Scaling Relationship of Complex Coacervate Core Micelles: Role of Core Block Stretching

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Abstract

The scaling relationship of complex coacervate core micelles (C3Ms) is investigated experimentally and theoretically. The C3Ms are formed by mixing two oppositely charged block copolyelectrolyte solutions (*i.e.*, AB + AC system) and are characterized by small-angle neutron (SANS) and X-ray scattering (SAXS). Scaling relationships for micellar structure parameters, including core radius, total radius, corona thickness, and aggregation number, all with respect to the core block length, are determined. Scaling theory is also proposed by minimizing the free energies per chain leading to four regimes depending on the core and corona chain conformations. Although the corona block is significantly longer than the core block, the structure of our C3Ms is consistent with the crew-cut I regime. A highly swollen core by water enables the core blocks to be stretched significantly and corona chains to be minimally overlapped.

Complex coacervation is a liquid-liquid phase separation (LLPS) driven by the electrostatic interaction between oppositely charged polyelectrolyte and the entropic gain of counterion release in an aqueous solution. Owing to the liquid-like interior, low interfacial tension, stimuli-responsiveness, and biological activity,¹⁻⁸ complex coacervates have been widely employed for industrial applications including encapsulation of biological macromolecules,^{9,10} underwater adhesion,¹¹ surface coating,^{12,13} and electrospun nanofibers.^{14,15} This LLPS can be manipulated to produce complex coacervate core micelles (C3Ms) through the use of block copolymers. In C3Ms, the coacervate cores are surrounded by hydrophilic neutral brushes such as poly(ethylene oxide) (PEO). C3Ms have been widely investigated as delivery vehicles of biological and pharmaceutical molecules, due to the hydrophilic and charged nature of the coacervate cores and the enhanced responsiveness to external stimuli including pH, ionic strength, and temperature in physiological conditions.¹⁶⁻¹⁹

Despite extensive experimentation on the structure and properties of C3Ms, much less is known about the relationship between the C3M structure and the molecular/environmental design parameters from both experimental and theoretical perspectives.²⁰⁻²³ Nonetheless, this relationship is crucial to predict and design the C3Ms for enhanced transportation and cellular uptake efficiency.²⁴⁻²⁶ Analogous to the self-assembly of amphiphilic molecules, the structure and morphology of C3Ms, including core radius (R_{core}) and aggregation number (N_{agg}), are primarily affected by the chain lengths, the ratio of core and corona blocks (N_{core} and N_{corona} , respectively), and the interfacial tension between coacervates and aqueous media.^{23,27-32} Rumyantsev *et al.* proposed a scaling theory of C3Ms prepared by mixing two oppositely and weakly charged diblock copolyelectrolytes (*i.e.*, AB + AC system, where A is the neutral hydrophilic block, and B and C are the oppositely charged blocks). They demonstrated the ionic strength-dependent structure and the morphological transition of C3Ms by considering two free energies contributed by the corona chain crowdedness and the excess energy at the core-corona interface.²² From an experimental perspective, Marras *et al.* investigated C3Ms formed by mixing diblock copolyelectrolytes and oppositely charged biomolecules (*i.e.*, AB + C system) and reported that the observed scaling relationships are universal for AB + C systems.²³ Although these works provide significant insights into the scaling relationship for C3Ms, a quantitative comparison between experimental data and theoretical description has not been fully established yet.

As of now, the AB + C system has been mostly studied experimentally as a representative of delivery vehicles, but the conformation and the spatial distribution of C homopolyelectrolytes produce an additional degree of freedom to determine the C3M structure.^{20,33} Also, the lack of experimental data on the AB + AC systems poses uncertainties to the theoretical prediction for their applications and scientific understanding. In this study, we present small-angle neutron (SANS) and X-ray scattering (SAXS) measurements on the AB + AC system. A series of precursor block copolymers is used to produce block copolyelectrolytes with different charge types and core block lengths but with a nearly identical number and position of the charged groups. This ideal synthetic route allows for regulation of the C3M structure exclusively by the core block length. Furthermore, we propose a scaling theory to quantify and understand the relationship between the C3M structure and the molecular design.



