Comparing Zwitterionic and PEG Exteriors of Polyelectrolyte Complex Micelles

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Comparing Zwitterionic and PEG Exteriors of Polyelectrolyte Complex Micelles

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ABSTRACT

A series of model polyelectrolyte complex micelles (PCMs) was prepared to investigate the consequences of neutral and zwitterionic chemistries and distinct charged cores on the size and stability of nanocarriers. Using aqueous reversible addition-fragmentation chain transfer (RAFT) polymerization, we synthesized a well-defined diblock polyelectrolyte system, poly(2-methacryloyloxyethyl phosphorylcholine methacrylate)-*block*-poly((vinylbenzyl) trimethylammonium) (PMPC-PVBTMA), at various neutral and charged block lengths to compare directly against PCM structure-property relationships centered on poly(ethylene glycol)-*block*-poly((vinylbenzyl) trimethylammonium) (PEG-PVBTMA) and poly(ethylene glycol)-*block*-poly(lysine) (PEG-PLK). After complexation with a common polyanion, poly(sodium acrylate), the resulting PCMs were characterized by dynamic light scattering (DLS) and small angle X-ray scattering (SAXS). We observed uniform assemblies of spherical micelles with a core diameter of ~40 nm when PMPC-PVBTMA was used, and smaller particles between 20-30 nm for PEG-PLK and PEG-PVBTMA via SAXS analysis. Additionally, PEG-PLK PCMs proved most resistant to dissolution by both monovalent and divalent salt, followed by PEG-PVBTMA then PMPC-PVBTMA. All micelle systems were serum stable in 100% fetal bovine serum over the course of 8 h by time-resolved DLS, demonstrating minimal interactions with serum proteins and potential as in vivo drug delivery vehicles. This thorough study of the synthesis, assembly, and characterization of zwitterionic polymers in PCMs advances the design space for charge-driven micelle assemblies.

1. Introduction

Polyelectrolyte complex assemblies have the unique ability to interface with an array of biologics [1-4]. This liquid-liquid phase separation process upon mixing oppositely-charged polyelectrolyte solutions can be advantageous for partitioning biomolecules into compartmentalized domains [5-8]. When the complex domain is confined to the core of polyelectrolyte complex micelles (PCMs) with neutral-charged block polycation and/or polyanion architectures, therapeutically relevant cargo can be captured, protected by the outer hydrophilic corona, and released in response to changes in pH or ionic strength. In terms of polymer selection for the outer stealth layer, poly(ethylene glycol) (PEG; synonymously polyethylene oxide or PEO) is one of the most common ways to facilitate steric stabilization from aggregation and facilitate biocompatibility. PEG-containing PCMs can self-assemble into discrete nanoparticles with controlled size and morphologies at dilute polymer conditions [9,10] and resemble ordered gels at higher polymer concentrations [11-14]. However, certain limitations of PEG have become identified in the past decade. For biological applications, this includes hypersensitivity after drug administration, deteriorating oxidative stability over time, and accelerated systemic clearance upon repeated dosage [15-17]. Thus, there has been substantial effort to explore PEG alternatives that can instill colloidal stability in PCM carriers in aqueous environments while mitigating some of these drawbacks [18-23].

One promising alternative includes polyampholytes, or polyelectrolytes with both cations and anions along the chain. Polyzwitterions are polyampholytes with paired cation and anion moieties per repeat unit along the electrostatically neutral chain [24,25]. The 2-methacryloyloxyethyl phosphorylcholine (MPC) methacrylate monomer, in particular, has become transformative as a building block for polyzwitterions in emerging biomaterials and

biodevices [26], as the phospholipid polar groups provide hydration but does not disturb the hydrogen bonding of water [27]. When polymerized to poly(2-methacryloyloxyethyl phosphorylcholine methacrylate) (PMPC), the zwitterionic phosphorylcholine moieties endow high resistance to nonspecific protein adsorption, cellular adhesion, and blood coagulation, as well as cell membrane penetration, molecular recognition, and underwater lubrication [28,29]. These bioinert “stealth” properties have been exploited in antifouling [30] and water treatment [31] materials, bioengineering [32], and nanomedicine applications [33]. Furthermore, the anti-polyelectrolyte effect, where certain polyzwitterion chains swell (increased coil size and viscosity) with added salt [34-36], has been shown to not affect the solution conformation of PMPC [37,38]. These combined attributes have allowed PMPC to emerge as a versatile material platform for a diverse array of bioapplications.

When adapted to hydrophilic coronas in nanocarriers, several reports that have directly compared PMPC to PEG have shown enhanced delivery for siRNA polyplexes [33], bovine serum albumin protein nanoparticles [39], α -chymotrypsin proteins [40], and papain enzyme bioconjugates [41]. These PMPC-containing block polymers are often synthesized using reversible-deactivation radical polymerization (RDRP). Lowe and McCormick provide an excellent review on the controlled synthesis of zwitterionic polymers [42], followed by more recent examples of PMPC systems in the literature prepared using atom transfer radical polymerization (ATRP) [43-46] or reversible addition-fragmentation chain transfer (RAFT) polymerization [33,47,48]. Thus, the use of RDRP to produce PMPC block polyelectrolytes for fundamental self-assembly investigations can lead to more efficacious PCM vehicles in terms of biomolecule storage and delivery. For example, Takahashi and coworkers have reported on how NaCl can trigger morphological transitions in PCM assemblies comprising RAFT PMPC block

polyelectrolytes [49]. These results have advanced our overall understanding of how to tailor the static and dynamic properties of polyelectrolyte complex assemblies.

The objective of this work is to evaluate and compare the formulation and stability of polyelectrolyte-based nanocarriers, comprising PMPC and PEG as hydrophilic coronas and chemically distinct charged cores. Using aqueous RAFT polymerization, we have previously established a synthetic framework to prepare designer block polyelectrolytes from PEG macromolecular RAFT chain transfer agents [50]. Specifically, the diblock polycation PEG-*block*-poly((vinylbenzyl)trimethylammonium chloride) (PEG-PVBTMA) has been paired with various synthetic polyanion architectures, including poly(sodium acrylate) (PAA) [50-53], PEG-*block*-poly(styrene sulfonate) [50,54], and DNA oligonucleotides [55,56], to form assemblies of varying size, shape, and responsivity to salt. Because of the ease and versatility of RAFT chemistry for the production of stimuli-responsive materials [57], these endeavors have enabled us to establish structure-property relationships with special attention to effects of modulating charged block length, chemical identity, and polyelectrolyte pairing. Figure 1 depicts the extension of this molecular engineering effort by comparing PCMs of PAA with three diblock polycations: PEG-*block*-poly(lysine) (PEG-PLK), PEG-PVBTMA and PMPC-PVBTMA. For ease of nomenclature and distinction, we refer to PMPC-containing systems as zwitterionic PCMs (zPCMs).

