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The Impact of Polymer Architecture on Polyion Complex (PIC) Micelles: When Topology Matters (and When It Doesn't)

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The influence of homopolymer architecture on the properties of polyion complex micelles is reported. Using a combination of dynamic and static light scattering, the authors show how the architecture is only relevant in kinetically trapped states of micelles formed by the electrostatic assembly of poly(*N*-isopropyl acrylamide-block-styrene sulfonate) (p(NIPAM-*b*-SS) and linear, 4-arm, 8-arm star quaternized poly(dimethyl amino ethyl acrylate) (PDMAEA) homopolymers or poly(amidoamine) (PAMAM) dendrimers. Interestingly, the micellar size and the aggregation number in these kinetically arrested states follow a clear trend with the number of arms but differ in the case of dendrimers possibly due to the distinct chemical nature of the monomers. The authors show that if the micelles are prepared in a weak polyelectrolyte pairing regime (i.e., high ionic strength), they all converge into similar structures. The presented findings represent a new way of controlling the properties of polyion complex micelles through kinetically trapped states.

1. Introduction

Polyion complex (PIC) micelles, also known as complex coacervate micelles (C3Ms[1]), are mixed association colloids formed upon the spontaneous assembly of a water-soluble neutral-charged block copolymer and an oppositely charged polyelectrolyte. The ubiquitous hydrophilic nature, their wide variety of components, and responsiveness to environmental changes make these supra-polymeric constructs versatile nanocompartments with modular properties and ideally suited for encapsulation of fragile and water-soluble (bio)macromolecules, such as biologics.[2]

What parameters control particle size, morphology, and polymer exchange dynamics have been studied extensively. This greatly advanced our fundamental understanding of the structure and dynamics of PIC micelles and provided design handles to optimize their performance in nanocarriers applications, such as compartmentalization, transport, delivery, and release of active (therapeutic) cargo. The size, morphology, and salt resilience of electrostatically driven micelles are tunable to a large extent and in a predictable manner through adjustments in the chemistry of the starting materials (e.g., the block length ratio, N_n/N_c, and overall molecular weight, M_{dbp}, of the neutral-ionic diblock copolymer) and physicochemical parameters, such as the ionic strength and pH of the polymer solution.[2–4] PIC micelles tend to be spherical for diblock copolymers (dbp) with a longer neutral block, N_n, than charged block, N_c, whereas worm-like or lamellar morphologies are obtained for $N_n < N_c$. The hydrodynamic radius of spherical micelles increases with increasing M_{dbp}.[5] Added salt weakens the attraction between the charged blocks and often reduces the solubility of the neutral chains in the corona. At modest salt concentrations, this induces swelling

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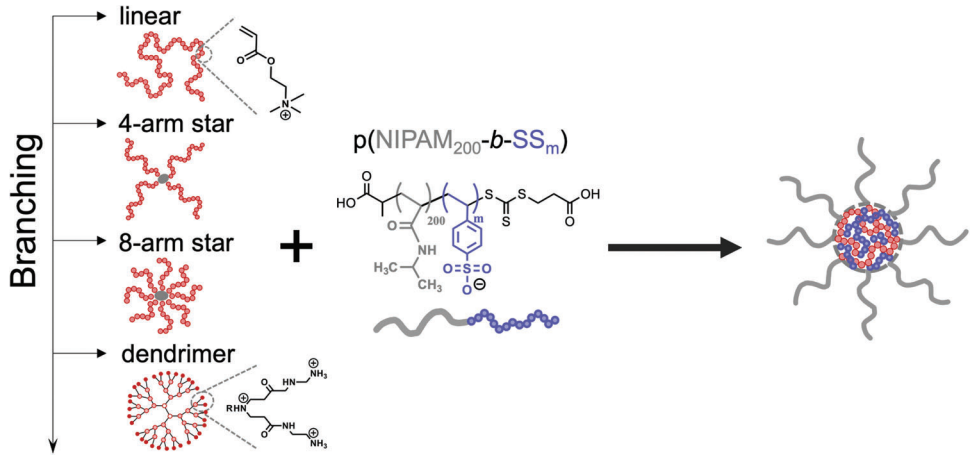

Scheme 1. Schematic representation of PIC micelle formation by self-assembly of polyanionic-neutral poly(*N*-isopropylacrylamide-*b*-styrene sulfonate (gray–blue, p(NIPAM200-*b*-PSS$_m$), m = 14–100) with a topological library of polycations, including a generation 3 PAMAM dendrimer, and quaternized poly(dimethylaminoethyl acrylate) (qPDMAEA) with a linear, 4-arm, and 8-arm star architecture.

of the polyion core and thinning of the micellar shell, while at elevated salt concentrations, micelles may undergo morphological transitions and/or disintegration.