Figure 1. Schematic representation for complex coacervate core micelles (C3Ms) prepared by mixing two oppositely charged block copolyelectrolytes; the complex coacervate cores are surrounded by hydrophilic and neutral PEO blocks. Since precursor PEO-PAGE block copolymers were functionalized for cationic ammonium (A) or guanidinium (G) groups, or anionic sulfonate (S) group, the degree of polymerization for core and corona blocks (N_{core} and N_{corona} , respectively) is exactly same for corresponding block copolyelectrolytes. The C3M structure characteristics including core radius (R_{core}), overall micellar dimension ($R_{micelle}$), corona thickness (L_{corona}), and aggregation number (N_{agg}) were characterized by small-angle neutron/X-ray scattering (SAN/XS) measurements.

Poly(ethylene oxide-*b*-allyl glycidyl ether) (PEO-PAGE) block copolymers with various molecular masses of PAGE block were synthesized using anionic ring-opening polymerization technique, as we reported earlier:²⁷ $N_{PEO} = 227$ and $N_{PAGE} = 30, 56, 69, 71, 81, 96, and 144$. The PAGE blocks were functionalized for cationic ammonium (A) or guanidinium (G) groups with chloride counterions, or anionic sulfonate (S) group with sodium counterions. The experimental method and molecular characteristics are described in Supporting Information. This molecular design provides the identical number and distribution of charged groups for both cationic and

anionic block copolyelectrolytes, and thus minimizes any side effects of charge density or length mismatch on the micellization.^{27,34} These block copolyelectrolytes were dissolved in deionized water separately, and complex coacervate core micelles (C3Ms) were formed by mixing two oppositely charged block copolyelectrolyte solutions (overall mass fraction of 0.005) of the identical degree of polymerization at a stoichiometric charge ratio (see Figure 1). Overall pH was adjusted to pH = 5 by adding HCl or NaOH to the solutions. Previously, we showed that these molecular configurations produce spherical C3Ms composed of A+S or G+S coacervate cores surrounded by hydrophilic PEO chains, which are referred to as A+S(*x*) or G+S(*x*), respectively, where *x* denotes the degree of polymerization of the core block (*i.e.*, $N_{core} = N_{PAGE}$).^{27,35} Hydrodynamic radii of the C3Ms measured by dynamic light scattering (DLS) increase gradually with increasing N_{core} as shown in Table S2. A micellar structure was not observed for A+S(30) solution at 25 °C due to the relatively short charged block and weaker bonding strength compared to the G+S pair.³⁶⁻³⁹



Figure 2. Small-angle neutron scattering (SANS) profiles for 0.5 wt% A+S C3Ms with N_{core} of

56, 69, 71, 81, 96, and 144 (from bottom to top) at 25 °C. Open symbols indicate the experimental data, and the solid curves are the best fit for the detailed micelle model. The SANS profiles are vertically shifted for clarity.

To characterize the C3Ms, small-angle neutron scattering (SANS) experiments were performed at 40 m SANS beamline in High-flux Advanced Neutron Application ReactOr (HANARO), Korea Atomic Energy Research Institute (KAERI) with a neutron wavelength of $\lambda = 6$ Å and a wavelength spread of $\Delta \lambda / \lambda = 0.12$.⁴⁰ All C3M specimens prepared in D₂O were loaded in quartz banjo cells and exposed to the beam at 25 °C. SANS profiles obtained from 0.5 wt% A+S C3Ms in an aqueous solution are illustrated in Figure 2. A distinct first minimum near $q \approx 0.04$ Å⁻¹ observed for the five longest polymers progressively shifts to lower q values as N_{core} increases, reflecting that the core dimension increases. The inter-micellar interaction is negligible due to the significantly low polymer concentration. Solid curves in Figure 2 represent the best fits to a model composed of polydisperse spherical cores with Gaussian corona chains attached to the cores as explained in Supporting Information.^{27,41-43}





