

Synthesis, Characterization and Self-assembly of Amphiphilic Block Copolymers

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Xiaojun Wang

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Abstract

This dissertation presents a review on state-of-the-art research of well-defined charged block copolymers, including synthesis, characterization, bulk morphology and self-assembly in aqueous solution of amphiphilic block polyelectrolytes.

In Chapter 1, as a general introduction, experimental observations and theoretical calculations devoted towards understanding morphological behavior in charged block copolymer systems are reviewed along with some of the new emerging research directions. Further investigation of charged systems is urged in order to fully understand their morphological behavior and to directly target structures for the tremendous potential in technological applications. Following this background, in Chapters 2, 3, 4 and 5 are presented the design and synthesis of a series of well-defined block copolymers composed of charged and neutral block copolymers with full characterization: sulfonated polystyrene-*b*-fluorinated polyisoprene (*s*PS-*b*-*f*PI) and polystyrene-*b*-sulfonated poly(1,3-cyclohexadiene) (PS-*b*-*s*PCHD). Their bulk morphological behaviors in melts and self-assembly of *s*PS-*b*-*f*PI, PS-*b*-*s*PCHD in water were investigated. Some unique behaviors of *s*PS-*b*-*f*PI were discovered. The mechanisms for formation of novel nanostructures in aqueous solution are discussed in details in Chapter 4. Spherical and vesicular structures were formed from strong electrolyte block copolymers, *e.g.* PS-*b*-*s*PCHD. Detailed light scattering and transmission electron microscopy were applied to characterize these structures. The abnormal formation of vesicles as well as microstructure effects on self-assembly is discussed in Chapter 5.

In Chapter 6, we describe the successful synthesis of a well-defined acid-based block copolymers containing polyisoprene while maintaining the integrity of the

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In the last Chapter 7, detailed synthesis and characterization of a novel conjugate: poly(L-leucine) grafted hyaluronan (HA) (HA-*g*-PLeu) are presented. This work describes a new method to synthesize HA-*g*-PLeu *via* a “grafting onto” strategy. Due to the amphiphilic nature of this graft copolymers, a “local network” formed by self-assembly which was characterized by atomic force microscopy and light scattering. The secondary structure of the polypeptide was revealed by circular dichroism.

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Chapter 1 Introduction: Morphologies of Ionic Block Copolymers

Abstract

This Introduction reviews current experimental observations and theoretical calculations devoted towards understanding micro-phase separation in charged block copolymer systems. The results for melt and solution phase morphologies as well as some of the new emerging research directions are presented. Overall, a comprehensive picture is beginning to emerge on the fundamental role of electrostatics in the micro-phase separation of charged block copolymers. However, further investigation for this particular charged system is urged for fully understanding of the morphology behavior and to direct targeted structures that endow the materials with desired properties that can have tremendous potential in technological applications.

1.1 Background

In the past decades, extensive scientific research¹⁻⁸ has been directed toward development of a molecular level understanding of micro-phase separation in block copolymers. With advent of robust synthetic methodologies and strategies, block copolymers with precisely controlled chain architectures can be synthesized and characterized.^{1-3, 6} Typical architectures include diblock copolymers, multiblock copolymers, branched copolymers, and dendrimers. Other than purely academic interest in these macromolecular systems, novel applications⁴ such as the development of advanced materials (e.g. thermoplastic elastomers),⁹ nanotechnology (e.g. lithography),¹⁰ ¹¹ green energy (e.g. fuel cells)¹² etc., have made these systems a major focus in soft matter research worldwide.

Micro-phase separation, ubiquitous in block copolymers, leads to a myriad of morphologies. The temperature and the intrinsic parameters such as block copolymer architecture and the interaction parameter (χ) determine the nature of the morphologies of these block copolymers.⁵ Classical experimental studies on these morphologies have focused on block copolymers comprised of monomers like styrene and derivatives, dienes, (meth)acrylates, ethylene oxide, and so on, which lead to flexible, uncharged polymer chains. A commonly studied system in this area of research is narrowly dispersed diblock copolymers in the concentrated solution regime or in melts. In particular, the morphology diagram for the flexible neutral diblock copolymer melts has been mapped out by both experiments and theoretical calculations.^{5, 8, 13} Furthermore, excellent agreement between the experiments and theory has been established.¹³ By manipulating interactions between the two phases (χN) with individual control of the Flory-Huggins interaction parameter of

the two blocks (χ) and the overall degree of polymerization (N), and volume fraction of each component, various morphologies (spheres, cylinders, gyroid, lamellae) have been revealed. Furthermore, morphological studies have been extended to architecturally complex polymers, for example, branched block polymers.⁷

In the last decade, block copolymers containing charged and neutral blocks have also attracted considerable attention^{12, 14-16} from polymer physicists and the polymer membrane community. This interest is largely motivated by the possible use of charged block copolymers as proton exchange membranes (PEM) in PEM fuel cell (PEMFC) applications.¹² It has been demonstrated that charged-neutral *block* copolymers have the potential to offer higher proton conductivity¹⁷⁻²⁰ than *random* ionic copolymers (for example, the commercialized product Nafion).^{21, 22} However, for the development of novel materials offering higher proton conductivity requires fundamental understanding of micro-phase separation in the presence of charges and this needs to be more thoroughly developed. Optimization of the proton transporting channels resulting from micro-phase separation and optimizing the mechanical properties of the materials are some of the directions^{5, 8, 23-26} that need to be pursued in order to achieve this goal. The early morphological knowledge was mainly extracted from studying physical properties of ionized multiblock thermoplastic elastomers.^{27, 28} Also Eisenberg *et al.*²⁹⁻³¹ and McGrath and Wilkes *et al.*,^{32, 33} performed structural investigations of block ionomers. The bulk morphologies mainly consisted of spherical or rod-like ionic domains of fully charged, short ionic blocks.^{29-32, 34, 35}

As an emerging area of research, block copolymers containing poly(ionic liquids) (PILs) as building blocks have attracted much attention due to applications of PILs in

catalysis,³⁶ gas separation media^{37, 38} and their use as polyelectrolytes in the fuel cell and battery industry.³⁹ Morphology controlled ionic conductivity is one of the fundamental issues that needs to be addressed for designing, developing and controlling advanced electrical materials that possess benefits of low glass transition temperature (T_g) (offering high ion conductivity) of PILs^{40, 41} and mechanical strength endowed by the nature of ionic block copolymers.²⁶ Such a combination could allow the development of mechanically tough and nano-structured electrochemical devices.¹²

Despite extensive research^{16, 21, 42} on charged polymers (polyelectrolytes and ionomers), thorough understanding of these systems is far from complete. The situation in the case of charged-neutral block copolymer melts is even worse. The fact is that studies on the behavior of these charged macromolecules in the melts are scarce. Only recently, several experiments^{43, 44} have been systematically carried out to understand the behavior of ionic block copolymers in the melts. Introduction of ionic /charged functional groups on one (or more) of the blocks of the polymers widens the parameter space for manipulating morphologies and complicates the phase behavior.⁴⁵⁻⁵² Furthermore, explicit Coulomb interactions between the charged species lead to complexities that have hindered theoretical and simulation advancements.^{43, 53, 54}

This introduction is motivated by the need to delineate the state-of-the-art in understanding structure-morphology relationships of charged block copolymers. Despite some of the complications with the experimental and theoretical developments, a comprehensive picture is beginning to emerge on the role of electrostatics in the micro-phase separation of charged block copolymers. It is hoped that this will attract additional researchers to this area and advance the understanding of these macromolecular systems.

This introduction is organized as follows: a simple method of synthesis of ionic block copolymers is presented in section 1.2, followed by the characterization of morphologies along with theoretical predictions for diblock copolymers in section 1.3. In section 1.4, I briefly discuss the solution self-assembly and end with the conclusions and some of the future directions of research

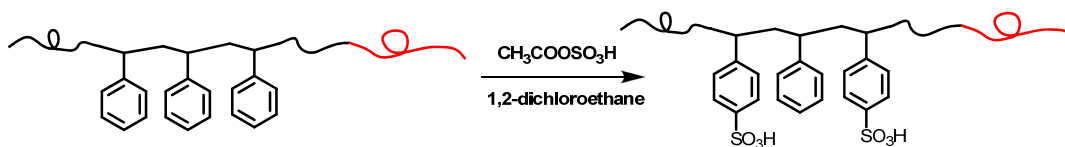
1.2 Synthesis of Ionic block copolymers

Considerable work has focused on the synthesis of charged block copolymers during recent decades, mainly *via* “living”/controlled radical polymerization (atom transfer radical polymerization (ATRP), reversible addition–fragmentation chain transfer (RAFT) radical polymerization, nitroxide-mediated polymerization (NMP)) and anionic polymerization combined with post-polymerization modification.^{2, 3, 55-57} The syntheses include direct polymerization of ionic monomers (cationic, anionic) consisting of amino-/ammonium type, sulfonate and carboxylate functionalized type, or modification of block copolymer precursors by sulfonation, carboxylation, quaternization and so on. Introduction of ionic liquid blocks into well-defined block copolymers has only recently emerged thanks to developments in controlled radical polymerization^{37, 58-62} and due to unusual and attractive physicochemical properties of ionic liquids.⁶³ Recently, phosphonium cation containing block copolymers have been also synthesized, and their morphologies have been studied.^{64, 65} We shall not review the synthesis of ionic block copolymers, but we will briefly describe one of the methods that is widely used to produce charged block copolymers from well-defined precursors synthesized by anionic polymerization, e.g. sulfonation of polystyrene (Scheme 1.1).

Precisely-defined narrowly dispersed block copolymer precursors can be obtained by anionic polymerization,^{3, 66} mainly of styrene and its derivatives, dienes, (meth)acrylates, vinyl pyridines, and so on. Anionic polymerization has the advantage of being the “gold standard” of all living polymerization methods, and thus it offers maximum control over block molecular weights, polydispersities, and overall control of macromolecular architecture.^{56, 67} Depending on the specific structure employed, chemical modification of one block, for example saturation of polydienes by methods such as hydrogenation, may need to be carried out before ionizing the other block, e.g. sulfonation of polystyrene (PS). These block copolymers would thus consist of a neutral hydrogenated polydiene block and anionic PS blocks bearing sulfonic acid or metal sulfonate functional groups. The advantage of this type of anionic block copolymer is that the charge contents/sulfonation degree (SD, percentage of sulfonated styrene in polystyrene block) can be adjusted from low (ionomers) to fully charged blocks for the purpose of investigating dependence of morphology on degree of charge. The ionic groups of sulfonated PS are believed to be approximately randomly distributed along the polymer backbone to which they are attached. These polymers have been widely applied in studies of charged block copolymer morphology. For this type of anionic block polymers, sulfonate functionalized monomers can also be employed directly in “living”/controlled radical polymerization, as well as other post-polymerization modification.⁶⁸

Scheme 1.1 A typical sulfonation reaction of polystyrene containing diblock copolymers.

Black, polystyrene block; Red, neutral block.



Although there are a variety of sulfonation methods described in the literature derived from chemistry of small organic molecules,⁶⁹ most of the sulfonated PS block copolymers studied to date have been prepared by a method developed by Makowski⁷⁰ using acetyl sulfate in 1,2-dichloroethane (Scheme 1.1), taking the advantage of mild nature of this reaction which minimizes side reactions and cross-linking, leaving the polymer backbone intact (no change in degree of polymerization). The sulfonating reagent, acetyl sulfate, is prepared from H₂SO₄ and acetic anhydride at ice bath temperature in a chlorinated solvent, such as dichloromethane, chloromethane or dichloroethane, which are inert to this reagent. The sulfonation degree can be well controlled by the ratio of acetyl sulfate to styrene units and reaction time. For block copolymers with high molecular weight, this method is not efficient enough to readily produce highly sulfonated products, thus stronger sulfonating agents like the complex of triethyl phosphate and SO₃ are needed to achieve high level of sulfonation.⁷¹

1.3 Morphology

1.3.1 Diblock copolymers

1.3.1.1 Experimental results: charged-neutral diblock copolymers

The morphology diagram of neutral-neutral flexible diblock copolymers (e.g. polystyrene-*b*-polyisoprene) has been mapped out by self-consistent field theory (SCFT).⁷²⁻⁷⁶ Furthermore, experimental studies^{5, 77} on these systems are in reasonable

agreement with the theoretical predictions.⁷²⁻⁷⁶ Recently, the theoretical morphology diagram is brought closer to the experimental findings by incorporating the effects of fluctuations.¹³ In particular, different morphologies evolve as a function of the thermodynamic mismatch between the two kinds of monomers (χN) and volume fraction of one of the blocks (f or $v\%$), through disorder to order, mirroring itself from sphere to lamellar structures as order-order transitions (OOT) as shown in Figure 1.1.

As shown in Figure 1.1, linear neutral diblock copolymer melts with narrow polydispersity exhibit spheres, cylinders, gyroids, lamellae morphologies in a specific window of f and χN . Also, the theoretical predictions and the experimental observations demonstrate good agreement, although there remain some discrepancies⁵ especially near the disorder-order transition.

In contrast to the morphology diagram for the neutral-neutral diblock copolymer melts as shown in Figure .11, such diagrams for charged-neutral diblock copolymers are not very well studied for the melts self-assembly or for self-assembly in the presence of solvent. Moreover, recent experimental studies⁵⁷⁻⁶⁰ on charged-neutral diblock copolymers have revealed unusual behavior in these charged systems. In particular, Balsara and co-workers^{44, 78} and Goswami *et al.*^{43, 53} have demonstrated that the morphology diagram for charged-neutral diblock copolymer melts and solutions is more complex than that shown in Figure 1.1.