Figure 1. Chemical structures of polyelectrolytes and illustration of the rational pairings to investigate the chemical effects of the complex core and neutral corona in complex coacervate micelles. Diblock polycations include poly(ethylene glycol)-*block*-poly(lysine) (PEG-PLK), poly(ethylene glycol)-*block*-poly((vinylbenzyl)trimethylammonium chloride) (PEG-PVBTMA), and poly(2-methacryloyloxyethyl phosphorylcholine methacrylate)-*block*-poly((vinylbenzyl)trimethylammonium chloride) (PMPC-PVBTMA). Poly(sodium acrylate) (PAA) was used as the polyanion for all cases. See Table 1 for polymer length and characterization.

2. Materials and Methods

2.1 Materials

Certain commercial equipment and materials are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendations by the National Institute of Standards and Technology (NIST) nor does it imply that the material or equipment identified is necessarily the best available for this purpose.

The following chemicals were reagent grade and used as received unless otherwise specified: poly(ethylene glycol)-*block*-poly(lysine) (Alamanda Polymers; Huntsville, AL, USA), poly(ethylene glycol) methyl ether (2-methyl-2-propionic acid dodecyl trithiocarbonate) (Sigma, Reported M_n 10,000 and 5000 g/mol; verified experimentally by ^1H NMR spectroscopy and SEC-MALS with $D = 1.02$ and 1.01 , respectively), 2-methacryloyloxyethyl phosphorylcholine (MPC, Sigma, 97%), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid N-succinimidyl ester (CPSE, Sigma), 2-(butylthiocarbonothioylthio)propanoic acid (BuPA, Boron Molecular, $\geq 95\%$), sodium acrylate (NaA, Sigma, 97%), (vinylbenzyl)trimethylammonium chloride (VBTMA, Sigma, 99%), 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044, Wako Chemicals, USA), acetic acid (glacial, Sigma, $\geq 99.85\%$), sodium acetate trihydrate (Sigma, $\geq 99\%$), sodium chloride (Sigma, $\geq 99.0\%$), trifluoroacetic acid (TFA, ReagentPlus®, 99%), sodium nitrate (NaNO_3 , ACS reagent, $\geq 99.0\%$), sodium azide (NaN_3 , ReagentPlus®, $\geq 99.5\%$), Fetal Bovine Serum (ES Cell-Qualified, Thermo Fisher, USA), pooled normal human AB serum (Innovative Research Inc., Novi, Michigan), and SnakeSkin dialysis tubing (MWCO 3.5K, 22 mm, Thermo Scientific).

For RAFT polymerizations, monomers VBTMA and NaA were used as received. MPC monomer was filtered with diethyl ether to remove trace inhibitor, vacuum dried, and stored under dried nitrogen until use. Acetate buffer solution (pH 5.2) was prepared from 0.1 M acetic acid and

0.1 M sodium acetate trihydrate (42/158, v/v). Water was filtered from a Milli-Q water purification system at a resistivity of 18.2 MΩ-cm at 25 °C.

2.2 RAFT Synthesis of PEG-PVBTMA, PAA, and PMPC-PVBTMA

We synthesized PEG-PVBTMA with aqueous RAFT polymerization from a PEG macromolecular chain transfer agent (macro CTA), according to parallel synthesis and characterization procedures described by Ting et al. [30] previously. PAA was synthesized using BuPA as a RAFT chain transfer agent (CTA) in aqueous settings and characterized. Detailed molecular characterization is provided in the Supplementary Materials Section S1.

Scheme 1 depicts the synthesis of PMPC and PMPC-PVBTMA. The following describes a representative RAFT protocol: To a dried round bottom flask, the chemical precursors (monomer, RAFT CTA, and VA-044 initiator) were combined in solvent and sealed. A ratio of 10:1 equivalence of RAFT CTA to initiator ($[CTA]_0/[I]_0 = 10:1$) was used. The flask was degassed for at least 30 min with dried nitrogen sparging, before running the reaction at 50 °C under constant stirring for >18 h. The final monomer conversion was confirmed by ^1H NMR spectroscopy before quenching by cooling to room temperature and opening the reactor to air. The crude red-orange mixture was dialyzed against Milli-Q water for 3 cycles of 8 h each, lyophilized, and characterized by size-exclusion chromatography with multiangle light scattering, ^1H NMR spectroscopy, and thermogravimetric analysis.

Scheme 1. Synthesis of PMPC-*b*-PVBTMA via RAFT Polymerization.

2.2 Size-Exclusion Chromatography with Multiangle Light Scattering (SEC-MALS)

SEC measurements were conducted on a Waters liquid chromatogram (well-stirred mobile phase reservoir, Waters In-Line Degasser AF, Waters 1515 Isocratic HPLC pump, Waters 2707 Autosampler) equipped with three Waters columns (Ultrahydrogel Column 500, Ultrahydrogel Column 120, and Ultrahydrogel Column 250) maintained at 35 °C with pore sizes suitable for materials with effective molecular weights from 1000 to 400,000 g/mol. The SEC is equipped with a Waters 2998 photodiode array detector, a miniDAWN TREOS II multi-angle laser light scattering (MALS) detector at a laser wavelength of 658 nm (3 angles), and a Wyatt Optilab T-rEX refractive index detector. Empower 3 and ASTRA® 6 Software were used for setting up the experiments and analyzing data, respectively.

Samples were dissolved and immediately run. For PEG and PMPC, 0.1 M NaNO₃ + 0.01% NaN₃ was run as the mobile phase at 1.0 mL/min at 35 °C. For PAA, 0.3 M NaNO₃ + 0.01 M NaH₂PO₄ at pH 7 was run as the mobile phase at 1.0 mL/min at 25 °C. For diblock cationic polyelectrolytes, a mixture of acetonitrile and water (40/60, %, v/v) with 0.1% trifluoroacetic acid was run as the mobile phase at 1.0 mL/min at 35 °C. The change in refractive index value (dn/dc) values were determined using an Abbe refractometer with a red light-emitting diode as a light

source in the appropriate SEC mobile phase at 25 °C, in which refractive index values at various polymer concentrations were collected in triplicate (Supplementary Figure S-X). The dn/dc of each block polyelectrolyte $(dn/dc)_{block}$ was calculated using Equation 1, which assumes that the $(dn/dc)_{block}$ is a weighted linear combination (i.e., the weight fraction of each block) of the change in each refractive index value of individual polymers A and B .

$$\left(\frac{dn}{dc}\right)_{block} = w_A \left(\frac{dn}{dc}\right)_A + (1 - w_A) \left(\frac{dn}{dc}\right)_B \quad (1)$$

2.3 Proton Nuclear Magnetic Resonance (1H NMR) Spectroscopy

1H NMR measurements were collected using a Bruker AVANCE III HD 400 Mhz NanoBay spectrometer with 16 transients to minimize signal-to-noise. 1H NMR spectra were processed and analyzed using iNMR (Version 5.5.7).