Figure 3. Dependence of (a) core radius (R_{core}) including the distribution of core (σ_{core}), (b) overall micelle dimension ($R_{micelle}$), (c) corona thickness (L_{corona}), and (d) average number of polymer chains per micelle (N_{agg}) for A+S (blue circle) and G+S (red square) C3Ms on the charged block length (N_{core}). Slopes indicate the scaling relationship of R_{core} , $R_{micelle}$, L_{corona} , and N_{agg} as a function of N_{core} as denoted in the main text.

Representative SANS results of micelle structure including the core radius (R_{core}), the distribution of R_{core} (σ_{core}), the overall micelle dimension ($R_{micelle}$), the corona thickness ($L_{corona} = R_{micelle} - R_{core}$), and the average number of polymer chains per micelles (N_{agg}) for A+S and G+S C3Ms are displayed as a function of N_{core} in Figure 3 (see also Table S4). As N_{core} increases, R_{core} , $R_{micelle}$, and N_{agg} show a notable increase, while L_{corona} decreases slightly. The scaling fits shown in Figure 3 were obtained by performing linear fits to the log-scaled data, taking the error bars into consideration. The fitting results are summarized in Table 1. Within the range of uncertainty, the scaling relationship of the structural parameters show reasonable agreement between A+S and G+S C3Ms. This implies that the scaling relationship of C3Ms is not affected by the ion pair, even though the actual values are dependent on the charged block chemistry.²⁷ Small-angle X-ray scattering (SAXS) was also conducted at beamline 4C SAXS-II of Pohang Accelerator Laboratory (PAL) to determine R_{core} , $R_{micelle}$, and L_{corona} for the corresponding C3Ms as described in Supporting

Information, which shows reasonable agreement with the SANS results (see Table 1). Slight discrepancy between SANS and SAXS results is attributed to different scattering contrast and uncertainty in the fitting process associated with polydisperse micelle system.

	System		$R_{\rm core}$	$N_{ m agg}$	$R_{\rm micelle}$	L _{corona}
Expt.	A+S C3Ms	SANS	0.63 ± 0.05	1.19 ± 0.10	0.41 ± 0.02	$- \ 0.25 \pm 0.20$
		SAXS	0.69 ± 0.01	_	0.38 ± 0.01	$- \ 0.05 \pm 0.04$
	G+S C3Ms	SANS	0.71 ± 0.03	0.95 ± 0.12	0.47 ± 0.11	$- \ 0.28 \pm 0.26$
		SAXS	0.62 ± 0.05	_	0.26 ± 0.03	$- \ 0.09 \pm 0.05$
Theory	Crew-cut I		0.67	1		0.6 (no overlap) - 0.11 (minimal overlap)
	Crew-cut II		1	2	_	0
	Star-like		0.6	0.8	_	0.16

Table 1. Summary of Scaling Relationship Exponents to $N_{\text{core.}}$ Error bars represent one standard deviation.

We explored the environmental and conformational aspects of the core and corona chains within C3Ms by considering the physical dimension of these micelles and the molecular characteristics of block copolyelectrolytes. This investigation is crucial for scaling analysis. First, the amount of water in the cores ($f_{water,core}$) is estimated as $(1 - f_{water,core})(4/3)\pi R_{core}^3 = N_{agg}v_{core}$, where v_{core} is the core block volume, resulting in $f_{water,core} \approx 0.82$ and 0.73 for A+S and G+S C3Ms, respectively (see Table S4). Notably we observed that $f_{water,core}$ remains nearly constant with varying N_{core} for both A+S and G+S C3Ms. This finding aligns with the theoretical prediction indicating that the water content in coacervates is nearly independent of polymer chain length beyond a critical value.^{44,45} This substantial presence of water in the cores reflects that the cores are highly swollen and hydrophilic, consistent with previous findings.^{27,35,46,47}