Substituting diblock copolymers of linear architectures by branched- and star-like counterparts influences micellar morphology as the polymer architecture impacts chain packing.[6–9] For example, increasing the mean number of arms in poly(ethylene oxide-block quaternized dimethyl aminoethyl methacrylate mikto-arms) (PEO_{114}-(qPDMAEMA$_{40}$)$_{4.3}$)/polystyrene sulfonate PIC micelles resulted in a morphological shift from spherical to elongated structures and further to vesicles.[8] Mixing cationic poly(ethylene oxide-block-methacryloxyethyl trimethylammonium chloride mikto-arms with linear poly([2-(methacryloyloxy)ethyl]trimethylammonium iodide) also resulted in unilamellar polymersomes.[6] Inspired by these studies, we wondered whether homopolymer (hp) architecture would also impact micellar properties. Recently developed scaling theories and empirical data suggest that this is not the case: that the architecture and chemical nature of the charged hp is irrelevant to the size and structure of PIC micelles when in thermodynamic equilibrium.[5,10] In contrast, other studies did observe a (strong) influence of homopolymer topology for PIC micelles comprising strong and relatively stiff polyelectrolytes, homopolymers with high molecular weights and/or alternating surface charge distributions (i.e., proteins).[11–13]

Aiming to reconcile these seemingly contradictory results and considering that most studies arriving at deviant conclusions use strong polyelectrolytes, we wondered whether kinetic factors could also play a role in addition to polymer topology. To examine when topology matters and when it does not, herein, we systematically study the influence of hp architecture on the properties of PIC micelles in different ion-pairing strength regimes (corresponding to different salt concentrations). We focus on chain architecture and selected for this purpose cationic homopolymers (hp) with ≈60 (permanently) charged amine moieties arranged in a linear chain, a 4-armed star, an 8-armed star, and a third-generation dendrimer. These four polyelectrolytes thus carry a similar number of chargeable groups, while varying in architecture (**Scheme 1**). As oppositely charged diblock copolymer for complexation, we opted for poly(*N*-isopropyl acrylamide-*b*-polystyrene sulfonate) (p(NIPAM$_{200}$-*b*-SS$_m$)) with a fixed length PNIPAM block and a variable length pSS block of m = 14, 30, 50, 70, and 100 repeating units. We study the properties of PIC micelles with pSS length m = 100 in a range of salt concentrations up to 500 mM, since it is well known that salt has a large influence on the ability of the micelles to reconfigure. Strong, solid-like complexes are consistently reported upon association of pSS with polyamines at low ionic strength,[14] while more liquid-like complexes form at elevated salt concentrations when the interaction strength of the SS/amine pairs is weakened. We hypothesize that hp topology influences the structural properties of PIC micelles under strong bonding conditions at low ionic strength due to the occurrence of kinetically trapped states. Conversely, and in line with recently developed polymer-scaling theories,[5,10] hp topology will have little impact under weak bonding conditions at high ionic strength when the interaction strength of the SS/amine pair is sufficiently reduced, so that relaxation and reconfiguration proceed more rapidly.

2. Results and Discussion

First, the p(NIPAM-*b*-SS) block copolymers were synthesized. Polymerizations were performed in aqueous media via a photo-induced reversible addition–fragmentation chain-transfer (RAFT) using a poly(*N*-isopropylacrylamide) (p(NIPAM)-based chain transfer agent (macro-CTA, $DP_{n,\mathrm{NIPAM}} \approx 200$) and sodium styrene sulfonate (1:15, 1:30, 1:50, 1:70, & 1:100 macro-CTA: monomer ratios). 2-Hydroxy-2-methylpropiophenone (HMPP) was added as a light-sensitive radical source, enabling the polymerization to run at room temperature to produce p(NIPAM$_{200}$-*b*-SS$_m$). Block copolymers with m = 14, 30, 50, 70, and 100, as determined with ^{1}H NMR (Figure S1, Supporting Information) were synthesized. PAMAM generation 3 was commercially available as a solution in methanol and was further dried and redispersed in the required buffer. The topological set of hp was completed by linear- and star-shaped PDMAEA synthesized via Cu(0)-mediated controlled radical polymerization.[15–17] For the linear- and star-shaped polymers, post-polymerization

quaternization was required to suppress auto-hydrolysis of the PDMAEA and safeguard polymer stability.[18,19] Quaternization yields polymers with pH-independent permanent charges (Figures S2 and S3, Supporting Information). The topological library and block copolymers used to create the PIC micelles are depicted in Scheme 1.