A representative 1H NMR spectrum of the PMPC homopolymer is shown in Figure 2. 1H NMR (400 MHz, D_2O) δ : 0.6-1.3 ppm (2H, $-CH_2-$), 1.6-2.2 ppm (3H, $-CH_3$), 2.9-3.2 ppm (9H, $-N-(CH_3)_3$), 3.3-3.6 (2H, $-CH_2-N-$), and 4.0-4.3 (6H, $-CH_2-O-$).

Figure 2. Representative 1H NMR of purified PMPC in D_2O .

A representative ^1H NMR spectrum of the PMPC-*b*-PVBtMA homopolymer is shown in Figure 3. The calculated chemical composition of VBTMA was determined to be 75%, in good agreement with the targeted chemical composition of VBTMA (68%). ^1H NMR (400 MHz, D_2O) δ : 0.7-2.1 ppm (alkyl backbone), 2.6-3.0 ppm (9H, $-\text{N}(\text{CH}_3)_3$), 3.0-3.3 ppm (9H, $-\text{N}(\text{CH}_3)_3$), 3.5-3.7 ppm (2H, $-\text{CH}_2\text{N}-$), 3.8-4.5 ppm (6 H, $-\text{CH}_2\text{O}-$; 2H, $-\text{CH}_2\text{N}-$), and 6.1-7.4 ppm (4H, ArH).

Figure 3. Representative ^1H NMR of purified PMPC-PVBtMA in D_2O .

2.4 Thermogravimetric Analysis (TGA)

TGA was conducted using a TA Instruments Discovery TGA equipped with an infrared furnace, auto-sampler, and a gas delivery module. Nitrogen was used as the purge gas set at a flow rate of 10 mL/min; a heating rate of 10 $^{\circ}\text{C}/\text{min}$ was set for all samples. TA TRIOS software (Version 2.2) was used to analyze the thermal transitions.

2.5 Micelle Preparation

PCMs were prepared via rapid mixing of water, NaCl or MgCl_2 , and polyelectrolyte solutions. Water and salt were first mixed with the neutral-cationic diblock polymer, mixed thoroughly with a vortexer, and followed by the polyanion and additional rapid mixing. The polyelectrolytes were added from a 5 mg/mL stock for a final concentration of 3 mM charge concentration for both the cation and anion. For PEG-PLK and PEG-PVBTMA systems, reversing the order had no effect on the final assembly structure. For PMPC-PVBTMA, we unexpectedly observed a dependency of adding the diblock polycation to the polyanion (or vice versa) on the micelle size and response to salt, as shown in Figure S-38. The origin of this phenomenon is the subject of ongoing investigation.

2.6 Dynamic Light Scattering (DLS)

DLS measurements were made using a Brookhaven Instruments BI-200SM Research Goniometer System with an incident laser ($\lambda = 637 \text{ nm}$) at room temperature. A dust-free decalin bath was used to match the refractive index of glass. The angular dependence of D was acquired by plotting q^2 versus decay rate Γ . A linearity over a range of scattering angles is a good indication of isotropic scatters. The average hydrodynamic radius of scatterers under Brownian diffusion was calculated by the Stokes-Einstein relationship. The correlation function at each angle was fitted to a first-order single exponential using a MATLAB code. The size distribution was obtained using the REPES algorithm. Kinetics experiments were done on the same instrument by recording data every 5 min for up to 16 h in a sealed sample container to minimize evaporation.

For the low salt (0-200 mM NaCl) screening studies, experiments were carried out on a 163.5° single-angle Wyatt Möbiuζ instrument (Santa Barbara, CA) with an incident laser ($\lambda = 532$

nm) at 20 °C. Prepared micelle solutions were directly transferred to quartz micro-cuvettes. The size and colloidal polydispersity index were analyzed using DYNAMICS software.

2.7 Small-Angle X-ray Scattering (SAXS)

SAXS measurements were made at beamline 12-ID-B of the Advanced Photon Source (APS) at Argonne National Laboratory (Lemont, IL, USA) and at beamline 4-2 of the Stanford Synchrotron Radiation Lightsource (SSRL) at SLAC National Accelerator Laboratory. Glycerol was added to all samples for a final concentration of 1% by volume to prevent sample degradation.

For APS experiments, micelle samples were irradiated in a thin-wall glass capillary flow cell with a photon energy of 14 keV. Data were reduced in MATLAB at the beamline. Background subtraction and fitting were performed using the multi-level modeling macros distributed with the Irena software package [58] for Igor Pro as described in Ref [56].

For SSRL experiments, the sample-to-detector distance was set to 3.5 m with measurements collected at a photon energy of 9 keV. Aliquots of 30 μ L of the polyelectrolyte micellar suspension were loaded onto the automated fluid sample loader at the beamline. At least 20 consecutive 1 s exposures were collected first from the buffer background (water, or water/salt mixtures), followed by samples. During data collection, solutions were oscillated in a stationary quartz capillary cell to maximize the exposed volume and reduce the radiation dose per unit volume. The SAXS data were radially integrated, analyzed for radiation damage, and buffer subtracted using the automated data reduction pipeline available at the beam line. Only data that did not show any evidence of radiation damage were included in the final average for each sample.

2.8 Micelle Salt Dependence

Micelles were prepared in numerous salt solutions and compared via SAXS and DLS. High salt concentrations disrupt complex formation as large amounts of counterions compete with ion pairing. This is noticeable as SAXS and DLS intensity decrease and nanoparticles lose shape and dissolve into solution. The salt resistance of a polyelectrolyte complex can be used as a measure of ion pair stability in the assembly material.

2.9 Micelle Stability Tests

Micelle solutions were prepared in water with no salt at 4.5 mg/mL of total polymer. These solutions were then diluted 5x in 100% Fetal Bovine Serum (FBS), and DLS measurements were taken at $\theta = 90^\circ$ scattering angle at 25 °C for 2 min collection time every 5 min. The autocorrelation function and light scattering intensity were recorded for an 8 hr period to observe changes in the size of detectable particles in the presence of FBS media.