The significant amount of water in the core provides an explanation for why R_{core} is larger than L_{corona} despite $N_{core} \ll N_{corona}$ (= N_{PEO}) for all C3Ms. This further indicates that the core blocks need to stretch toward the center of the cores because the polymer density in the cores is reasonably constant and charge neutralization is achieved.^{34,48} We compared the measured R_{core} to the unperturbed radius of gyration for PEO chains dispersed in water, $\langle R_{g,PEO} \rangle_0$, as PEO serves as a proxy for PAGE due to its similar backbone. This comparison results in a degree of core chain stretching (*s*) defined as $s \equiv R_{core}/(2\langle R_{g,PEO} \rangle_0)$ because s = 1 roughly indicates that the core chains are not stretched.⁴⁹⁻⁵¹ With increasing N_{core} , *s* increases from 2.6 to 3.1, based on $2\langle R_{g,PEO} \rangle_0 = 31$, 35, 35, 38, 41, and 50 Å for $N_{PEO} = 56$, 69, 71, 81, 96, and 144, respectively.⁵² This corroborates the notion that the core blocks in C3M cores are highly stretched.

Next, the dimensionless brush density on the core surface is calculated as the ratio of the projected surface area per PEO corona chain ($\equiv \pi \langle R_{g,PEO} \rangle_0^2$) to the available surface area per chain ($\equiv 4\pi R_{core}^2/N_{agg}$). The dimensionless brush density serves as a two-dimensional analog of the reduced concentration c/c^* , where c^* is the overlap concentration.^{27,41,51} As indicated in Table S4, the estimated values for the dimensionless brush density are approximately 1.5 and 2 for A+S and G+S C3Ms, respectively, and are roughly independent of N_{core} . This suggests that PEO corona chains on the core surfaces only exhibit weak overlap and are adequately hydrated, consistent with the previous findings.²⁷ Consequently, the corona blocks in C3Ms appear to be relatively relaxed when compared to traditional amphiphilic block copolymer micelles.^{53,54}

To understand these results, we develop a scaling theory for C3Ms starting with the free energy per chain:

$$\frac{F}{N_{\text{agg}}k_{\text{B}}\text{T}} \sim \frac{R_{\text{core}}^2}{N_{\text{core}}b^2} + \frac{N_{\text{core}}}{\phi}g(\phi,\psi) + \frac{N_{\text{core}}}{\phi R_{\text{core}}}\gamma(\phi,\psi) + f_{\text{corona}}(\phi,\psi,N_{\text{core}},N_{\text{corona}}), \quad (1)$$

where b is the Kuhn length and $\phi \sim N_{agg}N_{core}/R_{core}^3$ is the monomeric concentration in the core and ψ is the concentration of small ions.^{22,55-57} Consistent with our system, we have taken both the homopolymers and small ions to be symmetric such that we only need to consider total concentrations. The first term in Eq. 1 accounts for the stretching of the core block, while the second term represents the free energy of coacervation of the core. Here, we let the explicit form of $g(\phi,\psi)$, the intensive free energy of coacervation, remain unspecified for generality. The only constraints are (1) that it must be the same free energy as for bulk coacervation and (2) that it is only dependent on the monomer and small ion concentrations. The former constraint is consistent with prior work on the scaling of C3Ms^{21,22} and the latter is reasonable as long as the core block is long enough.⁴⁴ It should be noted that specific forms for $g(\phi,\psi)$ can be considered^{21,22} and that $g(\phi,\psi)$ contains both electrostatic and non-electrostatic contributions.⁴⁴ The third term in Eq. 1 accounts for the interfacial tension between the core and aqueous media. We leave the specific form of the interfacial tension, $\gamma(\phi,\psi)$, unspecified as it depends on $g(\phi,\psi)$. If it is assumed that $\gamma(\phi,\psi)$ can be approximated by the interfacial tension between the coacervate and supernatant, then recent work shows that $\gamma(\phi,\psi)$ contains no N_{core} dependence up to $O(1/N_{core})^{44}$, and thus can be approximated as a function only of the monomer and small ion concentrations. The final term in Eq. 1 accounts for the corona, for which either the crew-cut or star-like expressions can be used adjusting for appropriate solvent quality.⁵⁸⁻⁶⁰

