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In Situ Measurements of Polymer Micellization Kinetics with Millisecond Temporal Resolution

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Abstract

Utilizing synchrotron small-angle X-ray scattering (SAXS) integrated with a microfluidic device, micellization kinetics of a diblock co-polymer, poly(ethylene glycol)-*b*-poly(caprolactone) (PEG-*b*-PCL) was measured in situ with millisecond temporal and micrometer spatial resolution. The evolutionary regimes of polymer micellization – nucleation, fusion, and insertion were directly observed. The five-inlet microfluidic device provided steady continuous mixing of the polymer solution and the antisolvent. Solvent replacement was mainly dominated by lateral diffusion across the hydrodynamically focused central layer, whose thickness could be precisely designed and manipulated from mass balance of the partitioning streams. Knowing the micellization kinetics of the polymers is essential for design and optimization of self-assembled polymeric nanostructures. The technique of integrating SAXS with microfluidic devices can be translatable to other systems for a breadth of applications.

1. Introduction

Biocompatible and biodegradable polymeric micelles self-assembled from amphiphilic block co-polymers have attracted a great deal of attention due to their promise as reliable drug delivery carriers.^{1–3} Amphiphilic polymers with a lower critical micellization

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concentration (CMC) provide better micelle stability upon blood dilution after injection, protect drugs from premature degradation, and prolong circulation time for drugs in the bloodstream.^{4–6} With precisely controlled physicochemical and surface properties, these polymeric nanoparticles may be able to direct the release of the active pharmaceutical ingredients (APIs) to the target disease sites by means of passive-delivery mechanism due to enhanced permeation and retention or active-delivery mechanism by specific ligand binding.^{7–9} It has been demonstrated in small and large animals that polymeric nanoparticles could significantly enhance compound bioavailability and reduce drug toxicity, and several such systems are undergoing clinical trials.^{10–12} Despite extensive research in the field over the past three decades, polymeric nanoparticles encapsulating APIs are not yet commercially available on the market for clinical use in human. Although many of the polymers or building blocks of the polymers have been approved by FDA for both injection and oral administration, polymer-assembled micelles or nanoparticles need to be re-evaluated for clinical use, because nanostructural properties (such as particle size distribution and structure stability) in addition to material properties may significantly shift the pharmacokinetics and influence the *in vivo* behavior of APIs.¹³ To evaluate the toxicity and efficacy of this category of polymeric nanomedicines and to precisely determine drug dosage, formation of polymeric micelles and nanoparticles, which is kinetically controlled during the self-assembly process, has to be highly reproducible and scalable, with the final product possessing well-controlled physicochemical properties. The recently-developed flash nanoprecipitation technique employing a custom-designed multi-inlet vortex reactor allows for precisely controlled, sophisticated mixing schemes, providing rapid solvent exchange that is required to manipulate the nonequilibrium structures of the nanoparticles.^{14–15} The process of flash nanoprecipitation is continuous and scalable, which is a significant advantage for the potential commercial translation of the nanotechnology. However, in order to design the nanoparticle formulation for each API, empirical trials have to be conducted to decide essential parameters (such as the polymer-to-drug ratio, ratio of hydrophobic to hydrophilic blocks, ratio of the organic to inorganic solvent, and molecular length of the polymer) to best match the kinetics of polymer micellization and drug nucleation and growth.^{15–17} Because of the enormous variability of the available polymers and the many combinations of possible parameters, empirical trials are tedious and the optimization of the system is almost impossible. Therefore, a complete understanding of the competitive kinetics is necessary to guide the process of nanoparticle self-assembly.

