies have reported the Förster radius of the DiO-DiI pair to be 5–6 nm.^{47–48} The micelles utilized in our study have hydrodynamic radii in the range of 6.1 – 6.8 nm, and are separated by significantly larger distances (the polymer concentration was held constant in the micelle solution at 1 wt%). We therefore expect that fluorophores which are encapsulated within separate micelles will not be in close enough proximity to undergo FRET. However, fluorophores which are encapsulated within the same micelle core are expected to exhibit signatures of the FRET process. Using the estimated micelle concentration discussed above and the fluorophore concentrations employed in this study (19.3 and 3.9 $\mu\text{mol/L}$ for DiI and DiO, respectively), we calculated the average number of fluorophore molecules per micelle in our system, which was in the range of 1.3 – 2.6 for DiI and 0.3 – 0.5 for DiO. This overestimates the fluorophore concentration within a micelle, as fluorophores are likely also present within the bulk solvent. Further details of calculations used to estimate the number of fluorophores per micelle are provided in Tables S1 and S2. An excess of DiI was used (5:1 molar ratio of DiI:DiO) to ensure sufficient signal from the excited donor fluorophores. Data obtained at various DiI:DiO ratios are shown in Figure S4.

In time-resolved FRET experiments, emission spectra (at an excitation wavelength of 450 nm) of three types of micelle samples were monitored over time: 1) micelles containing only one type of fluorophore (either DiI or DiO), 2) “pre-mixed” micelles which contain both fluorophores that were added from the very beginning of the sample preparation process, and 3) “post-mixed” samples in which a micelle solution containing DiO was added to a

micelle solution containing DiI at $t=0$, and the emission spectra of the mixed solution were monitored over time. We note that both fluorophores are highly hydrophobic and exhibit low solubilities in water (without the presence of the polymer micelles). In water/THF mixtures with 5–20 vol% THF, the fluorophores were insoluble at all concentrations examined (up to two orders of magnitude lower than the fluorophore concentration used in micelle solutions), shown in Table 3. By contrast, the fluorophores are fully soluble in pure THF, and varying the THF % in the micelle solution is a means of varying the interactions between the fluorophore and the solvent, thus impacting the partitioning of the fluorophore within the micelle core and bulk solvent.

We begin our discussion with spectra obtained on the micelle solution containing 5 vol% THF (Figure 5a). Spectra obtained from micelle solutions containing either donor (DiO) or acceptor (DiI) fluorophores were consistent with the emission spectra reported in Figure 4. In Figure 5a, the use of an excitation wavelength of 450 nm greatly reduced the fluorescence intensity measured from the acceptor molecule (as this wavelength was chosen to provide high excitation of the donor and little excitation of the acceptor). The pre-mixed micelle solution (containing both DiO and DiI, added at the beginning of the sample preparation process) showed characteristic signals associated with both fluorophores, with a significantly higher intensity emission spectrum associated with the acceptor and a smaller yet non-negligible peak associated with the donor (consistent with prior FRET studies using the DiO-DiI pair^{45, 48}). As expected, the intensity of the peak associated with the acceptor DiI in the pre-mixed solution was also greater in magnitude as compared to the micelle solution containing DiI only (as the excitation wavelength of 450 nm was optimum for the donor and not the acceptor). In the pre-mixed solution, the large signal associated with the acceptor and diminished signal associated with the donor are signatures of the FRET process. In the post-mixed sample, measurements were obtained at various time-points following mixing of a micelle solution containing DiO with a second micelle solution containing DiI. In the initial measurement ($t = 0.25$ min was the shortest time point that could be obtained) noticeable changes were observed in the donor and acceptor peak maxima intensities relative to the micelles containing solely DiO or DiI (the donor peak intensity decreased while the acceptor peak intensity increased). These sudden changes are likely due to the presence of free DiO and DiI molecules in the bulk solvent, which encounter one another and undergo FRET when the micelle solutions are mixed at $t = 0$. Over time, the post-mixed acceptor peak maximum intensity was found to increase until at long times it coincided with that measured from the pre-mixed sample. Similarly, in the post-mixed sample the donor peak maximum was found to decrease in intensity over time until it reached that observed from the pre-mixed sample (Figure 5a). To ensure that the time-resolved experiments primarily probed the FRET signal from fluorophores within the micelle cores, we also conducted fluorescence experiments on fluorophore solutions that did not contain micelles (i.e. a DiI solution was mixed with a DiO solution and the fluorescence intensity monitored over time, shown in Figure S5). The measured fluorescence intensities from the fluorophores were significantly lower in the absence of micelles (Figure S5), consistent with prior literature showing that the partitioning of fluorophores increases the fluorescence intensity, due to a higher local concentration.⁴⁹

Spectra obtained from the micelle solution containing 10 vol% THF (Figure 5b) behaved in a similar fashion to that described above for the sample containing 5 vol% THF. A notable difference is the measurement time required to reach the values of the donor and acceptor peak maxima at which the post-mixed sample was identical to that of the pre-mixed sample. As the % THF in the solvent increased, the time required substantially decreased. Indeed, when viewing spectra obtained from the micelle solution containing 20 vol% THF (Figure 5c), the initial measurement obtained on the post-mixed sample (at 0.25 min) was already coincident with that of the pre-mixed sample.