Typically, PIC micelles form close to charge stoichiometry, namely, when the number concentration of positive and negatively chargeable monomers are balanced ($n^+ \approx n^-$). The optimal solution composition for micellization is referred to as the preferred micellar composition (PMC). Here, micellar complexes are often most abundant and largest in size. To assess whether the PMC coincides with charge stoichiometry ($f^+ = n^+/(n^+ + n^-) = 0.5$) for the micelles investigated in this work, we performed titrations by adding a hp solution into a solution of p(NIPAM$_{200}$-*b*-SS$_m$) containing a fixed concentration of negatively charged groups (0.5 mM). To ensure that all dendrimer amines are charged, we used a 20 mM glycine buffer at pH 3 as t continuous phase.[20] The titrations were monitored by measuring the scattered intensity at a fixed scattering angle of 90° throughout the experiment (Figure S4, Supporting Information). As expected, the scattered intensity initially increased after each hp addition until it reached a maximum corresponding to the PMC, which was located at $f^+ \approx 0.5$ PMCs for all homopolymer architectures. This suggests that all the chargeable monomers in the dendrimer, linear, and star polymers are accessible for neutralization by the charged dbp.

Next, we investigated the impact of topology on micellar size for micelles prepared upon direct mixing of a hp stock solution (5 mM, 100 µL) and a solution of p(NIPAM$_{200}$-*b*-PSS$_m$) solution (0.55 mM, 900 µL) in 20 mM glycine buffer at the PMC using dynamic light scattering (**Figure 1**a). An increase in hydrodynamic radius (R_H) was found upon an increase in PSS block length, which was particularly significant for the linear and multiarm stars. Moreover, micellar aggregation numbers, P_{agg}, estimated from static light scattering experiments also increased with increasing m from 14 to 100 (Figure 1b and Figure S5, Supporting Information). This behavior is in line with scaling theories, which predict an increase in P_{agg} and core size as the length of the core-forming block of the dbp increases.[5,10] The R_H and P_{agg} of all micelles remained stable for at least 3 weeks after preparation (Figure S6, Supporting Information). Interestingly, R_H and P_{agg} also depend markedly on hp architecture: at fixed PSS block length m, always 8-arm > 4-arm > linear > dendrimer, particularly when m is high. For example, at $m = 100$, we have $R_H = 22$, 34, 36, and 51 nm and $P_{agg} = 126$, 483, 605, and 1708 for PIC micelles comprising dendritic PAMAM, linear chains, 4-armed stars, and 8-armed stars, respectively. This is remarkable, especially because no dependence of micellar size and morphology on hp length (for sufficiently short hp's) has been found previously in a comprehensive study of poly(*N*-methyl-2-vinylpyridinium)-*b*-poly(ethylene oxide)/poly(acrylic acid) PIC micelles.[21] Notice also that while P_{agg} increased with increasing number of arms for the linear- and star-like architectures, P_{agg} was smallest for the dendrimicelles, where the hp is most branched.

One may wonder that the large influence of hp topology on micellar size and aggregation number at low ionic strength (20 mM) is due to an effect of topology on the formation of kinetically trapped states, which may arise due to strong ion pairing at low salt concentrations hampering reconfiguration toward optimal packing. Indeed, rheological experiments on bulk coacervates of linear polyelectrolytes established that the relaxation processes in these viscoelastic materials slow down dramatically at low salt concentrations.[22] Topology is also known to impact relaxation in, for example, thin polymer films. Molecular dynamics simulations on thin-film crystallization of star polymers revealed that an increase in topological complexity (i.e., an increase in the number of arms) decelerated and for arms ≥8 prohibitively inhibited crystallization, which was partially attributed to slower relaxation.[23] Another contributing factor to differences in relaxation may be the nature of the cationic charges. Only the dendrimer carries pH-dependent charges. All other homopolymers are quarternized. The quenched and presumably more