There are several theoretical models predicting micellization kinetics. The classical Aniansson-Wall theory considers micelle growth mainly in terms of individual polymer insertion.^{18–19} Johnson and Prud'homme divided the process into nucleation, fusion, and insertion stages based on experimental measurements of polystyrene-*b*-poly(ethylene oxide) (PS-*b*-PEO) micelle growth quenched after flash nanoprecipitation.²⁰ Most experimental studies have been focused on the kinetics near equilibrium such as chain exchange kinetics.^{21–23} Near-equilibrium measurements were conducted by quickly introducing sequential jumping conditions in temperature or pH.^{23–24} Direct *in situ* measurements of the nonequilibrium polymer micellization kinetics so far have been limited. Lund et al. utilized synchrotron X-ray scattering integrated with a stopped-flow apparatus to obtain poly(ethylene-alt-propylene)-poly(ethylene oxide) (PEP₁-PEO₂₀) micellization kinetics at a

temporal resolution of 100 ms.²¹ PEP₁-PEO₂₀ with a very small hydrophobic head and a relatively much longer PEO brush is highly asymmetric and forms starlike micelles.²¹ The kinetics of PEP₁-PEO₂₀ micellization may be expected, however, to be much slower than that of typical amphiphilic diblock co-polymers commonly used for biomedical applications. The latter usually consists of longer chains for both blocks to achieve the goals of long blood circulation time and high drug loading. With et al were able to observe the micellization kinetics in a continuous microfluidic channel, but the focus was on disorder-order transition into the liquid-crystalline phase after polymer micellization.²⁵ In this study, one of the most popular biodegradable and biocompatible amphiphilic diblock co-polymers, poly(ethylene-glycol)-*b*-poly(caprolactone) (PEG₁₁₄-*b*-PCL₃₂) with 114 repeating units of PEG and 32 repeating units of PCL (equivalent to molecular weight of PEG 5000 and PCL 3600), was used as a model system to demonstrate direct in situ measurements of the nonequilibrium micellization kinetics.

In the present work, micelle formation was initiated and driven by an interfacial energy change induced by solvent exchange. Mixing of the polymer solution with an antisolvent was realized in a crystal-quartz microfluidic device (Figure 1A) to provide consistent and controlled measurements with millisecond temporal resolution. The microfluidic device was integrated with the BioCAT high brilliance synchrotron X-ray beamline, 18ID, at the Advanced Photon Source, Argonne National Laboratory. Compared to a stopped-flow apparatus, this microfluidic device has better control of the exact flow patterns and potentially faster temporal resolution. The quartz microfluidic device is compatible with harsh organic solvents including tetrahydrofuran (THF) used in this study. The polymer solution was introduced as a thin sheet from the central inlet. The two angled streams from each side of the polymer stream contained pure solvent to prevent premature mixing. Deionized water as the antisolvent was pumped in through the two orthogonal streams. Solvent exchange by diffusion occurred laterally over a distance of a few micrometers of the central layer of the original polymer solution. The thicknesses of the layers were manipulated by the flowrates of the inlet streams. To detect micelle evolution kinetics, spatial positions along the straight channel could be translated into time span because of the simple steady flow pattern.

Small-angle x-ray scattering (SAXS) is a technique well-suited for quantitative analysis regarding nanostructures of micelles, such as morphology, core-shell structure, polydispersity, and aggregation number. Previously, we have reported static measurements of the core-shell structures of micelles formed from the linear diblock co-polymers, PEG-*b*-PCL and poly(ethylene glycol)-*b*-poly(lactic acid) (PEG-*b*-PLA), as well as novel brush co-polymers, at their metastable states.²⁶ In this study, the kinetics of PEG-*b*-PCL micelle formation was investigated by integrating the time-resolved SAXS (TR-SAXS) with the quartz microfluidic device to achieve high spatial and temporal resolution.

2. Materials and Methods

2.1 Materials and Reagents

PEG₁₁₄-*b*-PCL₃₂ was used as purchased from the Polymer Source (QC, Canada). THF was purchased from Sigma-Aldrich. Milli-Q water deionized to 18.2 MΩ was used for all

experiments. A five-inlet quartz microfluidic chip was custom-fabricated by Translume (Ann Arbor, MI).