We roughly assume that the monomer concentration in the core is identical to the bulk in the high molecular mass limit. Although C3Ms usually do not show a sharp interface at the corecorona boundary, this assumption is reasonable as the water fraction in the core remains nearly constant regardless of N_{core} . If a specific form of $g(\phi,\psi)$ is chosen, this would require solving for equilibrium between the core and surrounding media. Consequently, the monomer concentration in the core is determined by molecular chemistry and environmental conditions including small ion concentration. Up to this point, the theorical framework is mathematically identical to all prior predictions for C3Ms.^{21,22} Compared to amphiphilic micelles, the only distinction is that the monomer fraction in the core is held constant, but not 1. Therefore, the predictions for the core block dependence of C3Ms should be identical to amphiphilic micelles.^{55-57,61} These predictions can also be determined by minimizing Eq. 1 with respect to R_{core} and considering the corona conformations. Note that second term in Eq. 1 has no R_{core} dependence, and thus does not contribute.

We propose that C3Ms are categorized into four regimes depending on the conformations of the corona chains, which is analogous to amphiphilic micelles. In the first regime, the corona chains do not overlap at all. In the second regime, the corona chains start to overlap but only minimally. In both regimes, denoted crew-cut I, the contribution to the free energy from the corona is negligible and the chain stretching of the core block balances the interfacial tension, promoting the formation of larger micelles. In these two regimes, the predictions for R_{core} and N_{agg} are the same, while the predictions for L_{corona} depend on if the corona chains overlap or not.^{55,57,61} In the third regime, denoted crew-cut II, the corona chains overlap to a greater extent and the free energy contribution from the corona crowdedness becomes dominant over the core block stretching, in which the density of chains in the corona is considered as a constant.^{55,57,61} In the fourth regime, denoted star-like, the corona chains become long enough that the chain density is a function of distance from the core, and thus the corona free energy is treated using the model proposed by Daoud and Cotton.⁶² The predictions for the scaling behavior as a function of N_{core} are summarized in Table 1 assuming the corona chains are in a good solvent.

To compare our predictions with the experimental results, we must confirm that the C3Ms characterized in this study are in equilibrium as the scaling theory is based on equilibrium principles. It is worth noting that macromolecular micelle systems often exhibit a kinetically frozen state, leading to nonergodic behavior. This nonergodicity is primarily attributed to slow chain exchange dynamics due to significant incompatibility between the core blocks and the surrounding media, and glassy nature of the cores.⁶³⁻⁶⁷ In the case of C3Ms, coacervate cores usually show low interfacial tension and significant hydration, but the electrostatic interaction between oppositely charged moieties hinders chain dynamics. Our previous findings show that the chain exchange rate in A+S(52) C3Ms is appreciable and decreases exponentially with increasing $N_{\rm core}$.⁶⁸ Also, the structure of C3Ms prepared by experiencing high ionic strength and followed by dialysis (*i.e.*, salt

annealing) is nearly identical to that of C3Ms prepared by the current protocol (see Figure S7). Although these observations do not guarantee complete equilibration of C3Ms, we believe that under equivalent conditions C3Ms are reproducible and the equilibrium theory is justified.