Balsara and coworkers^{44, 78-83} studied bulk morphologies for a series of symmetric diblock copolymers containing sulfonated polystyrene and poly(methylbutylene) (sPS-*b*-PMB) with varying degrees of sulfonation and molecular weight ($MW \sim N$, we use MW

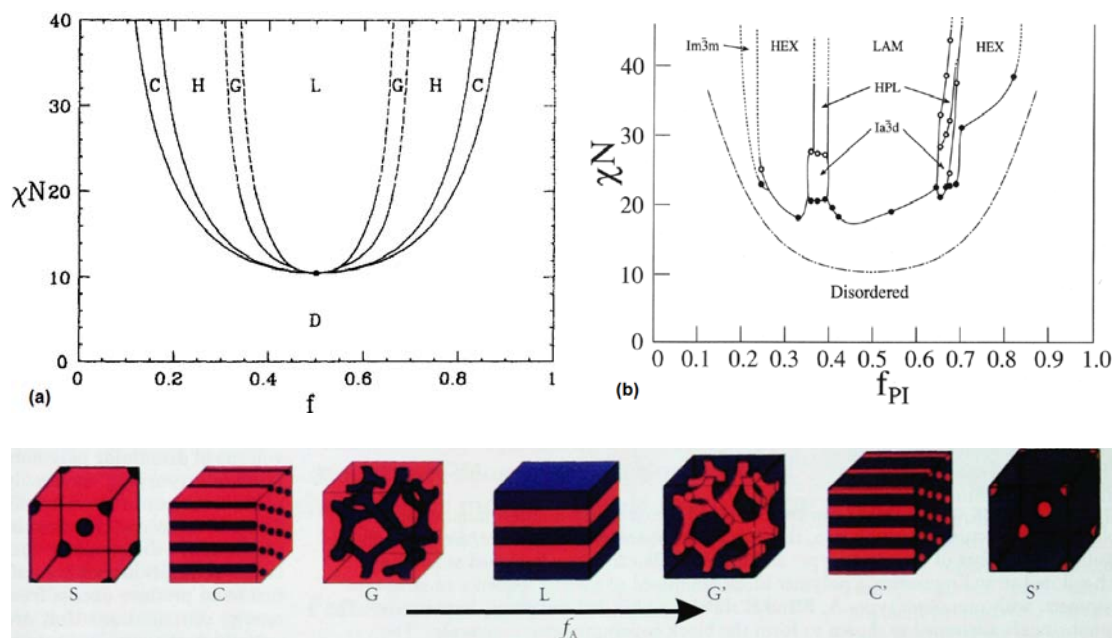


Figure 1.1 Phase diagram of linear diblock copolymers (A-*b*-B) as a function of χN and volume fraction (f). (a) Predictions of self-consistent mean-field theory;⁷⁶ (b) Experimental observations on diblock copolymers of styrene and isoprene.⁷⁷ C/Im3m, H/HEX, G/Ia3d, L/LAM denote spheres, hexagonally packed cylinders (cylinders), bicontinuous gyroid, and lamellae structures, respectively. (c, the bottom cartoon) Representation of phase structures in three dimensions as a function of volume fraction of one of the components (f_A) at fixed χN . The different components are represented by red and black. Figures are adapted from Ref 5, 76, 77.

instead of N for simplicity hereafter) at different relative humidities and temperatures. For low MW ($2.8\text{--}7.2\times 10^3$ g/mol) copolymers with volume fraction around 50% for each block, disordered morphologies were observed due to low segregation strength as characterized by χN . In experiments carried out on diblock copolymers of low MW, for example, of 7.2×10^3 g/mol, degree of sulfonation of the charged block has been shown to play an important role in determining their morphologies. For example, varying the sulfonation degree between 0 and 44.7%, the morphologies were found to change from disordered to gyroid to lamellae to hexagonally perforated lamellae (HPL) as demonstrated by transmission electron microscopy (TEM) and small angle X-ray scattering (SAXS) (Figure 1.2). In this range of sulfonation degrees, the volume fraction of each block remains nearly the same (from 0.457 to 0.476), and thus no morphological changes would be anticipated (cf. Figure 1.1). For high MW polymers, similar phase transitions occurred as a function of SD with appearance of a new morphology of HEX (hexagonally packed cylinders or cylinders) and disappearance of gyroid. However, no new morphologies other than classical morphologies were observed. MW was also found to affect the micro-phase separation even with the same SD and compositions. In particular, HEX and HPL morphologies were found for high and low MW copolymers, respectively. Also, temperature dependence of morphology was studied to obtain accessible disorder-order and order-order transition temperatures (DOT and OOT) in the range of MW ($2.8\text{--}43.6\times 10^3$ g/mol) that was investigated in this study. These transition temperatures were inaccessible when χN is large ($N > 141$).

It is worth noting the coexistence of two morphologies in the experiments⁴⁴ - HPL and lamellar for high MW copolymers. The authors attributed this to a distribution of

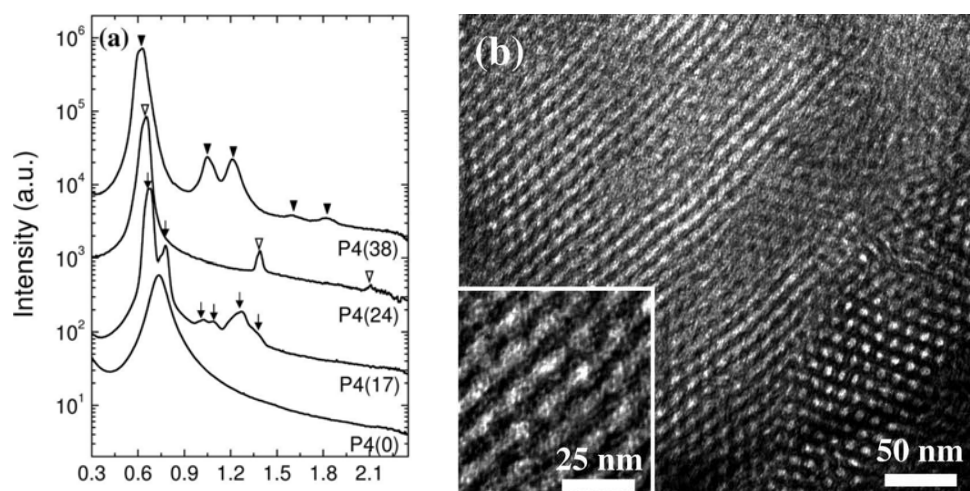


Figure 1.2 Morphologies of *sPS-b-PMB* (derived from *PS-b-PMB*, 3.5-*b*-3.7 kg/mol) at 25 °C. (a) SAXS profiles of evolution of morphology as a function of SD. P4(0), disordered; P4(17), gyroid; P4(24), LAM; P4(38), HPL. (numbers in parenthesis indicate sulfonation degrees in percentage) (b) Typical TEM image of P4(38), higher magnification is shown in inset.⁴⁴ PS domain was stained by RuO₄, appearing dark. Data were adapted from Ref 44.

sulfonation degree in terms of concentration of sulfonic acid groups and their position on each polymer chain, rather than traditional parameters: chain length and compositions which can, in principle, contribute to emergence of coexisting morphologies.⁸⁴⁻⁸⁶ Furthermore, the polymers used in these experiments were narrowly dispersed (made by living anionic polymerization) with controlled molecular compositions.

In addition to these puzzling experimental results, Goswami *et al.*^{43, 53} have reported the synthesis and morphology of model block copolymer melts derived from precursor poly(styrene)-*b*-poly(isoprene) (PS-*b*-PI), which was first fully fluorinated in the PI block (*f*PI) followed by partial sulfonation of the PS block (*s*PS). Due to its ionic nature (sulfonic acid/sodium salt which is highly hydrophilic) and fluorine content (which is strongly hydrophobic, even lipophobic), a high value of χ is expected, which leads to novel self-assembly behavior. In particular, it was found that diblock copolymers containing 75 v% *f*PI and 25 v% *s*PS, where the PS block was 50% sulfonated to the sulfonic acid form, when cast from anhydrous tetrahydrofuran (THF) formed “inverse morphologies”^{43, 87} as shown in Figure 1.3. The minority blocks (*s*PS) formed the continuous phase, due to charge percolation,⁴³ with hexagonally packed and well ordered cylinders of *f*PI. This is the inverse of the morphology exhibited for the precursor PS-*b*-PI of the same volume fractions (75 v% of PI), containing a continuous PI phase with dispersed PS cylinders. Thermal annealing or addition of water to the casting solvent disrupts the percolated structure and leads to a less-ordered system. Furthermore, a kinetically trapped morphology and its evolution as a function of temperature and hydration as well as microdomain orientation by external field had been studied.⁸⁸⁻⁹⁰

As mentioned earlier, in addition to the SD, the MW of diblock copolymers also

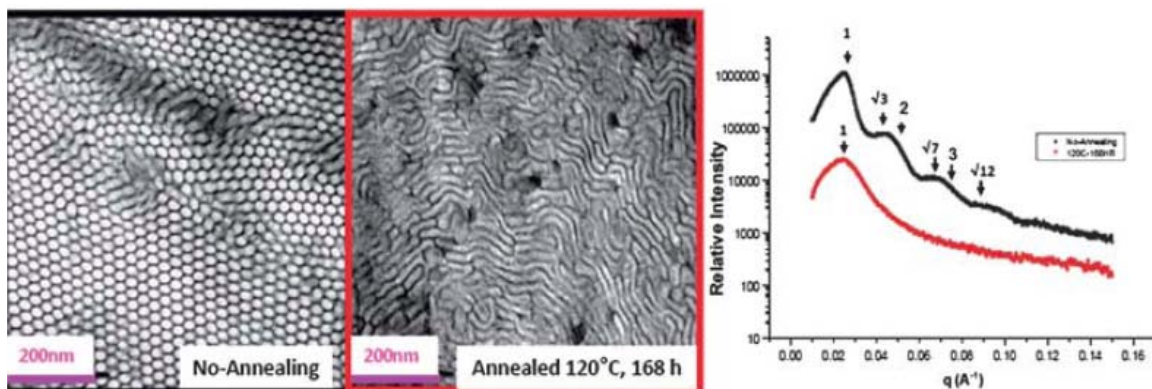


Figure 1.3 Demonstration of inversed morphology of *sPS-*b*-fPI* by TEM and SAXS and effects of annealing process. (a) No-annealing, as cast: well ordered inversed HEX structures; (b) same sample as in (a), but annealed at 120 °C for 168h: less ordered structures. (c) SAXS of sample in (a) and (b). PS domains were darkened by RuO₄ in TEM data.⁴³ Figures were adapted from Ref 43.

plays an important role in the stabilization of different morphologies. MW effectively acts as an independent parameter to describe the morphology diagram in the case of charged-neutral diblock copolymers. This is also indicated in the experiments reported by Chao *et al.*⁶⁸, where the morphologies showed a different order as observed by TEM and SAXS for polystyrene-*b*-sulfonated poly(hydroxystyrene) (PS-*b*-sPHS-NR₄(R=propyl)) having similar compositions (40 wt% of sPHS-NR₄, reasonably assuming approximately $f \approx \text{wt}\%$, same assumption discussed below) but different chain lengths (MW = ~46–230 kg/mol). In this composition and MW, from Figure 1.1, one may expect it to form well-ordered lamellae as suggested by the authors. Furthermore, for a composition of 62 wt% of sPHS-NR₄, a well ordered HEX structure was observed, in contrast to the expected lamellar morphology.⁶⁸

At this point, we highlight the observed similarities in the experimental results coming from different research groups on different charged-neutral diblock copolymers. It was found that the copolymers, sPS-*b*-PMB and PS-*b*-sPHS-NR₄ formed HEX structures^{44, 68, 79} in the composition region where lamellar morphologies are observed/anticipated in neutral-neutral diblock copolymer melts. PS-*b*-sPHS-NR₄ formed a “normal” HEX structure with the major ionic component forming the matrix of the morphology. However sPS-*b*-PMB with close to 50 v% of compositions had the charged block as the matrix as evidenced by TEM.⁷⁹ This phenomenon was also observed in the case of sPS-*b*-fPI, exhibiting inverse morphologies as discussed above. All samples with HEX structures have the ionic block as the matrix of the phase separated materials.^{43, 44, 79} It is also interesting to note, that high sulfonation degrees facilitate the formation of HEX structures (even though there are some exceptions which formed gyroid structure with

low MW polymers, the ionic part could still be considered as the matrix),^{44, 79} while polymers with low SD, tend to behave the same as neutral/non-charged precursors regarding phase separation behavior. In contrast to what was observed in *sPS-*b*-fPI*⁴³ case, asymmetrical *sPS-*b*-PMB*⁸⁰ with a similar *sPS* volume fraction of 25%, but a different sulfonation degree of 41% and different total molecular weight, a well-ordered lamellar structure formed as seen in SAXS, which is expected to form normal HEX structure with PMB as the matrix according to the phase diagram in Figure 1.1. All these experimental results point out that in addition to the chemical nature of the components (which determines χ), the MW and the SD play key roles in the micro-phase separation of charged-neutral diblock copolymers.^{44, 78}

Although the aforementioned sulfonated diblock copolymers exhibit unconventional morphological behaviors, there are diblock copolymers, which exhibit conventional phase separation as reported by Frisken *et al.*⁹¹ Sulfonated poly([vinylidene fluoride-*co*-hexafluoropropylene]-*b*-styrene diblock copolymers with sulfonation degrees the range of 22–40% (~44 v% of PS) showed formation of lamellar stacks, however, without long range order as demonstrated by small angle neutron scattering (SANS) with contrast variation, X-ray scattering and TEM. Furthermore, the morphology is in agreement with Figure 1.1 for non-ionic diblock copolymers.⁸ Also, the SANS and TEM results demonstrated improved ordering of lamellar structure with increase of SD in this range of sulfonation degree.⁹² However, for fully sulfonated samples (~10 v% of PS, comparable MW with partially sulfonated materials), a disordered phase separation was observed^{18, 91} in this combination of χ N and compositions. This might be attributed to the high polydispersity of the diblock copolymers and the thermodynamic equilibrium issues.