2.2 Integration of the Microfluidic Device with Synchrotron TR-SAXS

The microfluidic device was mounted laterally in the beamline path at a sample-to-detector distance of 2.5 m. Dual Harvard PHD 2000 infusion syringe pumps and a New Era NE-8000 syringe pump were used to control the flowrates of the polymer solution, the solvent, and the antisolvent streams (Figure 1A). For each test, THF without the polymer was introduced from the same central horizontal stream in the same flow pattern for background measurements and appropriate subtraction routines. Scattering patterns were acquired using a Pilatus3 1M detector (Dectris Inc.) with a given momentum transfer range of 0.00854-0.52842 Å⁻¹. Exposure time was 1 s to a beam of 12 keV X-rays focused to 5 × 20 μm² at the position of the microfluidic device. The incident flux was about 2 × 10¹² photons/s. A step size interval of 5 μm orthogonal to the flow direction was used for the measurements at each timepoint. An acquisition time of 1000 ms and a read-out pause of 5 ms between two sequential exposures was used to for all the measurements. Images were taken along the straight horizontal channel at 1 mm linear spatial intervals from the stream intersection (Figure 1C).

2.3 SAXS Data Analysis

Two-dimensional (2D) scattering data were azimuthally averaged to acquire one-dimensional (1D) curves for structure analysis. Each 1D curve was normalized by the beam transmission and scaled to absolute intensity based on the measurements of water scattering within the microfluidic chip.

For each 1D curve, the integrated scattering intensity as a scalar was projected to their corresponding position on the microfluidic detection channel to form a topological map (Figures 2A and S1A), which clearly indicated the position of the central polymer/micelle layer.

The pair distance distribution function (PDDF) and the radius of gyration (R_g) were calculated by fitting the 1D data using the ATSAS software program suite. The inlet polymer concentration was 50 mg/mL, which was in the dilute regime. Interparticle interference was neglected and the structure factor was set to unity.

The volume-of-correlation, denoted as V_c , was calculated as follows to obtain the molecular weights of the micelles,²⁷

$$V_c = \frac{I(0)}{\int_0^\infty qI(q)dq}, \quad (1)$$

where $I(0)$ is the scattering intensity at $q = 0$, $I(q)$ is the scattering intensity at q , and $q = 4\pi\sin(\theta/2)/\lambda$ is the wave vector. Here, θ is the scattering angle and λ is the X-ray wave

length. Integration of equation (1) was divided into two parts. The integral value from $q = 0$ to the Guinier region was calculated by extrapolating the Guinier plot to $q = 0$,

$$\int_0^{q_L} qI(q) dq \approx \int_0^{q_L} qI(0) \exp\left(-\frac{Rg^2 q^2}{3}\right) dq = \frac{3}{2} \frac{I(0)}{Rg^2} - \frac{3}{2} \frac{I(0)}{Rg^2} \exp\left(-\frac{Rg^2 q_L^2}{3}\right), \quad (2)$$

where q_L is the q value at the starting point of the Guinier region. The rest was directly integrated to the upper limit of the maximum measured q , because the integrand $qI(q) dq$ decayed to zero at high q for all scattering measurements.

A parameter defined as $Q_R = \frac{V_c^2}{Rg}$ was calculated. Molecular weights of the particles, despite folded-compact or unfolded flexible particles, have a linear relationship with Q_R under a log-log plot.²⁷ The molecular weight of PEG₁₁₄-*b*-PCL₃₂ micelle at quasi-equilibrium state and the corresponding Q_R were calculated from the previous static measurements.²⁶ The Q_R value for the polymer in a random-coil form was calculated from the first measurement in the microfluidic device, because at the first detection position, the calculated supersaturation ratio is much lower than unit. Molecular weights of micelles in the intermediate stage of evolution can be linearly interpolated using a $\log(Mw) - \log(Q_R)$ plot.

2.4. Calculation of the Polymer and THF Concentration Profiles Along the Detection Channel

In the flow direction, the Péclet number was calculated to be in the order of magnitude of 1000 for the solvent and two orders of magnitude higher for polymers or micelles, using typical flowrates and the X-ray beam width (20 μ m). Therefore, in the flow direction, mass transport was dominated by advection and diffusion was neglected, allowing the mass transport to be decoupled into convection in the flow direction and diffusion in the lateral direction. Thus, concentration spatial distributions of the components (concentration profiles) in the microfluid channel could be calculated by solving the one-dimensional unsteady diffusion problem (SI section S1).