3. Results

3.1 PMPC-PVBTMA Synthesis and Characterization

We have previously demonstrated the consequences of modifying the chemical nature of the core in PCMs for the complexation of oligonucleotides with PLK or PVBTMA units [55]. Other studies that employ block polyelectrolytes with PEG as the neutral block have also highlighted analogous design considerations [13,59]. However, far fewer PCM studies have focused on the fundamental PCM properties of zwitterionic block polyelectrolytes that form core-shell structured micelles. To this end, we sought to prepare direct polycation analogs to PEG-PLK and PEG-PVBTMA and evaluate aspects of PCM formulation with PAA as a model polyanion. For nomenclature throughout this work, subscripts next to the neutral and charged polymer block represent the molar mass and number-average degree of polymerization, respectively.

As shown in Scheme 1, the RAFT synthesis of PMPC-PVBTMA diblock polyelectrolytes were achieved using CPSE as a suitable RAFT CTA for MPC monomer in deoxygenated methanol at 50 °C, followed by chain extension of the PMPC macromolecular CTA with VBTMA monomer in deoxygenated acetate buffer/ethanol at 50 °C. Each reaction was run overnight (>18 h). The experimentally measured degree of polymerization (DP) of the charged block was similar to the targeted values, affording PMPC_{5K}-PVBTMA₅₀ and PMPC_{10K}-PVBTMA₉₇. ¹H NMR studies indicated that the RAFT synthesis in both steps was successful. Additionally, aqueous SEC-MALS showed distinct monomodal peaks of the PMPC precursor and PMPC-PVBTMA diblocks in the optimized mobile phase solutions. Representative SEC refractive index traces are shown in Figure 4. We observe a very small fraction of unreacted PMPC by SEC-MALS, which likely represents dead chains that lost end-group fidelity.

Figure 4. Representative SEC refractive index traces of PMPC_{10K} (blue curve, bottom) and PMPC_{10K}-*b*-PVBTMA₁₀₀ (red curve, top).

Table 1 summarizes the characterization of the polymer number-average molecular weight (M_n), dispersity ($\bar{D}$), and degradation temperature (T_d). Data on the PEG-PLK samples are reported as received. For all diblock polyelectrolyte systems, the M_n distribution remained relatively narrow ($\bar{D} < 1.2$). By thermogravimetric analysis, two distinct thermal degradations corresponded to each neutral and charged block (Supplementary Figure S-8). Thus, the RAFT chain extension protocols for PEG-PVBTMA and PMPC-PVBTMA were controlled and successful in generating neutral-charged diblock polyelectrolytes. The PAA polyanion was also synthesized with aqueous RAFT chemistry targeting a DP of 50 and fully characterized ($M_n = 4.9$ and 5.0 kg/mol by SEC-MALS and NMR end-group analysis, respectively; $\bar{D} = 1.11$). Details of the RAFT synthesis and characterization of PAA are described in the Supplementary Information. In short, these six uniform diblock polycations were used for complexation with PAA to prepare PCMs for solution studies.

Table 1. Summary of Polymer Characterization.

System ^a	$M_{n, SEC}$ ^b (kg/mol)	$\bar{D}$ ^c	T_d ^d (°C)
PEG _{5K} -PLK ₄₇	13.0	1.06	314, 420
PEG _{10K} -PLK ₉₃	25.6	1.06	320, 404
PEG _{5K} -PVBtMA ₅₃	18.3	1.12	199, 378
PEG _{10K} -PVBtMA ₁₀₀	29.1	1.17	212, 366
PMPC _{5K} -PVBtMA ₅₀	27.8	1.12	211, 461
PMPC _{10K} -PVBtMA ₉₇	40.0	1.10	218, 460
PAA ₅₀	4.9	1.11	N/A

^a Subscripts denote the molar mass of the PEG/PMPC block and the degree of polymerization of the PLK/PVBtMA block from ¹H NMR spectroscopy, respectively. Characterization of PEG_{5K}-PLK₄₇ and PEG_{10K}-PLK₉₃ are reported from the manufacturer. ^b Experimentally-measured number-average molecular weight, determined by SEC-MALS. ^c Dispersity ($\bar{D}$) = M_w / M_n . ^d Degradation temperature (T_d) by TGA.

3.2 Polyelectrolyte Complex Micelle (PCM) and Zwitterionic PCM (zPCM) Assembly, Salt Resistance, and Stability

In order to elucidate the effect these physical and chemical differences have on micelle structure, we used the six polymers from Table 1 to separately complex with PAA(50) as a common polyanion, to form micelles in water. PCMs and zPCMs were assembled via direct dissolution and rapid mixing into the desired salt solution. Three polymer chemistries are being compared (PEG-PLK, PEG-PVBtMA, and PMPC-PVBtMA) at two lengths each (i.e., a 10 kg/mol neutral block with ~100 unit cation versus a 5 kg/mol neutral block and ~50 unit cation). For nomenclature, these systems will be referred to as neutral(10k)-charged(100) and neutral(5k)-charged(50). First, we screened the assembly size and size distribution of PEG-PVBtMA and PMPC-PVBtMA systems as a function of salt with a single-angle dynamic light scattering (DLS)

instrument. In a low salt regime from 0-75 mM NaCl, no difference in salt resistance was detected (Supplementary Figure S-9). At 100 mM NaCl, we observed an increase in the polydispersity of the zPCMs for both neutral(10k)-charged(100) and neutral(5k)-charged(50) lengths. In comparison, the PCMs for both systems with an outer PEG corona exhibited no change in aggregation through 200 mM NaCl.

To better understand this salt-driven transition in PCM and zPCM assembly, small angle X-ray scattering (SAXS) was used to more precisely quantify the micelle shape and the size of the core and corona, providing complete structural morphology for each system. Figure 5 shows SAXS results for each neutral(10k)-charged(100) system in 100 mM NaCl. For all six systems, we observe micelle formation at 100 mM NaCl. PEG-PVBTMA and PMPC-PVBTMA systems form spherical micelles, apparent from the flat slope at low q , while PEG-PLK forms worm-like micelles (q^{-2} dependence) when complexed with PAA. We also notice a consistent core size difference where PMPC-PVBTMA is the largest and PEG-PLK is the smallest, determined by SAXS fitting results displayed in Table 2 in the next section below. This was consistent with the DLS screening results. Furthermore, longer cationic blocks form larger micelle cores throughout this study, consistent with previous reports [52,56] of micelle size being solely dependent on the charged block length of the block copolymer and independent of homopolymer length.