Furthermore, an important point to note from these experiments is that sulfonation at both low and high levels destabilize ordered structures resulting from micro-phase separation.^{18, 91, 92} The destabilizing effect of the sulfonation on the micro-phase separation was also observed in block copolymers of sulfonated polystyrene-*b*-poly(ethylene-*alt*-propylene), where primary domain spacing decreased as SD increases. In particular, an increase in sulfonation leads to the stabilization of the disorder phase over the lamellar morphology of unsulfonated block copolymers.⁹³

However, the literature also reveals instances where sulfonating one of the blocks stabilizes the ordered morphologies. As an example, we note the experiments carried out on sulfonated diblock copolymers of styrene and methyl methacrylate (MMA).²³ Disordered isotropic morphologies from all precursors copolymers PS-*b*-PMMA changed to well ordered lamellae (from precursor with ~50 v% of PS), HEX or HPL (~20 v% of PS) and HPL (~15 v% of PS) structures, dependent on the sulfonation degrees (0–35%). Authors have attributed the stabilizing effect of the sulfonation to the enhanced value of the chemical mismatch parameter (χ_N) between the charged and neutral components.²³ Furthermore, SANS experiments showed that the primary domain spacing increased with SD due to an increase in the molar volume of charged monomer upon sulfonation.²³ As another example, spherical and disordered morphologies changed to HEX and lamellae structures, respectively, on neutralizing poly(styrene)-*b*-poly(methacrylic acid) with lithium hydroxide. The experiments were carried out on partially neutralized lithium salts of poly(styrene)-*b*-poly(methacrylic acid), where spherical and disordered morphologies changed to HEX and lamellae structures, respectively, resulting from the complexation of acrylic acid groups with lithium ions. This complexation is believed to enhance the

effective chemical mismatch parameter (χ) between the two blocks.⁹⁴ It was found that domain volume normalized conductivity of protons monotonically increased as morphologies went through disorder-HEX-HPL-lamellar structures, which demonstrated the concept of morphology controlled proton conductivity.^{23, 83}

With regard to morphology controlled ionic conductivity, Mahanthappa and coworkers⁹⁵ very recently provided an example that established the relationship between morphology and ion conductivity. They investigated an anhydrous micro-phase separated film of diblock copolymers containing poly(ionic liquids), poly(styrene-*b*-4-vinylbenzylalkylimidazolium bis(trifluoromethanesulfonyl)imide) (PS-*b*-PVBn-(alkyl)ImTFSI; alkyl = -CH₃, *n*-C₄H₉, *n*-C₆H₁₃) (19–50 v% of PolyIL).⁹⁵ The well ordered lamellar forming diblock copolymers with ~ 50 v% of PolyIL exhibited ionic conductivity of about one order of magnitude greater than a block copolymer exhibiting coexistence of lamellar and HEX (34 v% of charged block); while for the same materials with 34 v% of the charged block, a well-ordered morphology (from solvent casting) exhibited an order of magnitude higher conductivity than less the ordered material (obtained from melt processing) as shown in Figure 1.4, Left. This highlights the importance of connectivity of the ionic domains and the need to minimize morphological defects leading to “dead ends”, which would seriously jeopardize material properties, e.g. ionic conductivity.

The polymer with $f = 0.5$ was expected to phase separate into a lamellar structure according to the phase diagram in Figure 1.1 for this molecular composition and molecular weight. However, the material with $f = 0.34$ exhibited a coexistence of two

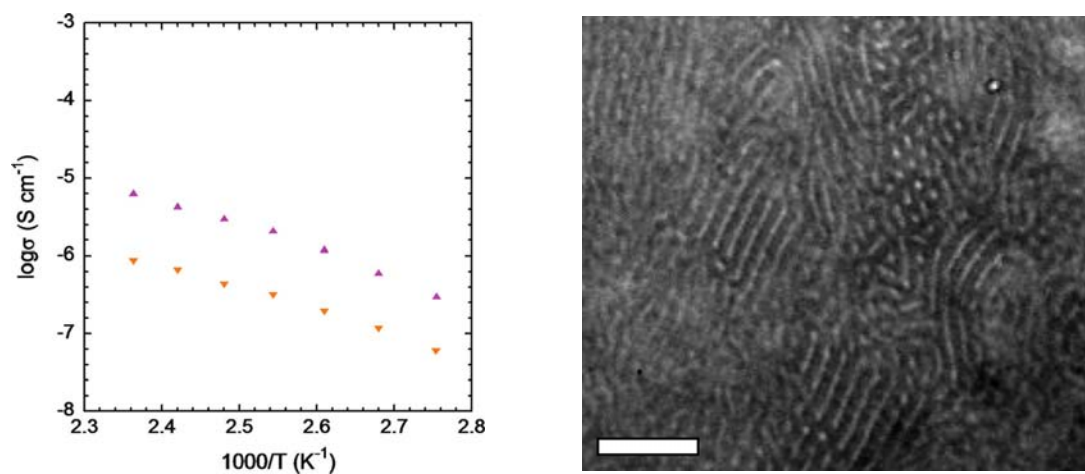


Figure 1.4 Temperature-dependent higher ionic conductivity is shown for the solvent-casted film (long range ordered) versus by melting processing (less ordered) for PS-*b*-PVBn-HexImTFSI-34 v% (Left). Coexistence of HEX and lamellar structure from the solvent casted film (Right).⁹⁵ Figures are adapted from Ref 95.

textures (Figure 1.4, right), clearly indicating ion-dipole interaction played an important role for perturbing the expected hexagonally packed structure.^{23, 44}

Well-defined PolyIL-containing diblock copolymers (like the one shown above) intrinsically have promising applications due to their unique properties.^{63, 96, 97} To date, the exploration of synthesis and morphology of block copolymers where one of the blocks is a polyIL is quite limited. Gin and coworkers synthesized charged polynorbornene-based block copolymers 1 (Figure 1.5) by polymerizing imidazolium and alkyl-substituted norbornene using a living ring opening metathesis polymerization (ROMP).⁹⁸ The covalent connectivity between two blocks was proven by multiple methods including surfactant behavior and solubility analysis, and diffusion-ordered spectroscopy, although the attempted direct proof by gel permeation chromatography (GPC) for chain extension was unsuccessful. SAXS, differential scanning calorimetry and rheological measurements demonstrated a long-range well-ordered lamellar structure in the range of compositions studied (~50 wt%) for the materials exhibiting a melt state at room temperature (Figure 1.5).⁹⁸

1.3.1.2 Theory, modeling and simulation developments: charged-neutral diblock copolymers

Theory: Along with immature systematic experimental studies, theoretical developments and simulation studies addressing the micro-phase separation in charged-neutral diblock copolymers are scarce. In contrast to the neutral-neutral diblock copolymer morphology studies which are well investigated using field theoretical approaches and with molecular dynamics (MD) and Monte Carlo (MC) simulations, understanding of charge block copolymer morphologies is still in a nascent stage due to the difficulties arising from the

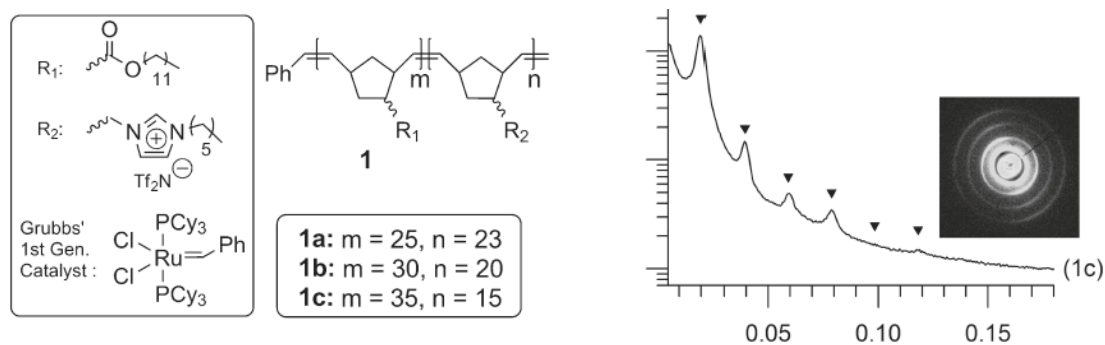


Figure 1.5 Structure of alkyl-imidazolium diblock copolymers and the catalyst used for ROMP (left); SAXS data of polymer 1c at room temperature showing well-ordered Bragg scattering at 1:2:3:4:5:6, representing lamellae morphology (right).⁹⁸ Data are taken from Ref 98.

presence of long range Coulombic interactions between the charged species. Here, we discuss the theoretical and simulation developments addressing charged-neutral diblock copolymer morphologies.

As mentioned earlier, a major bottleneck in the development of a rigorous theory and simulation techniques for charge-neutral diblock copolymers is the need to take into account the different types of interactions between the charged and neutral components. As an approximation, the charged and neutral components in the diblock copolymer systems can be assumed to interact by a charge-dipole interaction potential. Such an approximation is based on the fact that most of the neutral monomers have dipole moments, either permanent or induced. Furthermore, the theory needs to take into account the counter-ion adsorption on the charged block resulting into electric dipole formation and ion-pairing in the copolymer media, which has an ultra-low dielectric constant. To the best of our knowledge, no such theoretical treatment is available in the literature. However, there have been attempts to address some important issues concerning micro-phase separation in the context of charged-neutral diblock copolymers, which will be reviewed here.

Marko and Rabin⁹⁹ were the first to demonstrate the importance of the counter-ions and co-ions (resulting from added salt) in affecting the disorder-order transition boundary for the melts containing monodisperse charged-neutral diblock copolymer chains. They used a weak inhomogeneity expansion (also known as the Random Phase Approximation (RPA)) for the melts treating counter-ions and co-ions as point-like. For salt-free melts, it was shown that the translational entropy of the counter-ions and the

electrostatic screening tend to stabilize the disordered phase over the ordered morphologies.

For salt-free melts, it was shown⁹⁹ that the χ parameter for the disorder-order transition ($= \chi^*$) increases with an increase in the number of charges on the charged block. Also, as one adds more charges to one of the blocks, the critical wave-vector (q^*) characterizing the length scale of the ordered morphology increases and then stabilizes which represents the shortest length scale of the micro-phase separation. Addition of salt to this system and an increase in the salt concentration was shown to decrease both the χ^* and q^* . Experimentally χ is found to be inversely proportional to temperature. This, in turn, means that Marko and Rabin's theory predicts that the temperature at which an ordered morphology becomes stabilized over the disordered phase i.e., the disorder-order transition temperature (DOT), decreases with an increase in the number of charges on the charged block. An addition of the salt ions leads to an increase in the DOT. Furthermore, using $q^* \sim 1/D^*$, D^* being the domain spacing of the ordered morphology, one can infer, Marko and Rabin's theory predicts that D^* should decrease on charging one of the blocks and increase with the addition of the salt ions before saturating to the value corresponding to neutral-neutral diblock copolymer melts. In other words, the theory predicts that the domain spacing for the ordered morphology is always lower than that for the corresponding neutral-neutral diblock copolymer melts. Note that in the theory, ions were treated as point-like and the effect of the salt ions appears through electrostatic screening from all the ions. As such, all the aforementioned predictions can be interpreted as an outcome of the electrostatic screening. However, non-trivial effects such as counter-ion adsorption¹⁰⁰⁻¹⁰² in the concentrated regime and ion pairing in these systems were not

considered. This shortcoming of the theory limits a rigorous comparison of the theory and the experiments. Despite these limitations, stabilization of the ordered phase over the disordered phases is consistent with some of the experiments^{43, 53} as discussed in the previous section. Later on, Kumar and Muthukumar¹⁰³ studied the salt-free melts for the same model in the weak-segregation limit using the RPA and the lamellar morphology was studied using SCFT treating ionic interactions using the Poisson-Boltzmann equation. Predictions of Marko and Rabin's theory were confirmed and morphology diagrams similar to Figure 1.1, consisting classical morphologies (i.e., lamellae, cylinders and spheres), were constructed using the RPA for different number of charges on the diblock chains (see Figure 1.6). Inverse temperature dependencies of χ and the electrostatic interaction strength were taken into account while constructing the morphology diagram. It was shown that the morphology diagram for the charged-neutral diblock copolymers is asymmetric with respect to f , in contrast to the symmetric diagram for neutral-neutral diblock copolymer melts as shown in Figure 1.1. Furthermore, it was found¹⁰³ that the counter-ions tend to be located in the domains containing charged block and the effective segregation strength goes down on charging one of the blocks. Like Marko and Rabin's theory, this work also neglected ion pairing and the counter-ion adsorption on the charged blocks.