The binary diffusion coefficient of THF and water at room temperature is a function of THF mole fraction, varying from 0.21×10^{-9} m²/s to 4.15×10^{-9} m²/s.²⁸ For the calculation of the THF concentration profiles in the detection channel, polynomial functions were used to fit the experimentally measured binary diffusion coefficients reported by Leaist et al. (Figure S2).²⁸ At a THF molar concentration from 0 to 0.70 (corresponding to a mass concentration from 0 to 811.8 mg/ml), a fourth-order polynomial function was applied to fit the experimental data (Figure S2A),

$$D = 0.00162 c^4 - 0.02869 c^3 + 0.21202 c^2 - 0.77093 c + 1.32722. \quad (3)$$

For THF molar fractions from 0.70 to 0.99 (corresponding to a mass concentration from 811.8 to 887.0 mg/ml), a third-order polynomial function was used to fit the reported data (Figure S2B),

$$D = 18.55549 c^3 - 466.11097 c^2 - 3903.94161 c - 10900.87641. \quad (4)$$

For both polynomial fitting, c is the local mass concentration of THF in the unit of 100 mg/ml, and D is the diffusion coefficient of THF in the unit of $10^{-9} \text{ m}^2/\text{s}$. For molar ratios of THF from 0.99 to 1 (corresponding to a mass concentration from 887.0 – 889.0 mg/ml), diffusion coefficient was set as a constant number, $4.15 \times 10^{-9} \text{ m}^2/\text{s}$.

Precisely deciding self-diffusion of the linear diblock co-polymer PEG₁₁₄-*b*-PCL₃₂ and the micelles can be more complicated since this is a dynamic process. Initially, both blocks of the polymer were dissolved in a good solvent. The diffusion coefficient of PEG at room temperature was previously measured and reported to be about $5 \times 10^{-11} \text{ m}^2/\text{s}$ for PEG with molecular weight about 5000.²⁹ Based on Flory's scaling law, the diffusion coefficient of PEG₁₁₄-*b*-PCL₃₂ (Mw 5000-*b*-3600) was estimated to be about 25% lower. This molecularly dissolved stage was quickly followed by hydrophobic block aggregation due to solvent exchange (Figure 2E). The structure of this highly unsaturated micelle may be analogous to star-like or brush polymers, whose diffusion coefficient may be estimated using the scaling law with a power less than that for a linear polymer. After this transient state, much larger micelles were formed, with a self-diffusion coefficient estimated to be about $2.5 \times 10^{-11} \text{ m}^2/\text{s}$ according to the Stokes-Einstein equation,

$$D = \frac{RT}{N_A} \frac{1}{6\eta\pi r},$$

where R is the gas constant, T is temperature in Kelvin, N_A is Avogadro's Number, η is dynamic viscosity of water with a value of 0.89 mPa s, and r is the hydrodynamic radius of the micelle with an approximate value of 100 Å calculated from the radius of gyration for the micelles detected near the end of the microfluidic channel. For the calculation of the polymer and/or the micelle concentration profiles, a constant value of $4.0 \times 10^{-11} \text{ m}^2/\text{s}$ was used as the diffusion coefficient of the polymer and/or micelle, although the diffusion coefficient was smaller than this value and diffusion of the polymer and/or micelle could be even slower than being predicted.

Supersaturation ratio was defined as:

$$\text{Supersaturation Ratio} = \frac{\text{local polymer concentration}}{\text{local polymer CMC}}$$

Local polymer concentration was calculated as described above. Local polymer CMC was obtained by applying a plot of experimentally measured CMC of PEG₁₁₄-*b*-PCL₃₂ as a function of THF mole fractions in water.¹⁵

3. Results and discussions

We presented in situ measurements of polymer micellization kinetics by integrating the microfluidic device with TR-SAXS. Although micellization process was coupled with molecular transport due to concentration gradients, the flow pattern in the microfluidic channel was well-controlled to reveal the micellization kinetics. The initial thickness of the polymer layer in the segmented flow was calculated from the volumetric flowrates of the inlet streams, considering a parabolic velocity profile, which is applicable beyond the entrance region (Table 1 and SI section S5). The initial thickness of the central polymer organic stream layer was designed to be 8 μm or 4 μm . Linear spatial step (of 1 mm) motions were taken along the microfluidic channel by using a stepping motor. The total channel observation length was 30 mm. Due to the steady-state continuous flow in the microfluidic device, positions could be translated to time intervals by dividing distance by the average flow velocity.