Figure 5. Comparison of the A) apparent size hydrodynamic radius distribution of neutral(10k)-charged(100) micelles at 100 mM NaCl, analyzed by REPES analysis at the 90° angle, and the B) corresponding small angle X-ray scattering data (colored markers) and fits (black dashed lines). Intensity scaled for clarity; from bottom to up in both plots, the systems correspond to PEG-PLK (orange squares), PEG-PVBTMA (blue circles), and PMPC-PVBTMA (green diamonds).

Next, we aimed to evaluate the salt resistance and stability of the prepared PCMs and zPCMs with multi-angle DLS and SAXS modeling. We focused on the 0 mM and 100 mM NaCl conditions as a point of comparison. For both salt concentrations across all six systems, the hydrodynamic radius (R_h) and colloidal polydispersity ($PDI = \mu_2 / \Gamma^2$) were determined by fitting the autocorrelation function to a cumulant expansion [60] and via regularized positive exponential sum (REPES) analysis [61] (Supplementary Table S-1). Excellent agreement in micelle size and

low polydispersity between both methods were reported, again with a consistent hydrodynamic size difference where PMPC-PVBTMA was the largest and PEG-PLK was the smallest.

Recent reports have emphasized the importance of the charged group and neutral block chemistry for influencing complex micelle stability. In general, stability can be quantified by salt resistance – the critical salt concentration at which a complex assembly comes apart and dissolves into solution due to the abundance of ions in solution. In other words, electrostatic interactions are screened, thereby reducing the effective charge density holding the nanocarrier assembly together. In the individual polyelectrolyte solutions of PEG-PLK, PEG-PVBTMA, and PMPC-PVBTMA, there was no observed difference in the disappearance of the signature correlation peak that is prominent in the SAXS profile by adding up to 500 mM NaCl (Supplementary Figure S-40). This suggests that the conformation of the neutral-charged polycation chains respond to salt in a similar manner. In order to probe the stability of these systems complexed with PAA(50) and their behavior in higher concentrations of monovalent and divalent salts, we formed PCMs and zPCMs in 100, 250, and 500 mM NaCl, as well as 50 and 100 mM MgCl₂.

Figure 6 shows the SAXS results for the neutral(10k)-charged(100) cases, and fitting results are available in Table 2. The data for neutral(5k)-charged(50) systems show similar trends and can be found in Supplementary Figures S40-S45. For PEG-PLK PCMs, minimal structural differences or changes in scattering intensity were seen across all conditions, signifying strong complexation. PEG-PVBTMA formed PCMs at 100 and 250 mM NaCl and 50 mM MgCl₂ but not at higher salts. PMPC-PVBTMA exhibited the weakest complexation, with well-formed zPCMs only at 100 mM NaCl. Additionally, both PVBTMA systems revealed a loss in intensity and increase in size dispersity with increased salt, which was not present in the PEG-PLK system. PLK has been shown to form stronger ion pairs with DNA in previous literature [55], and here we

observe a consistent trend with PAA. We find a drastic discrepancy in salt stability due to the neutral versus zwitterionic block. PMPC-PVBTMA is unable to form micelles at 50 mM MgCl_2 and forms extremely polydisperse poorly formed micelles at 250 mM NaCl, conditions that are favorable for micelle formation when using the PEG variant. Additional SAXS data and models are available in Supplementary Figures S40-S45.

Figure 6. Small angle X-ray scattering data (color) and fits (black) for neutral(10k)-charged(100) micelles in a range of A) NaCl and B) MgCl_2 concentrations. PEG-PLK micelles are most resistant to salt, followed by PEG-PVBTMA, then PMPC-PVBTMA. Data are not scaled for this figure to show decreasing intensity with added salt.

Table 2. SAXS fit parameters summary with added NaCl salt.

Sample	[NaCl] (mM)	R ^a (nm)	PDI ^b	Aspect Ratio
PEG _{5K} -PLK ₄₇ / PAA ₅₀	100	7.2	0.14	-
	250	6.3	0.22	-
	500	8.7	0.13	-
PEG _{10K} -PLK ₉₃ / PAA ₅₀	100	10.8	0.08	-
	250	10.7	0.15	-
	500	11.8	0.10	-
PEG _{5K} -PVBtMA ₅₃ / PAA ₅₀	100	12.3	0.03	1.4
	250	9.8	0.23	1.03
	500	-	-	-
PEG _{10K} -PVBtMA ₁₀₀ / PAA ₅₀	100	13.7	0.01	1.65
	250	12.9	0.07	1.24
	500	-	-	-
PMPC _{5K} -PVBtMA ₅₀ / PAA ₅₀	100	17.7	0.03	1.69
	250	16.2	0.09	1.17
	500	-	-	-
PMPC _{10K} -PVBtMA ₉₇ / PAA ₅₀	100	19.4	0.05	1.36
	250	10.0	0.44	1.11
	500	-	-	-

^a Mean radius (nm) ^b Polydispersity index (σ^2/R^2). Models use Schulz-Zimm Spheroid and Unified Level fits, except for PEG_{5K}-PLK₄₇ / PAA₅₀ (flexible cylinder fitting) and PEG_{10K}-PLK₉₃ / PAA₅₀ (cylinder fitting).

3.3 PCMs and zPCMs in Biologically Relevant Media

Since micelle stability is crucial for systemic circulation and controlled release, we investigated the structural integrity of prepared PCMs and zPCMs in biologically relevant media, FBS. The temporal evolution of the micelles in full serum was monitored with static scattering intensity (I). As previously shown by Lin and coworkers [62], this technique provides a straightforward way to detect micelle-protein interactions shown in Equation 1:

$$I \sim KcM_w$$

[1]

K is the prefactor constant from the optics setup, c is the sample mass concentration, and M_w is the weight-average molecular weight of the suspended sample. The amount of light scattered is directly proportional to the molar mass and concentration of nanoparticles in solution. Because the pristine micelles that were prepared from all polyelectrolyte pairings are larger than the serum proteins in the FBS control sample (Figure S-46), the total I will increase if the M_w increases as a result of strong associative interactions between proteins and micelles.

For representative neutral(10k)-charged(100) PEG-PLK, PEG-PVBTMA, and PMPC-PVBTMA micelles in FBS, Figure 7 provides a summary of the average change in I over the course of 8 h, along with the apparent size distribution at 1 and 10 h. Experiments were conducted multiple times to ensure repeatability (see Figure S-46 for repeats). The FBS negative control showed no evidence of aggregation or sedimentation over 8 h. It should be noted that FBS media exhibits a broad bimodal distribution, so the PCMs cannot be distinguished from serum proteins. In the stability tests, the total I for both the PEG-PLK and PEG-PVBTMA systems remained relatively constant, while the PMPC-PVBTMA system increased by approximately 10% in I on average with greater variation over time. REPES analysis of the PMPC-PVBTMA system shows the growth of ~200 nm aggregates over 1 to 10 h. This slight stability issue may be mitigated by increasing the degree of polymerization of PMPC, as the total length of the zwitterionic corona in micelles have been previously shown to affect nanoparticle stabilization and delivery efficacy [33]. Nevertheless, because proteins adsorption onto nanoparticle carriers in biological settings is a known design consideration for micelle assemblies [63,64], the prepared PCMs and zPCM show good overall resistance to destabilization by serum.