It is well known that the RPA treatment is valid only in the weak segregation limit. Recently, Yang *et al.*¹⁰⁴ have performed SCFT calculations to construct morphology diagrams for the salt-free melts containing charged-neutral diblock copolymer chains. In this work, it was shown that different morphologies are stabilized as a result of charging

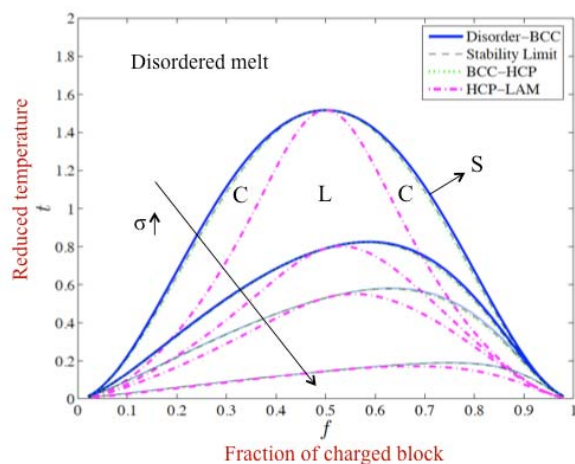


Figure 1.6 Morphology diagram for charged-neutral diblock copolymer melts constructed using the RPA for different degree of ionizations of the charged block (σ). Monodisperse copolymer chains contain $N=1000$ Kuhn segments and L/LAM, C/HCP and S/BCC stand for lamellar, hexagonally packed cylinders and body centered cubic spheres, respectively. Along with the order-order transition boundaries, the stability limit of the disordered phase is also plotted. Temperature dependence of the χ parameter and electrostatic interaction strength is used to define a parameter, t , which plays the role of temperature.¹⁰³ Figure is re-adapted from Ref 103.

one of the blocks, which is qualitatively in agreement with the experiments carried out by Balsara *et al.*^{44, 78}

Simulation: Theoretical studies often neglect minute details of a realistic system. For example, in the charged block copolymers system, the finite size of the ions/counterions, ion-pairing and charge states on the ion and model polymer structure, are not generally considered. To address these details, model based-simulation studies have been performed to help enhance the understanding of the experimental and theoretical studies. The work by Banaszak and Clarke¹⁰⁵ was one of the first to investigate the morphology of charged diblocks in solution (at a reduced density of 0.5) and their work helped explain micro-phase separation in ionic copolymers. Previous Monte Carlo (MC) studies by Knychala⁷⁸ showed different morphologies such as perforated lamellae, gyroid and hexagonally packed cylinders. These morphologies clearly indicated a marked difference between neutral diblocks and charged diblock copolymers. Coarse-grained (CG) molecular dynamics (MD) simulations have also been used to help develop an understanding of the morphologies. Pantano *et al.*,¹⁰⁶ used a coarse-grain model for poly(acrylic acid)-*b*-poly(butadiene) to simulate phases of these diblock copolymers in solution. The different morphologies observed were bilayers, cylinders (worm-like) and spherical micelles. CG studies also have been performed on much larger systems such as DNA condensation by charge-neutral block copolymers. These studies as shown by Ziebarth *et al.*,¹⁰⁷ are in solution and show core-corona structures with similarly ordered anionic and cationic beads. Coarse-grained MD for charged diblock copolymers have faced multiple challenges due to the presence of explicit Coulombic interactions between

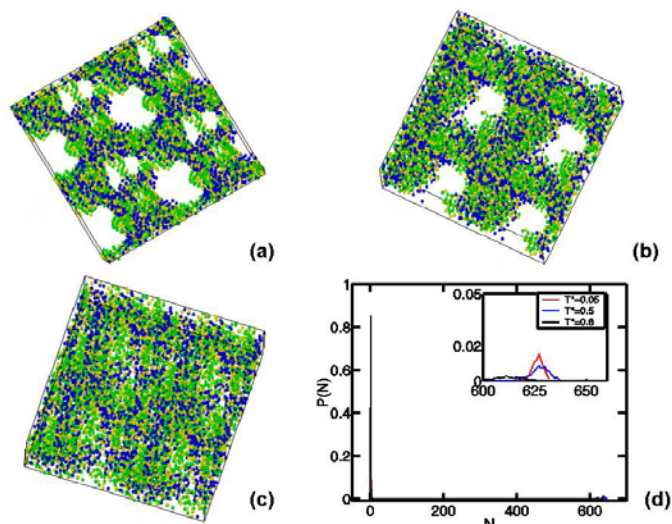


Figure 1.7 Morphology of a charged diblock copolymer at different temperatures in a melt. (a) $T^* (k_B T/\epsilon) = 0.05$, (b) $T^* = 0.1$ and (c) $T^* = 1.0$. (d) The cluster size distribution shows the presence of a large cluster indicating charge percolation. In the snapshots, colored sites are charged blocks and the empty sites are the uncharged blocks representing *s*PS and *f*PI respectively.⁴³ Figures are taken from Ref 43.

charge sites. Still it is these electrostatic interactions are considered to be responsible for the differences in self-assembly as compared to neutral diblocks.

To the best of our knowledge the first CG MD study of charged diblock copolymer melts were carried out by Goswami *et al.*⁴³ This molecular dynamics study showed that the counter-ion adsorption and charge aggregation in a low-dielectric copolymer media plays an important role in stabilizing ordered morphologies. In particular, it was demonstrated that charge agglomeration can lead to a percolated structure in a low dielectric constant polymeric media and stabilize “inverse” morphologies as observed in recent experiments (Figure 1.3 and Figure 1.7).⁴³ On increasing the dielectric constant of the polymeric media, conventional morphologies were observed (Figure 1.8).⁵³ The increase in dielectric constant effectively reduces the electrostatic interaction strength (a screening effect) thereby reducing the extent of the ion-counterion pairing and subsequently resulting in the weakly charged diblock behaving as a ‘neutral diblock’. Goswami *et al.* also performed Monte Carlo (MC) simulations on the charged diblock polymer melts showing how disk-shaped morphologies could be obtained, in agreement with experiments.¹⁰⁸

1.3.2 Triblock Copolymers

Besides ionic diblock copolymers, charged triblock copolymers have also been studied. In particular, ABA symmetrical structures with either A or B being sulfonated polystyrene. Actually, the studies of sulfonated block copolymer ionomers were first reported by Weiss *et al.* with triblock copolymers of sulfonated polystyrene-*b*-poly(ethylene-*r*-butylene)-*b*-polystyrene (*s*SEBS) with low sulfonation degree (0–18%, ionomers, 30 wt% of PS, $M_n = 50,000$ g/mol) in order to generate physically crosslinked

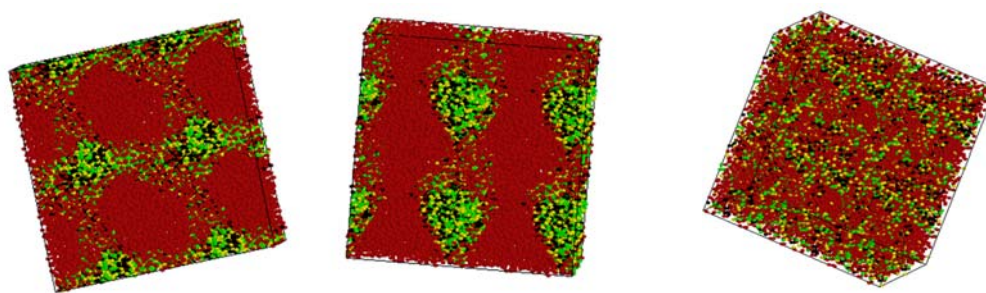


Figure 1.8 Morphologies of the 75-*b*-25 (*f*-*b*-*f*) charged diblock with 50% of the 25 block charged. (a) $T^*=0.05$, (b) $T^*=0.1$ and (c) $T^*=1.0$. Red dots represent the 75 neutral block, green and black represents the 25 block, yellow dots are counterions. Neutral diblock morphologies can be observed where the minority block forms the structure and the majority block forms the bulk.⁵³ Figures are adapted from Ref 53.

thermoplastic elastomers with properties of chemically crosslinked materials without losing processability at high temperature.^{25, 26, 109, 110} The general behavior of these ionomers was that two levels of phase separation existed, consisting of ionic domains of 3–4 nm size and microstructure of diblocks.^{25, 26} The extent of micro-phase separation decreased as sulfonation degree and ionic strength in terms of ionic dipole interactions of different counterions increased.¹⁰⁹ The ionic domain disassociated at lower temperature for block ionomers in a salt form (Zn salt) than that for similar ionic random copolymers; however, the dissociation temperature was hard to approach for relatively high SD polymers and polymers in the Na salt form before the functional groups of sPS began to decompose.^{24, 25} For this single architecture of triblock copolymers, no order-order transition was observed in this narrow SD range. However, sulfonation (SD5%) shifted the morphologies, which was in agreement of the results of Mauritz *et al.*¹¹¹ where well-ordered LAM structures were obtained upon sulfonation (SD12%), evolving from HEX structure of parent SEBS after a careful annealing process. The morphology studies were then extended to diblock and triblock copolymers of PS and poly(ethylene-*alt*-propylene) with different compositions (10–50 wt% of PS) with SD between 1–17% of polystyrene block.¹¹² The diblock with 20 wt % of PS changed the morphology from HEX to lamellar once sulfonated, the same as in the triblocks mentioned previously. This was expected from an increase of χN due to incorporation of sulfonate groups that results in higher χ and a potential increase of N due to hydrogen bonding interaction, which shifted the phase separation into the lamellar zone. This observation coincided with the lamellar structure obtained in asymmetric sPS-*b*-PMB (25 v% of PS) discussed above, although with different SD and molecular weight of the block copolymers.⁸⁰ For other diblocks

with high PS contents and triblocks with a range of PS compositions and SD studied, the parental morphologies were retained (spheres, HEX and lamellar) as demonstrated by SAXS. However, the sulfonation caused broadening of SAXS peaks due to the ion rich domain of sulfonated PS in the PS domain, which disturbed the micro-phase separation of block copolymers. Dynamic mechanical thermal analysis showed that the DOT had to be preceded by disassociation of ionic domain,^{24, 112} which potentially means high temperature has to be used to induce well-ordered phase separation and OOT²⁴(due to high T_g ¹¹³), before sulfonate groups of *s*PS potentially begins to decompose.¹¹⁴ Essentially, it is very difficult to push the phase separation into equilibrium by means of thermal annealing due to strong ionic interactions, especially for polymers with high SD.

Further, Kim *et al.*¹¹⁵⁻¹¹⁷ demonstrated the relationship between sulfonation degrees, solvents used to cast membranes and phase separation, proton conductivity and methanol permeability from *s*SEBS triblock copolymers ($M_w = 118,000$ g/mol, 28% wt of PS, SD8–47%) for the purpose of developing PEM for fuel cell application. It was shown by TEM and SAXS that well phase separated lamellar structure changed to disordered one as contents of methanol in THF and sulfonation degree increased, which is in agreement with that of block PS ionomers.^{25, 112} Due to the formation of disordered interconnected phase of *s*PS, both of proton conductivity and permeation of methanol were enhanced, however methanol was favored over water on contents uptake of these materials which indicates the difficulty to prepare direct methanol fuel cell (DMFC) from PEM of these materials. The disordered interconnected morphology was attributed to the favorable interaction of methanol and *s*PS chains during the film casting process.¹¹⁵

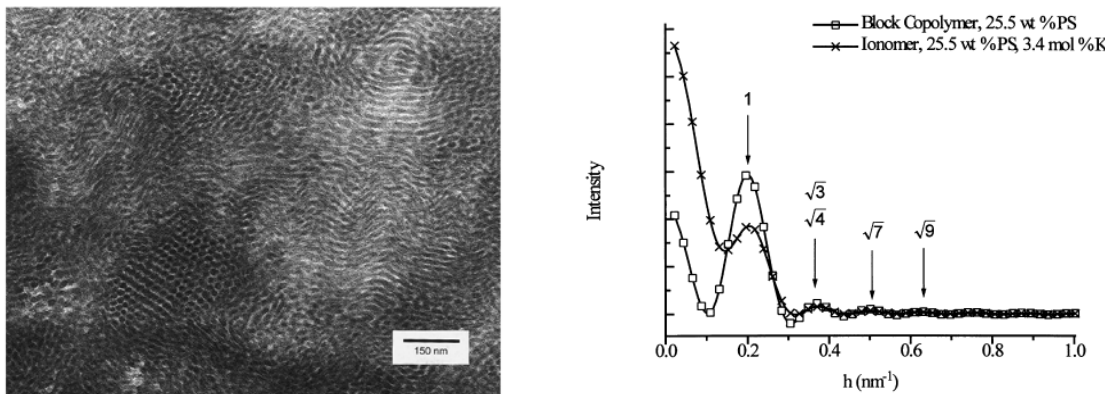


Figure 1.9 TEM image of the long range ordered morphology of *s*SBS ionomer (left); and SAXS profiles of precursor and ionomers showing HEX structures (right) (23 v% of PS; ionomer, SD3.4% in potassium salt form). Figures are adapted from Ref 118.