Based on the significant difference of the diffusion coefficients of THF, polymer, and eventually micelles within the detection channel, it is reasonable to assume that solvent exchange was mainly caused by binary diffusion of THF and water laterally across the channel - while polymer was primarily confined to the central segmented layer. The hypothesis was verified by both experimental measurements and theoretical analysis (Figures 2 and S1). Plots of the experimentally measured scattering intensity in the observation channel (Figures 2A and S1A) indicated that the device was tilted by a small angle when aligning it with the X-ray beam, which was corrected during data analysis. Concentrations of THF and polymer and/or micelles were numerically calculated. Polymers were determined to be mainly confined in the central segment (Figures 2C and S1C), while solvent exchange was relatively much faster (Figures 2B and S1B). The kinetic process of micellization highly depends on supersaturation, which was calculated and plotted (Figures 2D and S1D). With a small supersaturation ratio over 1, hydrophobic blocks of the polymers started to aggregate, initiating micellization which was termed nucleation. Higher supersaturation ratio downstream in the channel caused rapidly increased population of the nucleates, which collided and fused to generate bigger micelles until the repulsion force provided by the increased surface coverage of the PEG brushes prevented further fusion. Then through polymer insertion, micelles approached to their quasi-equilibrium stage (Figure 2E). The total possible experimental time window was 600 ms with the designed flow rate, which might not be long enough for the polymer to finish the insertion stage to reach a metastable structure. However, polymer insertion became much slower at later time points, which could potentially be interrogated using many other experimental techniques (such as neutron scattering and dynamic light scattering) and offline measurements. We postpone such interrogation to future work, since the focus of this study is on the earlier evolution stages to capture the structural transition kinetics.

A typical example of the temporal sequences of the scattering intensity as a function of wave vector q is presented in Figures 3A and S4A. The bright X-ray beam yielded reliable and clear scattering signals. An increase in intensity and a shift in the decay region indicated micelle growth over time (Figures 3A and S4A). The shift of the PDDF curves demonstrated large-scale structural and morphological changes (Figures 3B and S4B). Gaussian shaped

PDDF curves were evident in the early stages of the micelle evolution, indicating globular polymer structures³⁰ and likely formation of small polymer nucleates, with an R_g of approximately 35 Å. Dramatic changes then occurred in the structure due to the rapid merging of the nucleates. The dual-peaked distribution functions quickly transitioned to another group of PDDF curves with peaks at larger distances. At the end of the detection period, the micelles were about 90 Å, which would eventually grow over a much longer period of time into micelles at a metastable state by polymer chain insertion.

Micelle size evolution indicated by both PDDF and Guinier analysis were reasonably consistent, although R_g retrieved from the Guinier approximation was slightly larger than from the PDDF (Figure 4A). Three stages of micellization were captured: nucleation, fusion, and insertion. There was a rapid jump in size of the micelles at about 250 – 300 ms, indicating the primary growth by merging or fusion of the initially-generated, highly-unsaturated micelles. The bigger discrepancy for the calculated R_g by PDDF and Guinier analysis in the fusing regime was likely due to the large polydispersity of the micelles, including micelle populations before and after merging. Molecular weights of the micelles experienced an exponential jump in the abovementioned fusion regime as well (Figure 4). The micelle fusion regime corresponded with the position in the microfluidic channel with a maximum supersaturation ratio, at which nucleate population and collision rate were promptly increased and merging of the unsaturated micelles (or nucleates) were induced.