Though the partition coefficients have not been previously measured for DiI and DiO in PEO-PCL micelle solutions prepared in water/THF mixtures, a prior study has reported comparable partition coefficients for DiI and DiO in water/lipid mixtures (a partition coefficient of around 4 for both fluorophores).⁵⁰ Furthermore, Soo and coworkers have investigated DiI partitioning in PEO-PCL micelle solutions prepared in water/dimethylformamide (DMF) mixtures of varying DMF concentration (similar to THF, DMF is a good solvent for both the PEO and PCL blocks and is miscible with water: the DiI concentration increased in the solvent phase of the micelle solution as the DMF content increased).³³ Similar behavior is anticipated for our system of fluorophores in PEO-PCL micelles prepared in water/THF mixtures. We have examined the solubility of the fluorophores in water/THF mixtures, and determined that the solubilities are less than 0.193 and 0.039 $\mu\text{mol/L}$ for DiI and DiO, respectively, in the water/THF mixtures (containing 5–20 vol% THF), which is greater than two orders of magnitude lower than the fluorophore concentrations added to the micelle solutions. Both the DiI and DiO intensities at the peak maxima increased with decreasing THF content (Figure 5), which is consistent with an increase in the partition coefficient of the fluorophores as the THF content decreased.

To further explore trends highlighted in Figure 5, the data were quantified through the use of the FRET ratio, following previous studies:^{34, 45, 51}

$$\text{FRET ratio} = \frac{I_a}{I_a + I_d} \quad (2)$$

where I_a and I_d are the acceptor and donor intensities, respectively (determined at the peak maxima). The FRET ratio was determined for both the pre-mixed and post-mixed solutions over time (Figure 6). The FRET ratio was relatively constant for all three pre-mixed samples over time (as expected). However, significantly different trends are observed in the time-dependence of the FRET ratio measured on post-mixed solutions when comparing samples with differing THF content. In the micelle solution containing 5 vol% THF, the FRET ratio was found to increase over time and then plateau around the same value measured from the pre-mixed solution of the same THF content (Figure 6a). Similar behavior was observed in the post-mixed micelle solution containing 10 vol% THF (Figure 6b), though the time scale to achieve the plateau FRET ratio was greatly diminished as the THF content increased. Finally, the post-mixed micelle solution containing 20 vol% exhibited a time-independent FRET ratio which was commensurate with that observed in the pre-mixed micelle solution

(Figure 6c). The long-time FRET ratio was relatively constant as the THF content in the solvent varied (around 0.63–0.66). This indicates that the partition coefficients of DiI and DiO likely exhibit comparable changes as the THF content in the solution is varied (i.e. the ratio of fluorophores within the micelle core is insensitive to the THF content in the solvent).

The FRET ratios measured from post-mixed micelle solutions were normalized and fit using an exponential decay (shown as dashed curves in Figure 6) as previously described:^{51–52}

$$\frac{FR(t) - FR_o}{FR_\infty - FR_o} = 1 - \exp(-t/\tau) \quad (3)$$

where $FR(t)$, FR_o , and FR_∞ are the FRET ratio measured at time t , initial ($t=0$) FRET ratio, and long time ($t \rightarrow \infty$) FRET ratio, respectively (τ , FR_o , and FR_∞ were fitting parameters). The normalized curves are shown in Figure S6. From the fit of equation 3 to the data in Figures 6 and S6, time constants were extracted (summarized in Table 4): 1.2×10^4 and 1.9×10^2 min for post-mixed solutions containing 5 and 10 vol% THF, respectively. We also note that our inability to observe the rapid increase to the plateau FRET ratio for the post-mixed micelle solution containing 20 vol% THF indicates that this time constant is less than 2.5×10^{-1} min (the measurement time at which the first data point could be characterized). A clear trend was observed as the time constant increased as the vol % THF in the solvent decreased.

These results demonstrate the importance of the solvent-guest interactions and solvent-polymer interactions in tuning the release rate of an encapsulated species from the micelle core. The time constant describing the exchange of fluorophores between the micelles varied over five orders of magnitude through a relatively small change in the concentration of a polar additive to the bulk solvent (i.e. THF content varied from 5–20 vol%). In future work, we plan to investigate the physical mechanisms underlying this behavior. In particular, the question remains whether this drastic variation in the kinetics of guest exchange is mediated by diffusive processes or fusion-based processes, or micelle aggregation, all of which may be impacted by the solvent-fluorophore and solvent-polymer interaction strengths.