Storey *et al.*¹¹⁸⁻¹²⁰ and Elabd *et al.*¹²¹⁻¹²³ worked on a series of triblock copolymers of polystyrene-*b*-poly(isobutylene)-*b*-polystyrene (sSBS). The former focused on synthesis, mechanical properties^{119, 120} and morphology study by SAXS and TEM (Figure 1.9) with polymers of very low sulfonation ($M_n \sim 60$ kg/mol, ~ 23 v% of PS, SD <5%) showing a well-ordered HEX structure in equilibrium for ionomers for the first time by carefully controlling bulk film preparation conditions,¹¹⁸ while the latter extended sulfonation degrees to the range of 13–82% to elucidate the block copolymer morphologies as a function of SD as well as the subsequent effects on transport properties in terms of proton conductivity and methanol permeability ($M_n \sim 49$ kg/mol, 31 wt% of PS). The original cylindrical structure of the precursor was disrupted by low sulfonation (SD13%) when both samples were cast from toluene, which was consistent with other studies,^{93, 117, 118} as indicated by SAXS; lamellar structure as formed from casting solution in toluene/hexanol was disordered when PS was further sulfonated to 82% as shown by lack of high-order reflection peaks as well as peak broadening in SAXS.^{121, 123} The effect of the casting solvent was obviously inducing a cylinder structure to lamellar although all the samples were annealed at 50 °C for 2 weeks. As a further step, different solvents were used to study the morphology and proton conductivity. Although no conclusive solvent-morphology-conductivity relationship was drawn, the SAXS profiles of each sample cast from different solvents showed distinctly different scattering patterns indicating possible lamellar and cylinder structure (even coexistence) as shown in Figure 1.10 (left) below, which resulted in a 3 orders of magnitude of differences in proton transport.¹²³ The effects of solvent on morphology had been well investigated on neutral block

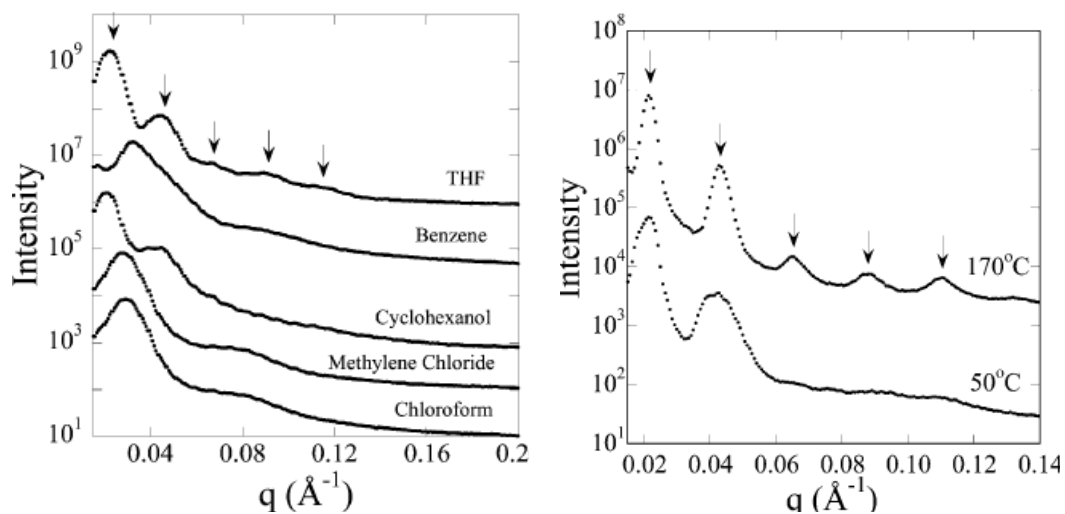


Figure 1.10 Left, SAXS profiles illustrating solvent effects on morphologies of s SBS (SD29%): Bragg scattering at $1:\sqrt{3}:\sqrt{7}$ (chloroform); $1:2:\sqrt{7}$ (methylene chloride); $1:2:3$ (cyclohexanol); $1:2:\sqrt{7}$ (benzene); $1:2:3:4:5$ (THF). Right, annealing effects on morphology of s SBS (SD22%). Thermal annealing helped promote long range ordered structure cast from THF, lamellae, $1:2:3:4:5$.¹²³ Figures are adapted from Ref 123.

copolymers;¹²⁴ however, its effects on ionic block copolymers have not been well explored.^{43, 116, 123} Interestingly, annealing at high temperature of 170 °C helped to form a long range ordered structure when cast from THF solution, as shown in Figure 1.10 (right); while no order was obtained from a solution in toluene when the film was annealed at same temperature for the sample with SD22%.¹²³ Goswami^{43, 53} showed that annealing would disorder long range order of morphology for sulfonated *sPS-*b*-PI* by experiment and simulation; however the differences here may be attributed to the architecture of the polymer and sulfonation degree. By performing conductivity and DMFC tests, the authors demonstrated transport properties are not only dependent on morphology but also on orientation of the structure in membranes.¹²¹⁻¹²³

Sulfonated poly(styrene)-*b*-poly(vinylidene fluoride)-*b*-poly(styrene) of high molecular weight (95,000 g/mol, 40 wt% of PS) was synthesized by Xu *et al.* with SD ranging from 13–100%.¹²⁵ Preliminary TEM and AFM images showed that low sulfonation disrupted the lamellar structure of non-sulfonated precursors;^{18, 125} while inter-connected large ionic channels developed as SD increased from SD > 23%. This was attributed to macro-phase separation driven by electrostatic interactions of ion pairs, which coincided with an abrupt increase of water uptake and proton conductivity. No long range ordered structures were observed, possibly owing to large PDI from the middle block prepared by conventional radical polymerization, or by low mobility of the fluorine containing components as suggested by Hickner *et al.*^{126, 127}

Hickner *et al.*^{126, 127} prepared and studied two series of triblock copolymers of poly(hexyl methacrylate)-*b*-sulfonated poly(styrene)-*b*-poly(hexyl methacrylate) (PHMA-*b*-*sPS*-*b*-PHMA) and poly(perfluorooctyl methacrylate)-*b*-poly(styrene)-*b*-

poly(perfluorooctyl methacrylate) (PFMA-*b*-sPS-*b*-PFMA) with different sulfonation degrees ranging from low to full sulfonation. None of these materials formed long range well-ordered morphologies. For the PHMA-*b*-sPS-*b*-PHMA series, as sulfonation level increased, the morphologies changed from local ordered lamellar to disordered to cylindrical structures as indicated by SAXS data. This data also showed that the primary domain spacing decreased since the primary scattering vectors shifted to high angles. The change of morphologies and the decrease of domain spacing with SD was explained by ion condensation and that the solution-state structure may template the bulk morphology.^{93, 127} However, the triblocks containing fluorine did not show any ordered structure due to low mobility of the chains and low solubility of the polymers during film casting process,¹²⁷ which prohibited the self-assembly process. That the ordered PHMA triblock showed better proton conductivity, indicates the importance of phase separation in the application of PEMFC, as mentioned above.²³

More recently, Long *et al.*⁶⁴ prepared two series of ABA type triblock copolymers containing poly(trialkyl-4-vinylbenzyl phosphonium chloride) as a charged outer block (alkyl = -butyl, -octyl) and poly(*n*-butyl acrylate) as the middle block. Thermal and mechanical properties (by DSC and dynamic mechanical analysis) studies showed that the incorporation of phosphonium cations into PS prompted good phase separation. This was determined by comparing to non-charged analogues containing PS as the outer block, as indicated by having the same well-defined T_g s of *Pn*BuA and the charged block with high ion contents as respective homopolymers. Lamellar morphology was observed in TEM and by SAXS for a sample containing 50 wt% of the ionic part with octyl as the alkyl group; while a possible cylinder structure existed as inferred from TEM image for

the sample containing 33 wt% charged contents with butyl as alkyl group. The longer chain of the octyl groups compared to butyl groups effectively results in a more dilute ion-dipole interaction between ion pairs, which decreased T_g and the relaxation time of hard/ionic domain of these thermoplastic elastomer like materials.⁶⁴

1.3.3 Other structures/graft copolymers

No systematic work on morphology had been conducted for charged copolymers with branched structures; such work has only been carried out on graft copolymers for the purpose of developing PEMs.^{128, 129} One of reasons is the synthetic challenge inherent in creating well-defined structures in terms of the contents of and the location of ions in addition to the difficulties involved in controlled graft copolymer synthesis.¹³⁰

Holdcroft *et al.*^{129, 131, 132} utilized stable free radical polymerization to prepare styrene functionalized macromonomer poly(sodium styrene sulfonate)(sPSNa) as grafts, followed by copolymerizing macromonomers with styrene by emulsion polymerization to obtain graft copolymers with controlled graft density and length of ionic grafts.^{129, 132} These materials demonstrated better proton conductivity with less water uptake as compared to random sulfonated homopolystyrene. This result was explained by using TEM to elucidate the morphology differences between the graft and random polymers. The graft polymers with high ion content and long grafts showed stronger phase separation and better connected ion channels than those with low ion contents and short grafts; while random sulfonated homopolymers were the poorest in terms of extent of phase separation and ion conductivity.¹²⁹ Whether these morphologies were kinetically trapped or at equilibrium is uncertain since the membrane for testing was made by compression molding at high temperature, as discussed above, and the texture of the bulk

morphology is still in question due to limited information only inferred from TEM. The blockiness of the charged part (100% sulfonation) endowed the morphology with typical block copolymer domain size, which increased the size of proton conducting channels.

In regard to the chemical ingredients, the graft polymers were extended to contain fluorine (partially sulfonated poly([vinylidene difluoride-*co*-chlorotrifluoroethylene]-*g*-polystyrene [P(VDF-*co*-CTFE)-*g*-sPS]) by the same authors^{49, 128} and Chung *et al.*¹³³, taking advantages of the stronger incompatibility and better chemical stability from the intrinsic characteristics of fluorine.¹³⁴ Generally, limited phase separation had taken place with cluster-network-like morphologies for high graft density and short grafts,⁴⁹ while polymers with low density grafts of long chains tended to micro-phase separate into lamellar- or cylinder-like structures.^{129, 133} The ionic domains were studied in terms of cluster size and density (number of clusters in 2-dimensional area) which were shown to be controlled by graft lengths and ion exchange capacity. This dependence could be understood by considering the sulfonation degree of the PS grafts. The proximity of the ionic groups determines the size, number density of clusters, and relative purity of ionic and hydrophobic domains. The closer the sulfonate groups are on PS grafts (high SD) gave larger and purer ionic domains. The size of clusters (2–4 nm) was typically smaller than that of Nafion (5–10 nm). Unfortunately, morphological information was only extracted on “pure” ionic domains that were stained by lead acetate in the TEM; while the bulk texture of phase separation between the fluorinated parts and sPS was unclear (although the stained structures may reflect the bulk morphology). As is well-known, ionic copolymers contain two levels of phase separation, as discussed above for charged diblock copolymers.

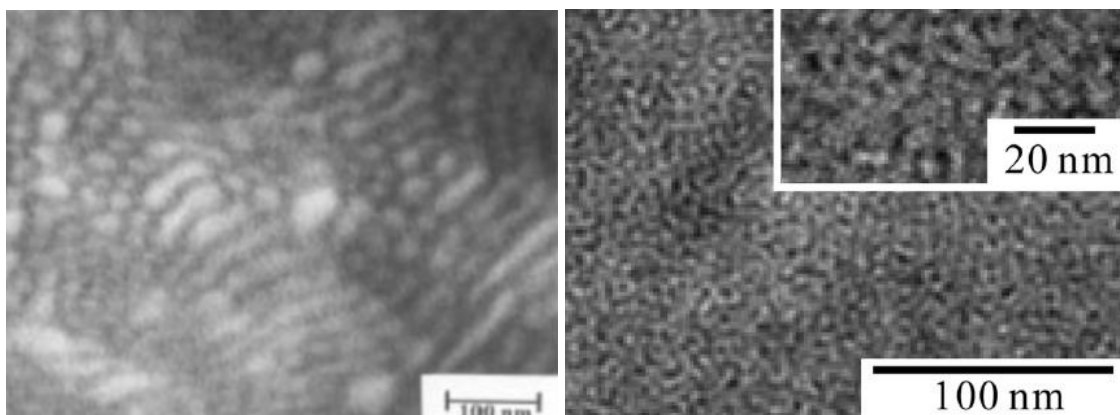


Figure 1.11 Morphology comparison (a, left) linear diblock copolymer (P[VDF-*co*-HFP]-*b*-PS), $M_{n,P[VDF-co-HFP]}=1.79 \times 10^4$ g/mol, $M_{n,PS} = 8.1 \times 10^3$ g/mol SD=22%;⁹¹ (b, right) graft copolymers, P(VDF-*co*-CTFE)-*g*-SPS, $M_{n, \text{back bone}}=3.12 \times 10^5$ g/mol, $M_{n, \text{graft polymer}} = 13.6 \times 10^5$ g/mol, graft density = 2.6%, SD = 41%.¹²⁸ Figures are adapted from Ref 91 and 128.

It is interesting to discuss the morphology behavior among fluorinated linear block copolymers, fluorinated graft copolymers, and non-fluorinated copolymers. The current observations^{91, 127, 128} show that it is difficult to achieve good long range ordered phase separation for copolymers that contain fluorinated contents, except the *sPS-*b*-fPI* case reported by Goswami *et al.*⁴³ Graft copolymers are even worse in regard to micro-phase separation in the range of compositions studied as compared to linear ones. A typical comparison is shown in Figure 1.11 for diblock and graft copolymers. Bear in mind that the morphology is determined by many factors such as sulfonation degree for linear block copolymer⁴⁴ and grafting density for graft copolymers.¹³⁵ As seen in Figure 1.11(a), linear ionic diblock copolymers containing a fluorine block and graft copolymers with long grafts (*sPS* for example) have good phase separation with limited long range order; while graft copolymers with short grafts and high graft density are more likely to exhibit connected cluster-like morphology (Figure 1.11(b)). The latter is due to the structural resemblance of two topologically different polymers. Graft copolymers with short graft length and high density of grafts would behave more like charged copolymers with a random distribution of ionic groups (or short ionic chains) attached to the backbone (e.g. sulfonated homopolystyrene).