The kinetics of micelle formation is mainly controlled by the lateral diffusion of solvents across the channel. Varying the initial thickness of the central polymer layer would affect THF concentration profiles and therefore the supersaturation ratio of the polymer and the nucleation kinetics. When changing the thickness of the central polymer layer from 8 µm to 4 µm, the rapid size jump of the micelles was shifted to an earlier time as the solvent lateral diffusion was faster (Figure 4B). By an order of magnitude analysis using $t \sim l^2/D$ (where t was the diffusion time, l was the characteristic diffusion length which in this case was the thickness of the polymer layer, and D was the binary diffusion coefficient of THF in water), when the thickness of the polymer layer was reduced by a half, micellization events such as jumps in size should occur at a quarter of the time. For example, fusion, which occurred at about 280 ms for the case with an 8-µm-thick central polymer layer, would be expected to shift to about 70 ms for the case with a 4-µm-thick central polymer segment. These obtained values roughly agreed with the experimental measurements. For both cases of different central polymer layer thicknesses, fusion happened at the timepoint with the highest supersaturation ratios in the channel. In the regime of polymer chain insertion, although R_g showed no apparently growth, molecular weights of the micelles kept increasing (Figure 4).

4. Conclusion

Polymer micellization kinetics have been historically challenging to measure at high temporal resolutions due to the rapidity of the events and non-linearity of the underlying phenomena. We have demonstrated that utilizing synchrotron SAXS integrated with a laminar-flow microfluidic mixing device allowed for increased control and observational tuning of the micellization process. Successful in situ probing of the three stages of PEG₁₁₄-*b*-PCL₃₂ micelle formation - nucleation, fusion, and insertion was performed at two different

polymer layer thicknesses. Being able to directly measure the micellization kinetics is essential for design and optimization of nanoparticle structures and for continuous scalable production of these polymeric nanoparticles, which is the key to overcome the hurdles of preparing polymeric nanoparticles for many biomedical applications.

The experimental technique and data analysis are translatable to other systems and not solely limited to studying polymeric micelle self-assembly. Kinetic measurements on other micelle and vesicle formation, protein folding, and biological molecule aggregation are possible, given the breadth of opportunities from tailoring microfluidic technologies integrated with synchrotron radiation.

Supplementary Material

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References

1. Cabral H; Kataoka K, Progress of drug-loaded polymeric micelles into clinical studies. *J Control Release* 2014, 190, 465–476. [PubMed: 24993430]
2. Gaucher G; Dufresne MH; Sant VP; Kang N; Maysinger D; Leroux JC, Block copolymer micelles: preparation, characterization and application in drug delivery. *J Control Release* 2005, 109 (1–3), 169–188. [PubMed: 16289422]
3. Rosler A; Vandermeulen GWM; Klok HA, Advanced drug delivery devices via self-assembly of amphiphilic block copolymers. *Adv Drug Deliver Rev* 2012, 64, 270–279.
4. Eetezadi S; Ekdawi SN; Allen C, The challenges facing block copolymer micelles for cancer therapy: In vivo barriers and clinical translation. *Adv Drug Deliver Rev* 2015, 91, 7–22.
5. Kakkar A; Traverso G; Farokhzad OC; Weissleder R; Langer R, Evolution of macromolecular complexity in drug delivery systems. *Nature Reviews Chemistry* 2017, 1 (8), 0063.
6. Wong PT; Choi SK, Mechanisms of Drug Release in Nanotherapeutic Delivery Systems. *Chem Rev* 2015, 115 (9), 3388–3432. [PubMed: 25914945]
7. Cabral H; Matsumoto Y; Mizuno K; Chen Q; Murakami M; Kimura M; Terada Y; Kano MR; Miyazono K; Uesaka M; Nishiyama N; Kataoka K, Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. *Nat Nanotechnol* 2011, 6 (12), 815–823. [PubMed: 22020122]
8. Hoang B; Ekdawi SN; Reilly RM; Allen C, Active Targeting of Block Copolymer Micelles with Trastuzumab Fab Fragments and Nuclear Localization Signal Leads to Increased Tumor Uptake and Nuclear Localization in HER2-Overexpressing Xenografts. *Mol Pharmaceut* 2013, 10 (11), 4229–4241.
9. Xu QG; Ensign LM; Boylan NJ; Schon A; Gong XQ; Yang JC; Lamb NW; Cai ST; Yu T; Freire E; Hanes J, Impact of Surface Polyethylene Glycol (PEG) Density on Biodegradable Nanoparticle Transport in Mucus ex Vivo and Distribution in Vivo. *Acs Nano* 2015, 9 (9), 9217–9227. [PubMed: 26301576]