Figure 7. A) Total light scattering intensity at 90° angle over 8 h of 100% FBS (gray circles) and 1:5 dilution of PEG-PLK (orange squares), PEG-PVBTMA (blue circles), and PMPC-PVBTMA (green diamonds) micelles with 100% FBS. Data points and error bars denote the average and standard deviation of 2 replicates; intensity is scaled for clarity. B) The apparent size hydrodynamic radius distribution of respective micelles at 1 h (solid markers) and 10 h (open markers), analyzed by REPES analysis at the 90° angle. Intensity is scaled for clarity.

4. Discussion

In alignment with greater efforts reported recently on elucidating the interplay between electrostatics and other non-covalent associations on polyelectrolyte complexation [65,66], this work provides a central platform for designing charged micelle-based nanocarriers with fundamental structure-property relationships. Neutral blocks in PCMs and zPCMs force nanophase separation and provide a protective corona around a charged polymer core, which often contains a sequestered cargo. Understanding the physical effects attributed to zwitterionic and neutral polymer coronas, and chemically distinct charged polymer cores, expands the design space

and versatility of PCMs and zPCMs. Altogether, we have prepared a series of polyelectrolyte-based nanocarriers from PEG-PLK, PEG-PVBTMA, and PMPC-PVBTMA with PAA, characterized the self-assembled micelle structure in water as a function of salt, and evaluated long-term stability in biologically relevant media. Aqueous RAFT polymerization was used to synthesize a well-defined zwitterionic diblock polyelectrolyte system PMPC-PVBTMA at various block lengths. Upon complexation with PAA, the zPCMs exhibited ~40 nm spherical micelles. These uniform assemblies were slightly larger than the PCMs formed from PEG-PLK and PEG-PVBTMA (~20-30 nm).

Characterizing micelle structure and disassembly in the presence of monovalent and divalent salts provide a measure of complex stability. Our results show that complexes formed by PEG-PLK are much more stable against salt, followed by PEG-PVBTMA which is slightly more resistant to salt than PMPC-PVBTMA, the least stable system. This result is consistent for both neutral(5k)-charged(50) and neutral(10k)-charged(100) block lengths. Overall, this study examines neutral and zwitterionic chemistries used as polymer coronas in PCMs to protect sequestered cargo and to control particle size, stability, and environmental responsiveness.

Supplementary Materials: The following are available online: polymer synthesis data, dynamic light scattering data and fitting results, and small-angle X-ray scattering data and fitting results.

Author Contributions: Conceptualization: A.E.M. and J.M.T.; Polymer synthesis and characterization: J.M.T. and J.D.M.; X-ray scattering and analysis: A.E.M. and J.M.T. Light scattering and analysis: A.E.M., J.M.T., J.D.M., and T.C. Research direction and funding: M.V.T. All authors participated in interpreting the data and preparing the manuscript.

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Comparing Zwitterionic and PEG Exterior of Polyelectrolyte Complex Micelles

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S1. Supplemental Polymer Synthesis Data

PMPC-PVBTMA Synthesis. Figure S-1 shows a representative crude ^1H NMR of PMPC, resulting in 81% total monomer conversion after 18 h. Figure S-2 shows a representative crude ^1H NMR of PMPC-PVBTMA, resulting in 93% total monomer conversion after 18 h. Figure S-3 shows the purified ^1H NMR of PMPC_{5K}-PVBTMA₅₀.

Figure S-1. Crude ^1H NMR of PMPC in D_2O .

Figure S-2. Crude ^1H NMR of PMPC-PVBTMA in D_2O .

Figure S-3. ^1H NMR of PMPC_{5K}-PVBTMA₅₀ in D₂O.

PAA Synthesis. Figures S-4 and S-5 show the ^1H NMR of the BuPA RAFT CTA and the homopolymer PAA prepared with aqueous RAFT polymerization, respectively. End-group analysis ($51 \times 94.04 \text{ g/mol} + 238.39 \text{ g/mol}$) resulted in a calculated $M_n = 5030 \text{ g/mol}$, in excellent agreement with SEC-MALS characterization. Figure S-6 shows the SEC refractive index trace of PAA, which consists of a monomodal peak exhibiting narrow dispersity in the M_n distribution.

Figure S-4. ^1H NMR of BuPA in CDCl₃.

Figure S-5. ^1H NMR of PAA in D_2O .

Figure S-6. SEC refractive index trace of PAA ($M_n = 4900$ g/mol, $D = 1.11$).

Refractive Index Measurements. To determine the absolute M_n of the polymers measured by SEC-MALS, we employed a refractometer to measure the dn/dc of polymers in their respective mobile phase. For PMPC-PVBTMA samples, Figure S-7 shows the dn/dc determination of the individual homopolymers. Using Equation 1 shown in the main manuscript, we calculated the dn/dc of the PMPC_{5K}-PVBTMA₅₀ and PMPC_{10K}-PVBTMA₁₀₀ to be 0.1661 and 0.1663 mL/g, respectively.

Figure S-7. Measured refractive index values versus polymer concentration of (A) PMPC and (B) PVBTMA. All measurements were taken using polymers completely dissolved in the cationic mobile phase solution at 25 °C. The circles on the left plot show the raw data; the circles on the right plot show the average of triplicate measurements with the dashed line denoting a linear regression to determine dn/dc .

Thermogravimetric Analysis Curves. Figure S-8 shows the TGA profiles of synthesized polyelectrolytes. All experiments were conducted at 15 °C/min.

(A) **(B)**

Figure S-8. TGA curves for block polymers (A) PEG_{5K}-PLK₅₀, PEG_{5K}-PVBtMA₅₃ and PMPC_{5K}-PVBtMA₅₀, as well as (B) PEG_{10K}-PLK₁₀₀, PEG_{10K}-PVBtMA₁₀₀ and PMPC_{10K}-PVBtMA₉₇. All experiments were conducted at 15 °C/min.

S2. Supplemental Dynamic Light Scattering Data

Figure S-9 shows a gallery of histograms for all prepared PCM samples from 0 mM NaCl to 200 mM NaCl. The autocorrelation functions for these samples are shown in the Figures S-35 through S-38 below.