Next, it is necessary to determine the appropriate regime for the C3Ms observed. Based on our findings that due to the significant water fraction in the cores, the core blocks are highly stretched with the s values ranging from 2.6 to 3.1 and the corona blocks are relatively relaxed with the dimensionless brush density ≈ 1.5 and ≈ 2 for our A+S and G+S C3Ms, respectively, we expect to find scaling consistent with the crew-cut I regime with minimal overlap of corona chains. This prediction shows reasonable agreement with our observed scaling behavior from both SANS and SAXS, although it is highly unexpected based on both intuition from amphiphilic micelles and prior studies on C3M scaling. Intuitively, one might expect C3Ms to exhibit the star-like regime or at least crew-cut II regime, considering that the corona block is significantly longer than the core block. It is noted that for traditional amphiphilic micelles, observing the crew-cut I regime is rare, but not impossible.⁶⁹ Regarding C3M scaling theories, Rumyantsev et al.²² explored the cases of crew-cut II and star-like regimes while Kramarenko et al.²¹ focused on the star-like regime. Both works considered specific forms of $g(\phi)$ that allow predictions for other parameters such as charge density. In the case of Rumyantsev *et al.* their scaling theory is limited to low charge densities, unlike our experiments. Furthermore, the comparison of scaling behavior between AB + AC and AB + C system is qualitatively described in Supporting Information. Although additional studies are necessary, one finds that the scaling for L_{corona} is different for the AB + C system. Assuming identical coacervate cores and the reduced number of corona chains, AB + C system may exhibit reduced chain crowding in corona, which suggests a promising future avenue for experiments.

In this Letter, the scaling relationship for structure parameters of C3Ms including core radius, overall micelle dimension, corona thickness, and aggregation number of the chain is investigated with respect to the charged core block length. As a model system, the C3Ms prepared by mixing

two oppositely charged block copolyelectrolytes (*i.e.*, AB + AC system) in aqueous media are characterized by small-angle neutron/X-ray scattering (SANS/SAXS). Furthermore, we proposed a scaling theory based on the balance of free energies, which considers four regimes: crew-cut I (corona chains do not overlap or minimally overlap), crew-cut II, and star-like. Although the corona block (*i.e.*, $N_{PEO} = 227$) is significantly longer than the core block (*i.e.*, $N_{PAGE} = 30$, 56, 69, 71, 81, 96, and 144), our experimental results showed the core radius is larger than the corona thickness due to the highly swollen complex coacervate core by water uptake; Consequently, the core blocks in the coacervate cores are extensively stretched and the corona chains are less crowded at the core-corona interface. This behavior indicates that the contribution to the free energy from the corona is negligible and our results consistently align with the crew-cut I regime. The observation of C3Ms in the crew-cut I regime with the AB + AC system is not what one would expect *a priori* based on amphiphilic micelles and prior literature.^{21,22} This finding highlights the importance of experimental characterization of micelle structure in advancing our understanding of the fundamental physics behinds C3Ms and establishing design principles for diverse applications.

Supporting Information

Additional materials are available free of charge at

http://pubs.acs.org/doi/X/X

Detailed experimental methods, molecular characteristics including ¹H NMR spectra and SEC traces, dynamic light scattering measurement, a detailed fitting model for SAN/XS, and structural characterization of C3Ms based on the sample preparation methods.

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T.Y.H. synthesized polymers and performed the X-ray/neutron scattering experiments. D.J.A developed the scaling theory. T.Y.H., D.J.A., and S.H.C. contributed to interpreting the data and wrote the manuscript. S.H.C. supervised the experimental work.

Notes

The authors declare no competing financial interest.

Certain equipment, instruments, software, or materials, commercial or non-commercial, are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement of any product or service by NIST, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

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Acknowledgements

This work was partially supported by the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MSIP) (No. NRF-2021R1A2C2011164 and NRF-2018R1A5A1024127). SANS experiments were conducted at the High-flux Advanced Neutron Application ReactOr at Korea Atomic Energy Research Institute. SAXS experiments were conducted at 4C SAXS-II located in Pohang Accelerator Laboratory.

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