10. Ahn HK; Jung M; Sym SJ; Shin DB; Kang SM; Kyung SY; Park JW; Jeong SH; Cho EK, A phase II trial of Cremophor EL-free paclitaxel (Genexol-PM) and gemcitabine in patients with advanced non-small cell lung cancer. *Cancer Chemoth Pharm* 2014, 74 (2), 277–282.
11. Kamaly N; Xiao ZY; Valencia PM; Radovic-Moreno AF; Farokhzad OC, Targeted polymeric therapeutic nanoparticles: design, development and clinical translation. *Chem Soc Rev* 2012, 41 (7), 2971–3010. [PubMed: 22388185]
12. Matsumura Y; Hamaguchi T; Ura T; Muro K; Yamada Y; Shimada Y; Shirao K; Okusaka T; Ueno H; Ikeda M; Watanabe N, Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin. *Brit J Cancer* 2004, 91 (10), 1775–1781. [PubMed: 15477860]
13. Elsabahy M; Wooley KL, Design of polymeric nanoparticles for biomedical delivery applications. *Chem Soc Rev* 2012, 41 (7), 2545–2561. [PubMed: 22334259]
14. Liu Y; Cheng CY; Liu Y; Prud'homme RK; Fox RO, Mixing in a multi-inlet vortex mixer (MIVM) for flash nano-precipitation. *Chem Eng Sci* 2008, 63 (11), 2829–2842.
15. Shen H; Hong SY; Prud'homme RK; Liu Y, Self-assembling process of flash nanoprecipitation in a multi-inlet vortex mixer to produce drug-loaded polymeric nanoparticles. *J Nanopart Res* 2011, 13 (9), 4109–4120.
16. Chow SF; Wan KY; Cheng KK; Wong KW; Sun CC; Baum L; Chow AHL, Development of highly stabilized curcumin nanoparticles by flash nanoprecipitation and lyophilization. *Eur J Pharm Biopharm* 2015, 94, 436–449. [PubMed: 26143368]
17. Pagels RF; Edelstein J; Tang C; Prud'homme RK, Controlling and Predicting Nanoparticle Formation by Block Copolymer Directed Rapid Precipitations. *Nano Lett* 2018, 18 (2), 1139–1144. [PubMed: 29297690]
18. Aniansson E; Wall S; Almgren M; Hoffmann H; Kielmann I; Ulbricht W; Zana R; Lang J; Tondre C, Theory of the kinetics of micellar equilibria and quantitative interpretation of chemical relaxation studies of micellar solutions of ionic surfactants. *The Journal of Physical Chemistry* 1976, 80 (9), 905–922.
19. Aniansson E; Wall SN, Kinetics of step-wise micelle association. *The Journal of Physical Chemistry* 1974, 78 (10), 1024–1030.
20. Johnson BK; Prud'homme RK, Flash nanoprecipitation of organic actives and block copolymers using a confined impinging jets mixer. *Australian Journal of Chemistry* 2003, 56 (10), 1021–1024.
21. Lund R; Willner L; Monkenbusch M; Panine P; Narayanan T; Colmenero J; Richter D, Structural observation and kinetic pathway in the formation of polymeric micelles. *Physical review letters* 2009, 102 (18), 188301.
22. Lund R; Willner L; Richter D; Dormidontova EE, Equilibrium chain exchange kinetics of diblock copolymer micelles: Tuning and logarithmic relaxation. *Macromolecules* 2006, 39 (13), 4566–4575.
23. Shen L; Du J; Armes SP; Liu S, Kinetics of pH-induced formation and dissociation of polymeric vesicles assembled from a water-soluble zwitterionic diblock copolymer. *Langmuir* 2008, 24 (18), 10019–10025. [PubMed: 18681410]
24. Weiss TM; Narayanan T; Wolf C; Gradzielski M; Panine P; Finet S; Helsby WI, Dynamics of the self-assembly of unilamellar vesicles. *Physical Review Letters* 2005, 94 (3).
25. With S; Trebbin M; Bartz CBA; Neuber C; Dulle M; Yu S; Roth SV; Schmidt HW; Forster S, Fast Diffusion-Limited Lyotropic Phase Transitions Studied in Situ Using Continuous Flow Microfluidics/Microfocus-SAXS. *Langmuir* 2014, 30 (42), 12494–12502. [PubMed: 25216394]
26. Szymusiak M; Kalkowski J; Luo HY; Donovan AJ; Zhang P; Liu C; Shang WF; Irving T; Herrera-Alonso M; Liu Y, Core-Shell Structure and Aggregation Number of Micelles Composed of Amphiphilic Block Copolymers and Amphiphilic Heterografted Polymer Brushes Determined by Small-Angle X-ray Scattering. *Acs Macro Lett* 2017, 6 (9), 1005–1012. [PubMed: 29308298]
27. Rambo RP; Tainer JA, Accurate assessment of mass, models and resolution by small-angle scattering. *Nature* 2013, 496 (7446), 477–81. [PubMed: 23619693]
28. Leaist DG; MacEwan K; Stefan A; Zamari M, Binary mutual diffusion coefficients of aqueous cyclic ethers at 25 degrees C. Tetrahydrofuran, 1,3-dioxolane, 1,4-dioxane, 1,3-dioxane, tetrahydropyran, and trioxane. *J Chem Eng Data* 2000, 45 (5), 815–818.