CONCLUSIONS

Fluorescence spectroscopy was employed to characterize the rate of guest exchange in PEO-PCL micelles formed in THF/water mixtures which encapsulated fluorophores (DiO, DiI, or both). In micelle solutions containing 5 and 10 vol% THF in the bulk solvent, an increase in the acceptor peak intensity maximum occurred over time in the post-mixed solution, accompanied by a decrease in the donor peak intensity maximum, as expected in the presence of FRET. At long times, the FRET ratios of the post-mixed solutions (acceptor peak intensity divided by the sum of the acceptor and donor peak intensities) became indistinguishable from those of the pre-mixed solutions of the same THF content. In the micelle solution containing 20 vol% THF, the FRET ratios measured from the post-mixed solution were commensurate to that measured from the pre-mixed solution, throughout the entire measurement time. A time constant describing the guest exchange process was

extracted from the time-dependence of the FRET ratio through fit of a typical exponential decay. An increase in the THF content in the micelle solution resulted in a decrease in the time constant, and the time constant varied over five orders of magnitude as the THF content was varied from 5–20 vol%, demonstrating the importance of the solvent-guest interactions and solvent-polymer interactions in the release rate of the fluorophore from the micelle core.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
 ^1H NMR spectrum obtained from the PEO-PCL diblock copolymer

Figure 2.
GPC refractometer signal for the PEO-PCL diblock copolymer

Figure 3. Dynamic light scattering data obtained from PCL-PEO micelles in a solvent mixture containing 5 vol% THF. Additional data obtained on all samples (containing 5, 10 and 20 vol% THF) are shown in Figures S1–S3). Solid red curve is a fit to the data using the method of cumulants.

Figure 4. Spectral overlap of the donor (DiO) and acceptor (DiI) fluorophores encapsulated in micelle solutions (solvent contained 5 vol% THF). Fluorescence intensities are normalized to have the same peak maximum.

Figure 5.

Fluorescence emission spectra (at an excitation wavelength of 450 nm) are shown for micelle solutions containing a) 5 vol% THF, b) 10 vol% THF and c) 20 vol% THF. The spectra for micelles containing either DiO or DiI at $t=0$ are indicated by solid blue and green curves, respectively. The pre-mixed micelle solution (containing both fluorophores) at $t=0$ is indicated by a solid red curve. Spectra obtained after mixing the DiO and DiI-containing micelle samples (i.e. post-mixed samples) are shown as dashed curves (time points provided in the legend).

Figure 6.

FRET ratio as a function of time for micelle solutions containing a) 5 vol% THF, b) 10 vol% THF and c) 20 vol% THF (pre-mixed micelle solutions: red $\square$, post-mixed micelle solutions: black $\blacktriangle$). Dashed curves represent the fit to the data obtained on post-mixed micelles (equation 3; described in main text) with the resulting time constants: a) 1.2×10^4 min (5% THF) and b) 1.9×10^2 min (10% THF).

Scheme 1.
PEO-PCL diblock copolymer synthesis

Table 1

Molecular characteristics of the PEO-PCL block copolymer

M_{n_PEO} (kg/mol)	1.9
M_{n_PCL} (kg/mol)	4.0
	1.06
PCL wt%	68

Table 2Characteristics of micelle solutions^a

Vol% THF in solvent	η (cP)	R_h (nm)	D (10^{11} m ² /s)	σ_D (10^{11} m ² /s)
5	1.415	6.6	3.2	0.7
5	1.415	6.8	3.1	1.1
10	1.704	6.1	3.1	0.3
10	1.704	6.5	2.9	0.3
20	1.715	6.5	2.3	<0.1
20	1.715	6.4	2.4	0.3

^a η is the solvent mixture viscosity at 25 °C determined from ref. ³⁶, R_h and D are the hydrodynamic radius and diffusion coefficient of the micelles, respectively, and σ_D is the standard deviation in the diffusion coefficient. Measurements were obtained on two samples for every solvent composition; all measurements are reported here and raw data is provided in Figures S1–S3.

Table 3

Fluorophore Solubilities

Fluorophore	Fluorophore Concentration ($\mu\text{mol/L}$)	Vol% THF in solvent	Optical Quality
DiI	19.3	100	Fluorophore fully dissolved
DiI	19.3	20	Fluorophore not fully dissolved
DiI	1.93	20	Fluorophore not fully dissolved
DiI	0.193	20	Fluorophore not fully dissolved
DiI	0.193	10	Fluorophore not fully dissolved
DiI	0.193	5	Fluorophore not fully dissolved
DiO	3.9	100	Fluorophore fully dissolved
DiO	0.039	20	Fluorophore not fully dissolved
DiO	0.039	10	Fluorophore not fully dissolved
DiO	0.039	5	Fluorophore not fully dissolved

Table 4Time constant of fluorophore exchange^a

Vol% THF in solvent	τ (min)
5	1.2×10^4
10	1.9×10^2
20	$<2.5 \times 10^{-1}$

^aCharacterized through fit of equation 3 to data obtained on post-mixed micelle solutions in Figures 6 and S6. R^2 values for each fit are provided in Table S3. Error in time constant determination was estimated to be $< 1\%$ of the value, based on observed variations in the fluorescence intensity measured from pre-mixed micelle solutions over time ($< 2\%$ variation in the fluorescence intensity was observed over the measurement time).