Considering the intrinsic nature of branching (like graft copolymers), e.g., the spatial position of components, the ionic functional groups can be controlled in location of the branched structures by synthetic methods, by which the effects of site-specific charged groups on morphology behavior could be explored. Long and coworkers¹³⁶ prepared sulfonated graft copolymers of methyl methacrylate (MMA), styrene (S) and *tert*-butyl styrene(*tBS*), in which MMA composed the backbone and the grafts consisted

of PtBS as the main content with short sulfonated PS as ionic component. *s*PS was either located at the conjunction between backbone and braches or at the terminus of PtBS braches, and these two architectures were compared in terms of thermal behavior and phase separation. The graft copolymers with the charged part in either acid or neutralized Na form locating at the end of branches showed higher glass transition temperatures as compared to those with ionic groups at the junctions for which T_g s were higher than that of uncharged precursors.¹³⁶ The ionization interrupted the micro-phase separation of the precursor neutral graft copolymers;^{109, 112} while it was not strong enough to change the texture of the lamellar structure as expected from the compositions, due to low ionic contents (~5 wt%). Furthermore, the ionic graft copolymers with *s*PS located at the terminus of PtBS presented the typical ionic clusters of around 5 nm in size.^{113, 137, 138} The reason for higher T_g and ionic cluster formation from graft polymers with *s*PS at the end of PtBS is due to the improved mobility of the terminus over the junction, which benefits the ion-dipole interaction between sulfonate groups.^{25, 136}

1.3.4 Block copolymers in Ionic Liquids (ILs)

As one of the strategies to obtain high conductivity at high temperature, and good mechanical strength for PEM materials, block copolymers conjugated with ionic liquids is very promising considering the combination of matrix supporting ability of block copolymers and the intrinsic nature of ILs, e.g., high temperature stability and conductivity, extremely low vapor pressure.^{39, 63, 139} In recent years, much attention has been devoted to understanding and developing composite materials containing polymers and ILs, for green energy applications such as fuel cells, and most of these studies have focused on random copolymers.^{140, 141} Morphological understanding of diblock/IL

composites is one of the critical underpinnings for achieving designed PEMs but work in this area has just begun, as one of the aspects in the investigation of lyotropic phase transition in systems of block copolymers/molecular solvent.^{142, 143}

Well-defined block copolymers of sulfonated styrene and methylbutylene have been investigated in terms of proton conductivity and morphology as discussed above.^{44,}
⁴⁵ Thus composite PEMs composed of *s*PS-*b*-PMB and ILs could be considered a good candidate for morphology studies. The ILs integrated *s*PS-*b*-PMB copolymers exhibited a variety of phase behaviors as a function of type of ILs, concentration of ILs in the bulk, and sulfonation degree.¹⁴⁴ For example, the filling with 1-ethyl-3-methylimidazolium tosylate in a range of concentrations induced lamellae to HPL morphology transition of block copolymers as evidenced by SAXS and TEM. Transitions of self-assembly of block copolymers caused by different kinds of ILs was also demonstrated (imidazolium as the same cation, tosylate(Tos), methanesulfonate (MS), tetrafluoroborate (BF₄) as anions) as shown Figure 1.12. Systematic investigation of this particular composite revealed the phase diagram, and showed that the compatibility/absorbance of ILs within *s*PS domains leads to volume fraction changes, imposing a critical effect on phase behavior. The morphology-conductivity relationship was also revealed by normalization of conductivity as a function of multiple factors (essentially morphology).¹⁴⁴

Lodge *et al.*^{145, 146} systematically investigated the lyotropic phase diagrams of ionic composites of block copolymers (PB-*b*-PEO, PS-*b*-PEO) of different compositions with 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([EMI][TFSI]) and 1-butyl-3-methylimidazolium hexafluorophosphate ([BMI][PF₆]) over a range of

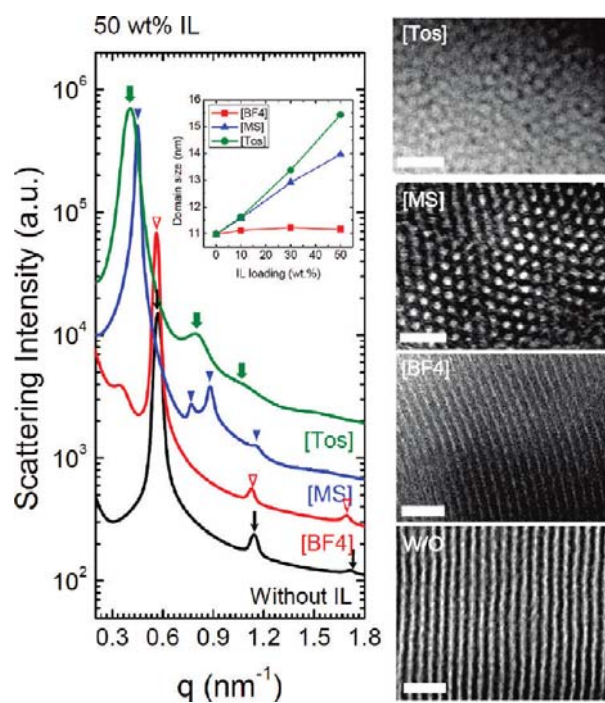


Figure 1.12 SAXS and TEM characterization of morphology transitions of composites of *sPS-*b*-PMB* (5.9kg/mol-*b*-5.1kg/mol, SD21%) as a function of ILs at 50 wt% filling rate. From bottom to top: lamellae (neat polymers without ILs), lamellae (BF4), HEX (MS), spherical (Tos). Inset in SAXS data shows primary domain spacing as a function of ILs loading rate with different ILs.¹⁴⁴ Data were adapted from Ref 144.

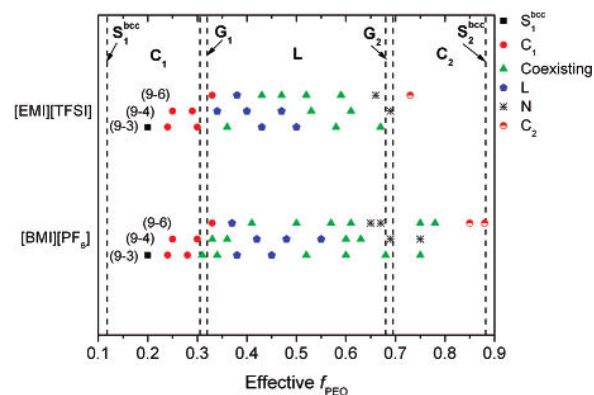


Figure 1.13 Phase diagram of PB-*b*-PEO/ILs. *x*-axis is volume fraction of PEO/ILs; *y*-axis corresponds to different ILs, and shifted in different PS-*b*-PEO for clarity. S, C, Coexisting, L, N denote sphere, cylinder (HEX), coexisting of C and L, lamellae, disordered network microstructures, respectively. The vertical dashed lines represent theoretical phase boundaries.^{146, 147} Data were adapted from Ref 147.

concentrations as shown in Figure 1.13. It was found that phase progression determined by SAXS qualitatively matched well with theoretical predictions¹⁴⁷ for diblock copolymers and was generally observed to progress from sphere to HEX to LAM to HEX to sphere depending on the concentration of ILs which had high selectivity for PEO domains. Interestingly, there existed disordered network microstructure and coexistence of LAM and HEX microstructures in PB-*b*-PEO composites.¹⁴⁶ The former was speculated to be analogous to behavior of PB-*b*-PEO in water;^{146, 148} while the latter was due to the increased segregation between two blocks caused by the incorporation of ILs, which alleviated frustrated packing involved in gyroid structures.¹⁴⁶ These materials showed promising conductivity as a function of ILs concentration and molecular length of PEO if the microstructure domains could be aligned properly.^{145, 149}

Segalman, Balsara and co-workers¹⁵⁰⁻¹⁵² characterized the lyotropic and thermotropic phase behavior as well as a domain spacing scaling analysis of poly(styrene)-*b*-poly(2-vinylpyridine) (PS-*b*-P2VP) copolymers in an ionic liquid, imidazolium bis(trifluoromethane)sulfonamide ([Im][TFSI]), selective for P2VP. Characterization was achieved with SAXS, SANS, DSC and optical transmission, as a function of multiple variables, e.g., compositions, molecular weights and polymer volume fractions. For simplicity, one example of a polymer of PS-*b*-P2VP (13 kg/mol-*b*-15 kg/mol) is considered here. As a function of concentration of IL and temperature, the morphology experienced ODT and OOT transitions, specifically between lamellae, HEX, coexisting morphology of lamellae and HEX, disorder, liquid like-micellar structure, as shown in Figure 1.14 below.

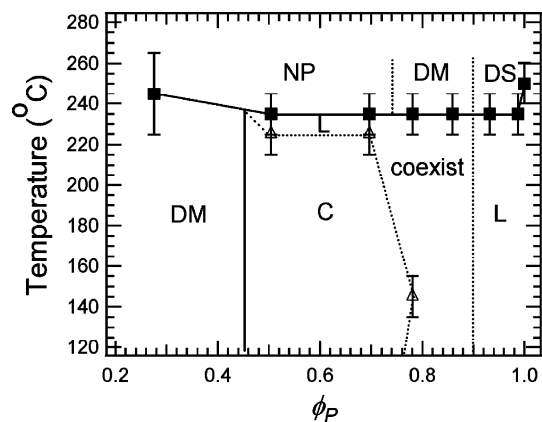


Figure 1.14 Phase diagram of PS-*b*-P2VP in ([Im][TFSI]) as a function of temperature (y -axis) and polymer volume fraction in solution (x -axis). DM, C, coexist, L, NP, DS denotes disordered micelle with liquid-like local structure, cylinder, coexistence of C and L, lamellae, nonperiodic disordered, disordered (reversible with L), respectively.¹⁵⁰

Figure was adapted from Ref 150.

The reversibility of phase separation was also studied by thermal annealing, showing that at high volume fraction of polymer (> 93 vol %) ODT is reversible while at low concentration it is irreversible or that a long annealing time is needed.^{150, 153} The study of lyotropic phase behavior generally concluded with the similarity between system of block copolymer/selective molecular solvents and mixtures of block copolymer/ILs; the addition of ILs strongly selective for one block into block copolymers increases segregation between blocks (χ).^{142, 143, 146, 152, 154, 155}

1.4 Morphology of Ionic Block Copolymers in Aqueous Media

Due to promising applications such as cargo delivery, biomedical/pharmaceutics, nanotechnology, self-assembly of amphiphilic block copolymers in water is a frequently pursued topic in polymer science.^{156, 157} As is well-known, the incompatibility between two covalently linked blocks drives each to form its individual domains. Similarly, in a selective solvent (e.g., water) for one block, amphiphilic block copolymers self-organize into nanometer-sized aggregates (micelles, vesicles, *etc.*), with the hydrophobic components forming the core of the micelles and the hydrophilic block forming the corona. The formation of aggregates is driven by the minimization of free energy of the system. Though sharing this common driving force, they produce different morphologies of aggregates which are mainly determined by factors including chain conformation, interfacial energy, volume fraction of each component, Flory-Huggins interaction (χ) between two blocks, *etc.*¹⁵⁸

Most of the amphiphilic block copolymers consist of charged block copolymers or block polyelectrolytes. Since the pioneering work of Selb and Gallot on pyridium type block copolymers in aqueous media,^{14, 28} numerous papers have been published on the

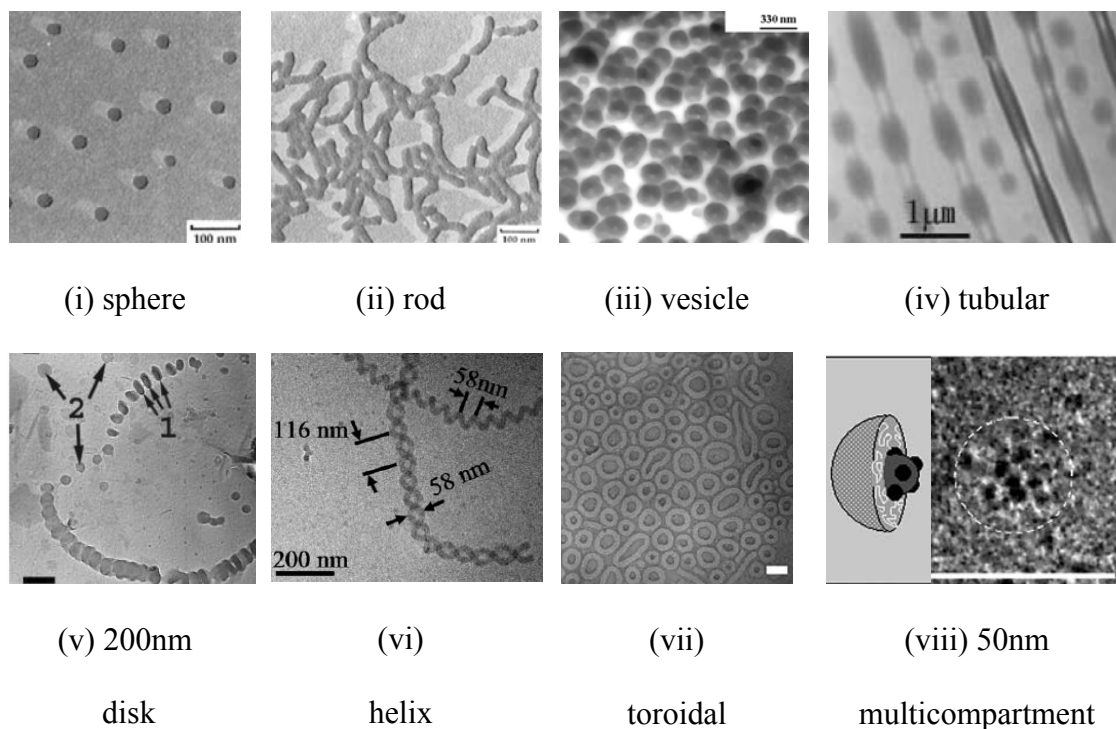


Figure 1.15 Various morphologies of block polyelectrolytes in aqueous media. (i)(ii), polystyrene-*b*-polyacrylic acid in H₂O.^{158, 162} (iii) poly(butadiene)-*b*-poly(γ -L-glutamic acid) in H₂O.¹⁶³ (iv) poly(L-lysine)-*b*-poly(tetrahydrofuran)-*b*-poly(L-lysine) in H₂O.¹⁶⁴ (v)(vi)(vii) Complex of poly(acrylic acid)-*b*-poly(methyl acrylate)-*b*-polystyrene and ethylenedioxy-bis-ethylenediamine in water.¹⁶⁵⁻¹⁶⁷ (viii) poly(4-methyl-4-(4-vinylbenzyl)morpholin-4-ium chloride)-*b*-polystyrene-*b*-poly(pentafluorophenyl 4-vinylbenzyl ether).¹⁶⁸ Refer to the corresponding references for detailed information about the micelle structures and experiments. Figures are adapted from corresponding references.

subject of micelles (in water) and reverse micelles (in organic solvent) of block polyelectrolytes, in terms of characterization, mechanisms, theoretical calculations, as well as applications.^{42, 156, 157} Nevertheless, from reviewing the literature it is clear that the morphologies exhibited by block electrolytes, to the best of our knowledge, reveal limited varieties as is shown in Figure 1.15. Morphologies illustrated include spheres (star-like, crew-cut), rod/cylinders/worm-like, vesicles (complicated vesicles), tubes (hollow cylinders), disks, helix, toroidal structures, and multicompartment structures.¹⁵⁹⁻