29. Waggoner RA; Blum FD; Lang JC, Diffusion in Aqueous-Solutions of Poly(Ethylene Glycol) at Low Concentrations. *Macromolecules* 1995, 28 (8), 2658–2664.
30. Svergun DI; Koch MHJ, Small-angle scattering studies of biological macromolecules in solution. *Rep Prog Phys* 2003, 66 (10), 1735–1782.

Figure 1:

(A) Images of the five-inlet microfluidic chip (left) and integrated setup of the microfluidic device with the synchrotron X-ray beam (right). The inlet streams were arranged such that the polymer solution was introduced from the central stream, next to the two angled streams with pure solvent (such as THF) to prevent premature mixing. The antisolvent (water) was introduced via the two orthogonal inlet streams. For measurements of the background scattering, pure THF was introduced from the central stream. (B) Three-dimensional sketch of the configuration and dimensions of the microfluidic device in units of millimeter. All

channels were 0.2 mm wide with a standard height of 0.6 mm at the inlet and a stepped-height increase of the orthogonal streams to 1.0 mm. (C) Typical 2D SAXS images captured along the straight channel after subtracting the background solvent scattering. More intense scattering could be observed at the downstream of the channel.

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Figure 2.

(A) Topological plot of SAXS intensity. Each point on the plot corresponds to the 1-D data with intensity integrated over the first 100 points in the q -range from 0.00854 – $0.0678 \text{ \AA}^{-1}$. Data was used after subtraction of the corresponding background scattering by the solvent mixture. (B) Calculated THF concentration distribution in the channel. (C) Calculated polymer concentration distribution in the channel. (D) Calculated supersaturation ratio distribution in the channel. (E) Schematic drawing of the three stages of polymer self-

assembly into micelles - nucleation, fusion, and insertion. For Figure 2A–2E, the initial polymer layer thickness was manipulated by the inlet flowrates to be 8 μm .

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Figure 3:

(A) One-dimensional absolute scattering intensity (after the subtraction of the background solvent scattering) at various time points during PEG₁₁₄-*b*-PCL₃₂ micellization. The initial thickness of the polymer layer was 8 μm . (B) Calculated PDDF from Figure 3A. The colors of the curves are consistent for both figures.

Figure 4:

Fitted R_g obtained via Guinier approximation (black) and PDDF (red), and micelle molecular weights in a log scale (blue) for the cases with initial thicknesses of the central polymer layer of (A) 8 μm and (B) 4 μm for PEG₁₁₄-*b*-PCL₃₂. The initial polymer concentration was 50 mg/mL. Very small error bars, corresponding to the fitting errors, were covered by the symbols.

Table 1.

Summary for experimental flowrates and initial polymer layer thickness

Flowrate ($\mu\text{L}/\text{min}$)				Average velocity (mm/s)	Polymer segment thickness (μm)
Polymer stream	Separation stream	Anti-solvent stream	Total		
36	4.5	277.5	600	50	8
18	4.5	286.5	600	50	4