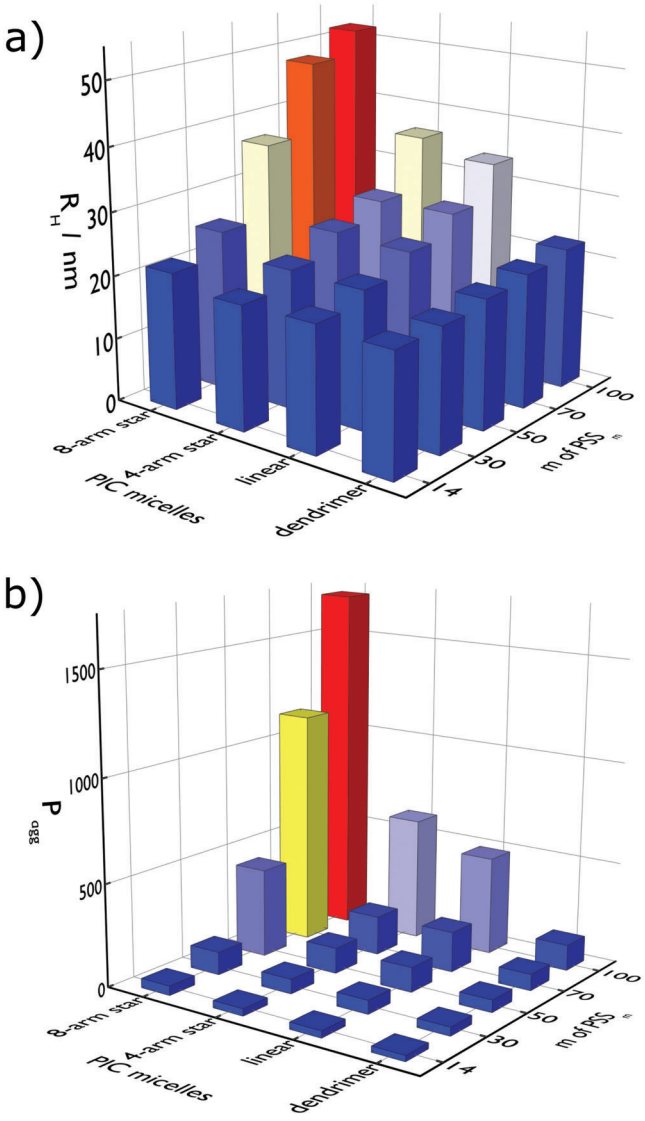

Figure 1. a) Hydrodynamic radii (R_H) and b) micellar aggregation numbers (P_{agg} = number of polycations + polyanions) from the self-assembled micellar structures formed by mixing polycations with various architectures.

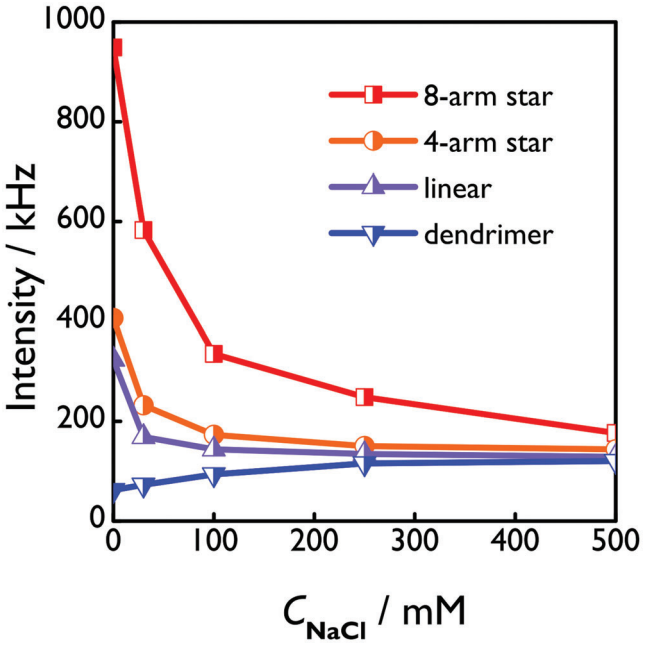

Figure 2. Scattered intensity at 90° of PIC micelles composed of $PNIPAM_{200}$-*b*-PSS_{100} with a topological library of polycations (dendrimer, linear, 4-arm star, and 8-arm star) as a function of NaCl content at the moment of sample preparation.

hydrophobic nature of the cationic monomers of the linear- and star-like hp's may also inhibit relaxation.