Figure S-9. Apparent size hydrodynamic radius distribution of (A) PEG_{5K}-PVBtMA₅₃ / PAA₅₀, (B) PEG_{10K}-PVBtMA₁₀₀ / PAA₅₀, (C) PMPC_{5K}-PVBtMA₅₀ / PAA₅₀, and (D) PMPC_{10K}-PVBtMA₉₇ / PAA₅₀ as NaCl salt is increased from 0 mM to 200 mM (left to right).

Table S-1 summarizes the multi-angle DLS experiments for all prepared PCMs at 0 mM NaCl and 100 mM NaCl. The autocorrelation functions were analyzed with a cumulant expansion as well as REPES analysis at the 90° scattering angle.

Table S-1. DLS summary of all prepared PCMs at 0 mM NaCl.

Sample	R_h^a (nm)	PDI ^b	Mean $R_{h, \text{REPES}}^c$ (nm)	SD $R_{h, \text{REPES}}^d$ (nm)
PEG _{5K} -PLK ₄₇ / PAA ₅₀	16.4 ± 0.1	0.18 ± 0.03	18.6	2.8
PEG _{10K} -PLK ₉₃ / PAA ₅₀	21.1 ± 0.2	0.11 ± 0.03	22.8	1.3
PEG _{5K} -PVBtMA ₅₃ / PAA ₅₀	20.9 ± 0.8	0.28 ± 0.07	24.1	4.1
PEG _{10K} -PVBtMA ₁₀₀ / PAA ₅₀	27.2 ± 0.7	0.26 ± 0.04	29.4	6.1
PMPC _{5K} -PVBtMA ₅₀ / PAA ₅₀	40.0 ± 1.4	0.22 ± 0.01	50.7	11.4
PMPC _{10K} -PVBtMA ₉₇ / PAA ₅₀	42.0 ± 1.6	0.23 ± 0.03	52.9	11.7

^a Mean hydrodynamic radius (R_h) ± 95% CI from a cumulant expansion across 60-120°. ^b Mean colloidal polydispersity (PDI = μ_2 / Γ^2) ± 95% CI from a cumulant expansion across 60-120°. ^c Average R_h determined by REPES analysis at the 90° scattering angle. ^d Standard deviation of R_h distribution from REPES analysis at the 90° scattering angle.

The detailed multi-angle DLS analysis of the PCMs is provided below. For each polymer system, Figures S-9 through S-32 show the measured angular dependence of the autocorrelation functions between 60° and 120° fitted by a cumulant expansion, as well as the linear regression of Γ vs q^2 between 60° and 120°.

Figure S-10. Measured angular dependence of the autocorrelation function for PEG_{5K}-PLK₄₇ / PAA₅₀ at 0 mM NaCl between 60° and 120° fitted by cumulant expansion.

Figure S-11. Linear regression of Γ vs q^2 for PEG_{5K}-PLK₄₇ / PAA₅₀ at 0 mM NaCl over 5 angles between 60° and 120°.

Figure S-12. Measured angular dependence of the autocorrelation function for PEG_{5K}-PLK₄₇ / PAA₅₀ at 100 mM NaCl between 60° and 120° fitted by cumulant expansion.

Figure S-13. Linear regression of Γ vs q^2 for PEG_{5K}-PLK₄₇ / PAA₅₀ at 100 mM NaCl over 5 angles between 60° and 120°.

Figure S-14. Measured angular dependence of the autocorrelation function for PEG_{10K}-PLK₉₃ / PAA₅₀ at 0 mM NaCl between 60° and 120° fitted by cumulant expansion.

Figure S-15. Linear regression of Γ vs q^2 for PEG_{10K}-PLK₉₃ / PAA₅₀ at 0 mM NaCl over 5 angles between 60° and 120°.

Figure S-16. Measured angular dependence of the autocorrelation function for PEG_{10K}-PLK₉₃ / PAA₅₀ at 100 mM NaCl between 60° and 120° fitted by cumulant expansion.

Figure S-17. Linear regression of Γ vs q^2 for PEG_{10K}-PLK₉₃ / PAA₅₀ at 100 mM NaCl over 5 angles between 60° and 120°.

Figure S-18. Measured angular dependence of the autocorrelation function for PEG_{5K}-PVB₅₃ / PAA₅₀ at 0 mM NaCl between 60° and 120° fitted by cumulant expansion.

Figure S-19. Linear regression of Γ vs q^2 for PEG_{5K}-PVBtMA₅₃ / PAA₅₀ at 0 mM NaCl over 5 angles between 60° and 120°.

Figure S-20. Measured angular dependence of the autocorrelation function for PEG_{5K}-PVBtMA₅₃ / PAA₅₀ at 100 mM NaCl between 60° and 120° fitted by cumulant expansion.

Figure S-21. Linear regression of Γ vs q^2 for PEG_{5K}-PVB₅₃ at 100 mM NaCl over 5 angles between 60° and 120°.

Figure S-22. Measured angular dependence of the autocorrelation function for PEG_{10K}-PVB₁₀₀ / PAA₅₀ at 0 mM NaCl between 60° and 120° fitted by cumulant expansion.

Figure S-23. Linear regression of Γ vs q^2 for PEG_{10K}-PVB₁₀₀TMA₁₀₀ / PAA₅₀ at 0 mM NaCl over 5 angles between 60° and 120°.

Figure S-24. Measured angular dependence of the autocorrelation function for PEG_{10K}-PVB₁₀₀TMA₁₀₀ / PAA₅₀ at 100 mM NaCl between 60° and 120° fitted by cumulant expansion.

Figure S-25. Linear regression of Γ vs q^2 for PEG_{10K}-PVB₁₀₀ / PAA₅₀ at 100 mM NaCl over 5 angles between 60° and 120°.

Figure S-26. Measured angular dependence of the autocorrelation function for PMPC_{5K}-PVB₁₀₀ / PAA₅₀ at 0 mM NaCl between 60° and 120° fitted by cumulant expansion.

Figure S-27. Linear regression of Γ vs q^2 for PMPC_{5K}-PVBtMA₅₀ / PAA₅₀ at 0 mM NaCl over 5 angles between 60° and 120°.

Figure S-28. Measured angular dependence of the autocorrelation function for PMPC_{5K}-PVBtMA₅₀ / PAA₅₀ at 100 mM NaCl between 60° and 120° fitted by cumulant expansion.

Figure S-29. Linear regression of Γ vs q^2 for PMPC_{5K}-PVBtMA₅₀ / PAA₅₀ at 100 mM NaCl over 5 angles between 60° and 120°.