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The majority of morphologies that amphiphilic block polymers (including neutral and charged block polymers) experimentally display are classical structures, e.g., spheres, cylinders, and vesicles. It is interesting to note that the nano-structures shown above in Figure 1.15 are mostly derived from weak electrolyte block copolymers consisting of acrylic acid, and protonated amine type materials. That is due to the feasibility of tunable solubility (pH sensitivity) and ample interaction of hydrophilic blocks as well as the compositions. In contrast, strong electrolyte block copolymers (ionic groups can fully dissociate in all range of pH such as sulfonic acids, quarternized amines, hydrophilic PILs) tend to only form spherical micelles, although these systems have received only limited morphological exploration.^{60, 169-173}

Very recently we observed that amphiphilic block copolymers based on strong polyelectrolyte sulfonated polystyrene-*b*-fluorinated isoprene (*s*PS-*b*-fPI) form ribbon-like micelles and tapered rod/cylinders in aqueous media as shown in Figure 1.16.¹⁷⁴ The structures were confirmed by atomic force microscopy, dynamic light scattering and TEM. The formation of these novel structures must be attributed to the inhomogeneity of

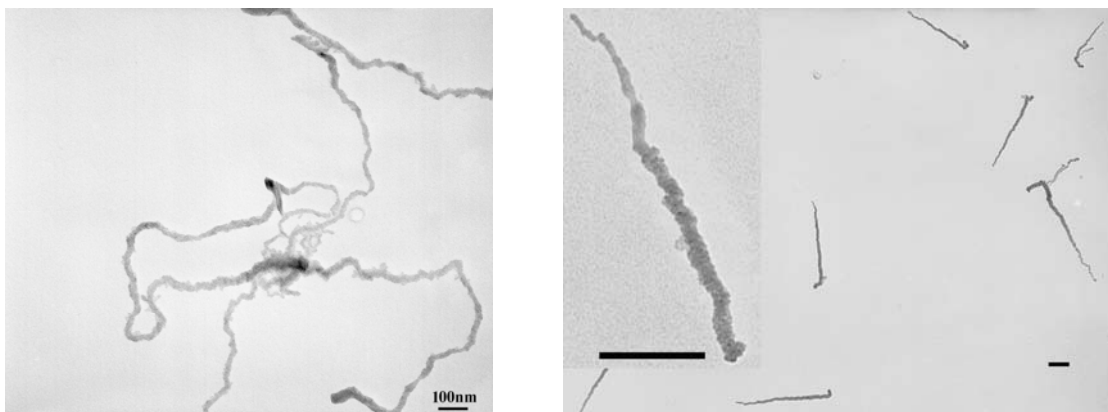


Figure 1.16 Morphologies of *sPS-b-fPI* in water. Left, ribbon like structures; Right, tapered rod (scale bar 250 nm).¹⁷⁴ Figures are adapted from Ref 174.

sulfonation of polystyrene that occurred when polymers were sulfonated, which leads to a distribution of sulfonation for all the polymer chains. The high Flory-Huggins interaction parameter (χ) between *s*PS and *f*PI imparts chain stretching of core forming *f*PI of the block copolymer chains having different SD, which was further illustrated by computational simulation. Moreover, intra-micelle phase separation caused the formed cylinder structure to be tapered in order to minimize free energy of the system.¹⁷⁴ Thus, the intrinsic nature of partially ionized PS and strong aggregation of different components causes this unusual self-assembly. This type of information may be useful for understanding and developing new nano-structures.

1.5 Conclusions and Outlook

The fundamental role of electrostatics in the micro-phase separation of charged block copolymers has been explored and the results were discussed in this introduction. To date it has been shown that charges can give rise to unique and tunable polymer and solution phase morphologies. Based on this understanding, a new dimensionality for block copolymer morphologies has been demonstrated and this expands the possibilities for target-controlled self-assembly processes that can allow for broad technological applicability of block copolymer assemblies.

Although considerable insights into the morphological behavior of charged block copolymers have been accomplished, more efforts are clearly needed in order to provide a complete or universal picture in terms of their structure-property relationships. By synthesizing well-defined ionic block copolymers with different structures and systematic characterization of these materials, combined with theoretical modeling/calculation development, predictive capabilities may be developed for these materials. Furthermore,

the structure and topology of the charged block polymers can be taken into account as a new parameter to understand complex systems.

One notable emerging area of research is block copolymers containing poly(ionic liquids) (PILs) as building blocks. The marriage of diblock copolymers and poly(ionic liquids) combines their individual respective natures, in which block copolymers with the ability of micro-phase separation provide a mechanical scaffold for the materials while poly(ionic liquids) endow the materials with broad applications such as in catalysis, gas separation media and energy resources (e.g. fuel cell and batteries). The fundamental understanding of the role of morphology in controlling material properties (for example, ionic conductivity) has to be addressed for the purposes of targeting/designing, development and utilization of such type of block copolymers.

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Chapter 2 Synthesis and Characterization of Fluorinated and Sulfonated Block Copolymers

Abstract

Well-defined block copolymers of isoprene and styrene were synthesized by sequential polymerization of monomers *via* anionic polymerization using high vacuum techniques. The polyisoprene (PI) segments were fluorinated first by addition of difluorocarbene, followed by sulfonation of polystyrene. The reaction conditions for fluorination of polyisoprene and sulfonation of polystyrene were optimized to fulfill the requirements of full saturation of PI followed by introduction of a variety of ionic contents into the PS block. All the materials were carefully characterized by gel permeation chromatography, ¹HNMR, and elemental analysis.

2.1 Background

Proton exchange membranes (PEM), a key to hydrogen fuel cells - a potential green energy source in the future, have been extensively studied and developed.¹ In the past decades, numerous materials designed for high proton conductivity, chemical stability and good mechanical properties have been developed.¹⁻⁴ Although Nafion, a classical commercial product developed by Dupont, has been applied in areas such as fuel cells, liquid and gas separation due to its unique ionic and fluorine containing structure, new materials for PEMs are still highly desirable because of Nafion's high cost. Thus designing and developing new PEM materials with better performance and lower cost than Nafion^{1, 4} is highly desired. As discussed in Chapter 1, block copolymers with micro-phase separation can offer better proton conductivity and function as the matrix of PEM systems, as compared to random ionomers such as Nafion. However, the study of well-defined charged block copolymers is limited and mainly focused on hydrogenated materials, thus incorporation of new ingredients (components) into block copolymers may help to elucidate understanding of behavior of proton transport, morphology, mechanical properties and the relationship between them.⁵⁻⁷ Here, we develop a new well-defined block copolymer material which can potentially be used as a PEM material or serve as a model block copolymers to understand and build structure-morphology-property relationship for understanding the behavior of Nafion and guide the design of new materials. Having this motive, we present our synthesis of model fluorinated and sulfonated block copolymers from the precursor poly(styrene)-*b*-poly(isoprene) which is first fully fluorinated followed by controlled sulfonation.^{8, 9} Due to its ionic nature (sulfonic acid/sodium salt, highly hydrophilic) and fluorine containing contents (strongly

hydrophobic, even lipophobic), a high value of Flory-Huggins interaction parameter χ between the blocks is expected, which can lead to interesting self-assembly behavior.^{8,9} To the best of our knowledge, we are the first to develop well-defined diblock copolymers containing both fluorine contents and ionic groups on the different blocks.

With these novel materials we initiate a series of investigation designed to achieve fundamental understanding of these novel block copolymers, as described in the following chapters. The micro-phase separation behavior in the melt will be presented in Chapter 3, and aqueous self-assembly of these block copolymers will be discussed in Chapter 4.

2.2 Experimental Part

2.2.1 Materials

Styrene (Aldrich, 99%), isoprene (Fisher, 98%), cyclohexane (Fisher, 99%) and methanol (Fisher, ACS certified) were purified according to standard procedures¹ for anionic polymerization. *sec*-Butyllithium was synthesized¹ and its concentration was determined by anionically polymerizing styrene and measuring the number-average molecular weight of the product. Hexafluoropropylene oxide (HFPO, Aldrich, 98%), 2,6-di-*tert*-butyl-4-methylphenol (BHT, Aldrich, > 99.0%), 1,2-dichloroethane anhydrous (DCE, Aldrich, 99.8%), acetyl anhydride (Aldrich, 99.5%) concentrated sulfuric acid (Aldrich, ACS reagent 95 to 98%), sulfur trioxide (Aldrich, 99%), triethyl phosphate (99.8%, Aldrich) were used as received.

2.2.2 Synthesis of Diblock Copolymer of Styrene and Isoprene

The diblock copolymers of styrene and isoprene were prepared by sequential polymerization of these monomers *via* high vacuum techniques.^{10, 11} The main reactor

was washed with *n*-butyllithium solution in cyclohexane to remove impurities on the glassware, followed by rinsing with distilled cyclohexane from a purge flask. Solvents were finally collected from purging solution of *n*-butyllithium. A typical procedure for preparation of diblock copolymer PS-*b*-PI is as follows.

All reagents in ampoules, including styrene, isoprene, initiator and methanol, were attached to the reactor and introduced at the appropriate time by breaking a break-seal. Polystyryllithium was generated by reacting *sec*-BuLi with styrene in cyclohexane. The immediate red color of the solution indicated successful initiation followed by propagation of styrene. This reaction solution was left overnight at room temperature to ensure complete consumption of styrene. Small aliquots of solution were sampled before isoprene was charged for characterization of the polystyrene block. Upon addition of isoprene, the color changed from red to pale yellow indicating the initiation of the isoprene block. Isoprene polymerization was allowed to proceed overnight at room temperature and was quenched by addition of methanol. Finally, diblock copolymer products were recovered by pouring the reaction solution into a 10-fold excess of methanol containing BHT (0.1%, w/v), filtered, dried in vacuo at 50 °C.

2.2.3 Fluorination of Block Copolymer of Styrene and Isoprene

Fluorination of polyisoprene blocks of block copolymers was conducted in a high pressure Parr reactor at 180 °C under magnetic stirring. A typical fluorination reaction is described as follows. 4.06 grams of PS-*b*-PI block copolymers (isoprene units, 1.89g, 0.028 mol) were added into the reactor along with 0.19 grams of BHT (~10 wt % of polyisoprene), both of which were dissolved in 200 ml of purified cyclohexane. The reactor was assembled, purged with pure N₂ for about 0.5 hours to remove oxygen, and

then was sealed. After the system was frozen, the reactor was degassed for about half an hour in order to remove residual traces of oxygen, more importantly, to create a vacuum for readily charging the fluorination reagent hexafluoropropylene oxide (HFPO, 51.8g, 0.31mol) through the inlet of the reactor. The amounts of HFPO added were monitored during the process of transferring HFPO into reactor. The accurate mass added into reactor was obtained by weighing the HFPO tank before and after transferring. The solution was thawed using a hot water bath to room temperature, followed by heating to 180 °C, and was maintained at this temperature for 4 hours. The reaction was quenched by cooling the system with ice water bath to room temperature. The pressure and the excess of HFPO were released by bubbling through a 10 wt % NaOH aqueous solution before the reactor was opened. The reaction solution was filtered, concentrated by rotary evaporation, and precipitated into large amounts of methanol or a mixture of methanol/2-propanol (1/2, v/v). The recovered polymers were dried under vacuo at 50 °C overnight. Yield, 5.05 g, 92.7 %.

2.2.4 Sulfonation of Polystyrene Blocks of Block Copolymers

Two methods were applied for obtaining different degrees of sulfonation. One method, utilizing acetyl sulfate developed by Makowski,¹² was applied to sulfonate polystyrene blocks. Low and medium sulfonation degrees (SD, molar percent of sulfonated styrene units, 0 – 60%) were achieved by this method. For high degrees of sulfonation (60% – 100%), the complex of sulfur trioxide and triethyl phosphate was used as sulfonating reagent.^{13, 14} Typical reactions were run as follows.

Acetyl sulfate method (Table 2.3, Run 4): A three-necked round bottom flask dried at 80 °C was equipped with an inlet of high purity nitrogen, a condenser with outlet

of N₂ to bubbler, magnetic stirring bar, and the third neck was sealed with a septum and was easy to open for the purpose of sampling the reaction solution. Block copolymers (1.0 g, styrene units: 0.40 g, 3.85 mmol) and 10 ml of 1,2-dichloroethane were added into the reactor after the empty reactor was purged with N₂ for about 20 min. The reactor then was immersed into a water bath thermostated to 50 °C and was stirred for about 30 – 60 min. Acetic anhydride (3.63ml, 38.4 mmol) was injected drop-wise into the solution, which was then stirred for 10 min, followed by drop-wise addition of sulfuric acid (2.0g, 20 mmol) under a slight flow of N₂ over 10 min with strong stirring. After a couple of minutes, the solution changed from colorless to yellow-brownish; no polymer was precipitated out of the solution during the entire period of reaction. Generally, after stirring at 50 °C for 2 – 5 hours, depending on the sulfonation degree desired, the reaction was quenched by cooling the reactor with ice water and by injection of several milliliters of methanol into the solution. For each reaction, the solution was sampled and tested by ¹H-NMR to confirm the desired SD before quenching. Sulfonation degree was monitored by sampling the solution out over a period of time (in this reaction). Solid polymers for ¹H-NMR were recovered by precipitating the sampled solution into hexane, followed by dissolution in *d*-DMSO. Yield after purification (see below), 1.01g, 85.1 %.

Sulfur trioxide/triethyl phosphate complex method for high SD: TEP (0.2 equiv, 0.14g) in DCE (7 ml) was placed in a flask equipped with two addition funnels (one of them was connected to N₂ outlet to bubbler), a condenser with N₂ inlet, and a magnetic stirring bar and was cooled to 0 °C using an ice bath. Copolymer (1.0 g, 1.0 equiv and 0.4g of PS, Table 2.1 No. 5) was dissolved in DCE (20 ml) and placed in one addition funnel. SO₃ (1.5 equiv. 0.45g) in DCE (7 ml) was placed in the other addition funnel.