Many studies on polymeric amphiphiles with high glass transition temperatures established that kinetic arrest can induce dispersity in and coexistence of various (exotic) micellar morphologies.[24] Transmission electron microscopy (TEM) revealed globular PIC micelles for all systems except for those incorporating the 8-armed stars, which deviated slightly from sphericity (Figure S7, Supporting Information). This is in line with the determined ratios of 0.6–1 (Table S2, Supporting Information) between the radius of gyration (*R*g) and R_H.

To assess if the micelles formed at 20 mM correspond to arrested states which may be "plasticized" upon an increase in salt concentration, we first examined the solubility of p($NIPAM_{200}$-*b*-SS_{100}) with increasing NaCl concentration at 20 °C, and found it remained entirely soluble up to 1 M NaCl (Figure S8, Supporting Information). Next, we measured the structure of PIC micelles prepared at various elevated salt concentrations up to 500 mM NaCl (**Figure 2** and Figure S10, Supporting Information). Interestingly, the scattering signal of the dendrimicelles is only slightly salt-dependent, while the forward scattering and hydrodynamic radii of all other micelles decrease strongly with increasing salt concentration up to 250–500 mM NaCl at which point the intensities essentially converge. More specifically, both the forward scattering and the apparent hydrodynamic radii of PIC micelles comprising linear hp chains, 4-armed stars, and dendrimers are essentially congruent around 250 mM NaCl with a $R_H \approx 25$ nm (Figure S10, Supporting Information), while micelles with 8-armed stars scatter more strongly up to 500 mM NaCl. Apparently, the strong impact of hp architecture on the micellar structure at low ionic strength vanishes at high ionic strength. Intuitively this can be attributed to faster structural relaxations caused by elevated salt concentrations weakening the ion pair strength. The findings also imply that hp topology impacts the relaxation of PIC micelles, with dendrimicelles only weakly arrested and micelles with 8-arm stars strongly arrested under conditions of strong ion pairing. Indeed, such an interplay between topology and relaxation behavior was recently reported by some of us, demonstrating that pSS-PEO-pSS triblock/PAMAM micelles relaxed faster compared with the analogous pSS-PEO/PAMAM micelles at comparable salt concentrations.[14] Note that no time-evolution of the forward scattering was observed for any of these samples for at least 3 weeks after sample preparation (Figure S9, Supporting Information).

3. Conclusions

In summary, through light scattering experiments, we have demonstrated that the architecture of cationic homopolymers used to create PIC micelles significantly impacts the micellar size and aggregation number in the strong ion-pairing regime, whereas the effect becomes vanishingly small at elevated salt concentrations. We attribute this to the impact of salt and topology on relaxation processes in polyelectrolyte complexes. Salt has a well-documented and marked impact, strongly decelerating relaxation at low ionic strength when the ionic bonds are strong. The impact of topology is less understood. In line with star polymer literature, our findings indicate that the micelles with 4-armed stars relax more easily than those containing 8-arm stars. Dendrimicelles appear the least arrested, which is presumably related to the more particle-like character of the dendrimers in comparison to the more polymer-like character of the linear hp and 4-arm and 8-arm stars. Another contributing factor may be the nature of the cationic charges. While the dendrimers are equipped with pH-dependent charges, the cationic monomers in the linear hp and stars are quenched; i.e., these quarternized polyamines carry pH-independent charges. Importantly, since both salt and topology impact kinetic arrest of the association colloids, PIC micelles with a range of sizes and aggregation numbers can be prepared from essentially identical chemical units by modulation of salt concentration and/or chain architecture. This reproducible and robust structural diversity originates from the interplay between reconfiguration, arrest, topology, and the complexity of the coassembly. The presented findings thus open up a new way of forming kinetically trapped PIC micelles with control over their size and aggregation number. We hypothesize that other (bio)polymers with complex topologies, such as proteins and polynucleotides, can be exploited in a similar manner to create with the same components PIC micelles with distinctly different structures. We further envision that our findings open up new avenues to tailor the structure and morphology of polymer colloids made through polymerization-induced electrostatic self-assembly (PIESA), wherein the molecular topology of the homopolymer can be used as a handle to tune the degree of structural complexity.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

block copolymers, complex coacervate core micelles, dendrimers, kinetic trapping, polyelectrolytes, polyion complex micelles, self-assembly

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[1] Although PIC micelles and C3Ms are terms that are frequently used interchangeably, the term C3Ms is strictly applicable to micelles with a coacervate (i.e., liquid-like) core. The term PIC micelles is less restrictive as to the nature of the micellar core. Since the nature of the core of the micelles investigated herein has not been determined, we use the term PIC micelles for the remainder of this paper.

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