Figure S-30. Measured angular dependence of the autocorrelation function for PMPC_{10K}-PVBtMA₉₇ / PAA₅₀ at 0 mM NaCl between 60° and 120° fitted by cumulant expansion.

Figure S-31. Linear regression of Γ vs q^2 for PMPC_{10K}-PVBtMA₉₇ / PAA₅₀ at 0 mM NaCl over 5 angles between 60° and 120°.

Figure S-32. Measured angular dependence of the autocorrelation function for PMPC_{10K}-PVBtMA₉₇ / PAA₅₀ at 100 mM NaCl between 60° and 120° fitted by cumulant expansion.

Figure S-33. Linear regression of Γ vs q^2 for PMPC_{10K}-PVBtMA₉₇ / PAA₅₀ at 100 mM NaCl over 5 angles between 60° and 120°.

Figures S-34 through S-37 show the autocorrelation functions that correspond to Figure S-9 for all investigated micelle systems from 0 to 200 mM NaCl.

Figure S-34. Summary of the autocorrelation functions of PCM assemblies PEG_{5K}-PVBTMA₅₃ at (A) 0 mM, (B) 25 mM, (C) 50 mM, (D) 100 mM, and (E) 200 mM NaCl.

Figure S-35. Summary of the autocorrelation functions of PCM assemblies PEG_{10K}-PVBTMA₁₀₀ at (A) 0 mM, (B) 25 mM, (C) 50 mM, (D) 100 mM, and (E) 200 mM NaCl.

Figure S-36. Summary of the autocorrelation functions of PCM assemblies PMPC_{5K}-PVBTMA₅₀ at (A) 0 mM, (B) 25 mM, (C) 50 mM, (D) 100 mM, and (E) 200 mM NaCl.

Figure S-37. Summary of the autocorrelation functions of PCM assemblies PMPC_{10K}-PVBtMA₉₇ at (A) 0 mM, (B) 25 mM, (C) 50 mM, (D) 100 mM, and (E) 200 mM NaCl.

Figure S-38. REPES fits for DLS of PCMs assembled in different orders. All micelles in this work were assembled with the cation added to the solution first, followed by the anion (C→A). When this order is reversed (A→C) the size distribution is different, suggesting that the system may be kinetically trapped.

Figure S-39. Comparison of the apparent hydrodynamic radius versus charged block length of PVBtMA in PCM assemblies.

S3. Small-Angle X-Ray Scattering

Figure S-40 shows the intensity versus q profiles of the PEG-PLK, PEG-PVBTMA, and PMPC-PVBTMA polyelectrolyte solutions with increasing NaCl salt. At 0 mM NaCl, the correlation peak can be observed at $q = 0.4\text{--}0.5 \text{ \AA}$ for PEG-PLK and $q = 0.2\text{--}0.4 \text{ \AA}$ for PEG-PVBTMA and PMPC-PVBTMA. Table S-2 contains the fitting for the SAXS data in Figure 7 of the main manuscript with added MgCl_2 salt.

Figure S-40. SAXS profiles of polyelectrolyte solutions of PEG-PLK, PEG-PVBTMA, and PMPC-PVBTMA at polymer concentrations of 5 mg/mL with increasing added NaCl salt. Intensity scaled for clarity.

Table S-2. SAXS summary with added MgCl₂ salt.

Sample	[MgCl ₂] (mM)	R ^a (nm)	PDI ^b
PEG _{5K} -PLK ₄₇ / PAA ₅₀	50	8.0	0.16
	100	8.6	0.14
PEG _{10K} -PLK ₉₃ / PAA ₅₀	50	11.2	0.15
	100	10.6	0.09
PEG _{5K} -PVBtMA ₅₃ / PAA ₅₀	50	10.7	0.09
	100	-	-
PEG _{10K} -PVBtMA ₁₀₀ / PAA ₅₀	50	19.8	0.15
	100	-	-
PMPC _{5K} -PVBtMA ₅₀ / PAA ₅₀	50	-	-
	100	-	-
PMPC _{10K} -PVBtMA ₉₇ / PAA ₅₀	50	-	-
	100	-	-

^a Mean core radius (nm) ^b Polydispersity index (σ^2/R^2). Models use Schulz-Zimm distribution flexible cylinder and Unified Level fits, except for PEG_{10K}-PVBtMA₄₇ / PAA₁₀₀ (spheroid) and PEG_{10K}-PLK₉₃ / PAA₅₀ (cylinder fitting).

For each polymer system, Figures S-40 through S-41 show the small angle X-ray scattering profile in water with 100, 250, and 500 mM NaCl and 50 and 100 mM MgCl₂.

Figure S-40. SAXS profiles for PEG_{5K}-PVBTMA₅₃ / PAA₅₀ with 100, 250, and 500 mM NaCl (left), as well as 50 and 100 mM MgCl₂ (right).

Figure S-41. SAXS profiles for PEG_{10K}-PVBTMA₁₀₀ / PAA₅₀ with 100, 250, and 500 mM NaCl (left), as well as 50 and 100 mM MgCl₂ (right).

Figure S-42. SAXS profiles for PMPC_{5K}-PVBTMA₅₀ / PAA₅₀ with 100, 250, and 500 mM NaCl (left), as well as 50 and 100 mM MgCl₂ (right).

Figure S-43. SAXS profiles for PMPC_{10K}-PVBTMA₉₇ / PAA₅₀ with 100, 250, and 500 mM NaCl (left), as well as 50 and 100 mM MgCl₂ (right).

Figure S-44. SAXS profiles for PEG_{5K}-PLK₄₇ / PAA₅₀ with 100, 250, and 500 mM NaCl (left), as well as 50 and 100 mM MgCl₂ (right).

Figure S-45. SAXS profiles for PEG_{10K}-PLK₉₃ / PAA₅₀ with 100, 250, and 500 mM NaCl (left), as well as 50 and 100 mM MgCl₂ (right).

S4. Supplemental Polyelectrolyte Complex Micelle Stability Data

Kinetic stability tests of the micelles were examined in FBS. Figure S-46 shows the apparent size distribution of PEG-PVBTMA and PMPC-PVBTMA at 1 and 10 h, repeated three times independently. The overall size distribution of particles appears to be consistent, with evidence of PMPC-PVBTMA micelle aggregation over time.

Figure S-46. Three replicates of the apparent size hydrodynamic radius distribution of PEG-PVBTMA and PMPC-PVBTMA micelles at 1 h (solid markers) and 10 h (open markers), analyzed by REPES analysis at the 90° angle. Intensity is scaled for clarity.

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