The SO₃ solution was added first at about 1 to 2 drops per second. Polymer solution was added a few seconds later at the same rate with stirring. Then both solutions were alternatively added, while keeping the solution at 0 °C. After completing addition of reagents, the solution was kept at 0 °C and stirred for another hour, followed by injecting several milliliters of methanol to quench the reaction. Yield after purification, 1.23g, 100%; SD: 98% by ¹H-NMR.

2.2.5 Purification: Dialysis of Polymer Solution

After the reaction was quenched, the dichloroethane solvent was removed by rotary evaporation until residuals in flask stopped bubbling, followed by dissolving the residuals in about 5 to 10 ml of THF, forming a clear transparent solution. About an equal volume of distilled water was added into this solution (or the THF solution was slowly dropped into water). If the solution formed in this way was milky and opaque, it was subjected to rotary evaporation again to remove possible traces of DCE until either cloudiness disappeared or the solution stopped boiling. In the case of the latter situation, about an equivalent amount of THF was added into the solution in order to cause the milky solution to become clear and transparent. The polymer solutions in water/THF mixtures were dialyzed against deionized water over 72 hours with regular changes of water in order to remove small molecules such as sulfuric acid. As the dialysis proceeded slowly, it was observed that the solution in the dialysis tubing became clearer and clearer, in some cases solutions undergoing gel formation, followed by reverting back to a readily flowing solution. Finally, polymers were recovered by lyophilization. The yield was obtained basing on SD which was measured by ¹H-NMR prior to calculation.

2.3 Characterization

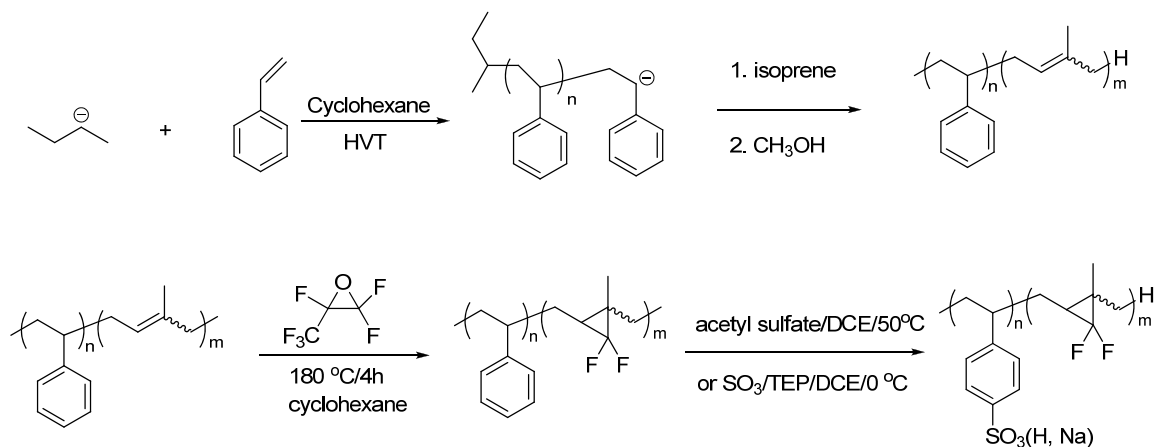
Number-average molecular weights M_n and polydispersity indices M_w/M_n (PDI) of all samples before sulfonation were determined by size exclusion chromatography using a Tosoh EcoSEC instrument which was equipped with two TSKgel Super Multipore HZ-V columns calibrated using standard polystyrenes with M_n from 580 to 7.5×10^6 g/mol. The polymer was eluted in THF at 40 °C at a flow rate of 0.35 ml/min.

^1H -NMR spectroscopy was performed on Varian Mercury 300 MHz spectrometer with deuterated dimethylsulfoxide ($\text{DMSO}-d_6$) and CDCl_3 as solvent. Element analysis (EA) was conducted by Galbraith Laboratories Inc for sulfur contents.

2.4 Results and Discussion

The synthesis of fluorinated and sulfonated block copolymers of styrene and isoprene mainly consists of two steps: fluorination of polyisoprene and sulfonation of polystyrene. The polyisoprene block was first saturated by *in-situ* generated difluorocarbene, followed by sulfonation using different methods for achieving low to medium to high sulfonation degrees. A representative reaction scheme is shown below in Scheme 2.1:

Scheme 2.1 Synthesis of PS-*b*-PI, PS-*b*-fPI and sPS-*b*-fPI



2.4.1 Synthesis of PS-*b*-PI

All the procedures for purifying monomers and solvents, preparing custom-built all-glass apparatuses, and conducting polymerization were performed according to standard protocols for high vacuum techniques in anionic polymerization.^{10, 11} Block copolymers were designed to have different volume fractions (f) of polyisoprene in each block copolymer with different chain lengths. These block copolymers have been shown to exhibit morphologies that are directly correlated with their compositions. The volume fraction of each block before and after modifications is expected to be slightly changed, but this volume variation will not change the morphologies of precursors.

A series of block copolymers of styrene and isoprene were synthesized having different compositions and molecular weights. Styrene was polymerized prior to addition of isoprene. Each stage of polymerization was controlled to insure complete consumption of the first monomer before addition of the second one, or termination by methanol, in order to obtain desired compositions having controlled structures. Typical GPC traces are shown in Figure 2.1 for two block copolymers. As one can see, a shift to lower retention time indicates chain growth from homoPS to a diblock of PS-*b*-PI. The well-defined structures of both PS and diblocks were demonstrated by symmetrical profiles of GPC peaks and narrow molecular weight distributions. Molecular weights of PS were determined using GPC calibrated with standard PS, and the results are in excellent agreement with material feeds ($M_{n \text{ cal.}}$) (Table 2.1). The compositions of PS-*b*-PI copolymers were obtained using ¹HNMR by integration of aromatic peaks of PS and vinyl peaks of PI (Figure 2.2). All block copolymers synthesized are listed in Table 2.1 with macromolecular characteristics indicated.

2.4.2 Fluorination of PS-*b*-PI

The fluorination of polyisoprene was accomplished by the addition reaction between *in-situ* generated difluorocarbene and the PI double bonds (Scheme 2.1). This fluorination method was first reported by Hillmyer et al.^{15, 16} for polyisoprene and polybutadiene, and further developed by our group for polycyclohexadiene.^{9, 17} Our purpose in this study is to achieve full fluorination to prevent sulfonation from taking place on double bonds in the PI blocks. Thus, reaction conditions were optimized to achieve this goal.

A series of reaction conditions were attempted with polymers in Table 2.1 in terms of fluorination time, solvents, HFPO/C=C ratios, and concentrations of BHT. The efficiency of the reactions was evaluated by the state of the resulting reaction solutions (by examining if there existed gels, color, transparency, and so on), and products (recovered polymers), which were carefully characterized using GPC and ¹HNMR. As shown in Table 2.2, a low ratio of HFPO/C=C results in low fluorination degrees (F) (Run 2). Long reaction times and low concentration of BHT lead to crosslinking with gel formation in the reaction solutions or multiple peaks in the GPC profiles (Run 3 and 6). Use of cyclohexane as solvent gives better fluorination efficiency over benzene (Runs 7-10). With all these parameter under consideration, reactions in Runs 19-21 gave excellent fluorination in terms of F content, polydispersity, and yields. As one can see in Figure 2.1, GPC traces of PS-*b*-PI show very symmetrical peak profiles and narrow molecular weight distributions (low PDI), indicating negligible crosslinking or chain degradation during the fluorination reaction. An obvious shift of retention time to lower position from PS-*b*-PI indicates an increase of the hydrodynamic volume of the diblock copolymers due

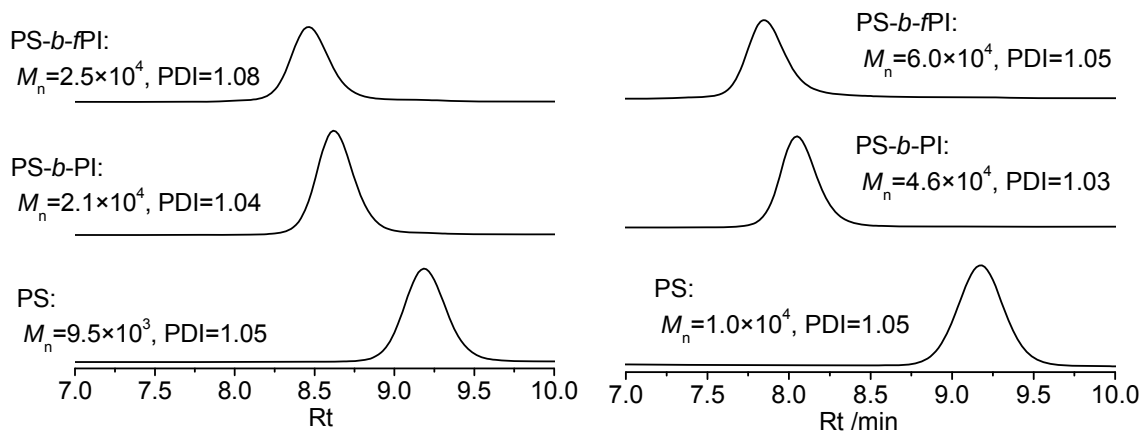


Figure 2.1 Size exclusion chromatography of PS, PS-*b*-PI, PS-*b*-fPI. Left, sample No. 5 in Table 2.1; Right, sample No.4 in Table 2.1.

Table 2.1 Characteristics of polystyrene (PS) and polystyrene-*b*-polyisoprene (PS-*b*-PI)

No	PS			PS- <i>b</i> -PI			
	$M_{n,cal.}$	M_n /g/mol (GPC)	PDI (GPC)	M_n /g/mol (GPC)	PDI (GPC)	St/Isoprene (m/m, ^1H NMR)	PS/PI (v/v, ^1H NMR)
1	1.1×10^4	1.0×10^4	1.11	4.6×10^4	1.09	1/2.89	31/69
2	1.1×10^4			2.7×10^4	1.06	0.8/1	51/49
3	2.6×10^4	2.5×10^4	1.08	3.0×10^4	1.04	1/0.2	87/13
4	1.0×10^4	9.8×10^3	1.05	4.6×10^4	1.03	1/4.46	23/77
5	1.0×10^4	9.5×10^3	1.05	2.1×10^4	1.04	1/1.31	50/50

to incorporation of $-\text{CF}_2-$ groups into the PI backbone. The complete disappearance of vinyl signals around 4.5-5.5 ppm in ^1H NMR of PS-*b*-*f*PI demonstrates full saturation, as shown in Figure 2.2(b). Also the aromatic peak patterns before and after fluorination exactly match each other, strongly inferring that the PS block remains intact.

2.4.3 Sulfonation of PS-*b*-*f*PI

Mild sulfonation reactions were chosen to sulfonate the polystyrene blocks of block copolymers in order to avoid possible chain crosslinking and degradation.^{12, 18, 19} The well documented method of sulfonation using acetyl sulfate was employed for this purpose.

Generally, the sulfonation degree (SD, percentage of sulfonated styrene repeating units in PS block) can be controlled by two parameters: (1) the molar ratio of acetyl sulfate and styrene repeating units; (2) the reaction time. Commonly used reaction conditions were applied for PS-*b*-*f*PI as described in the Experimental section. However, the molar ratio of acetyl sulfate and PS repeating unit was manipulated to achieve different sulfonation degrees.¹⁹ As shown in Table 2.3, sulfonation degree (SD is obtained by ^1H NMR as discussed below) increases with molar ratios of acetyl sulfate to PS repeating units for both of block copolymers having different volume fractions of fluorinated polyisoprene. However, with comparable reaction conditions (Run 1 and 6 in Table 2.3), polymers with high contents of *f*PI gave higher sulfonation degree; while both showed SDs were lower than stoichiometric values, indicating the low efficiency of sulfonation by acetyl sulfate.^{18, 19} The reason for higher SDs for polymers with greater *f*PI contents may be attributed to better solubility endowed by longer *f*PI chains (PS having

Table 2.2 Reaction condition study for fluorination of PS-b-PI

Run	Polymer No.	Solvent ^a	Reaction Time (hours)	HFPO/C=C (mol/mol)	BHT (wt% of PI)	F ^b (%)	Yield (%)	GPC Pattern	Reaction solution
1	4	CH	4	6	4.6	89.1	66.9	multi-peak no R _t shift	gel floated
2			16	1.3	15.8	36.6	62.0		gel floated
3			17	4.3	15.6				gelled
4	5	CH	4.5	5.8	15	58.4	72.1	single peak	CY-NoG ^c
5			5.5	7.4	7.0	62.9	82.8	shoulder at	CY-NoG
6			4	6.1	0	73.9	77.6	low R _t	CY-NoG
7	1	BZ	4	7.6	2.4	65.4	95.6	single peak	CY-NoG
8		BZ	4	12.3	2.4	100	46.6	single peak	CY-little gel
9		CH	4	8.4	15	99.2	92.8	shoulder at low R _t	CT ^c
10		CH	4	12.7	2.3	67.8	73.1	shoulder at low R _t	CY-little insol. gel
15	4	CH	4/175 ⁰ C	12.2	10	35.7			
16			4	11.9	10.2	100	97	good, PDI is retained	
17	3	CH	4	15.6	9.6	68.6	88.8	good	
18			4.3	>>10	~5	100	90.6	good	
19	5	CH	4	11.2	9.9	100	92.7	good	
20	4	CH	4	8.1	10	100	>90	good	
21	5	CH	4	10.6	10.1	100	95.1	good	

Notes:

^a, CH, cyclohexane; BZ: benzene. ^b, F: degree of fluorination, percentage of double bonds saturated by fluorination determined by HNMR. ^c, CY-NoG: clear solution, yellowish, no gel.; CT: clear, transparent.

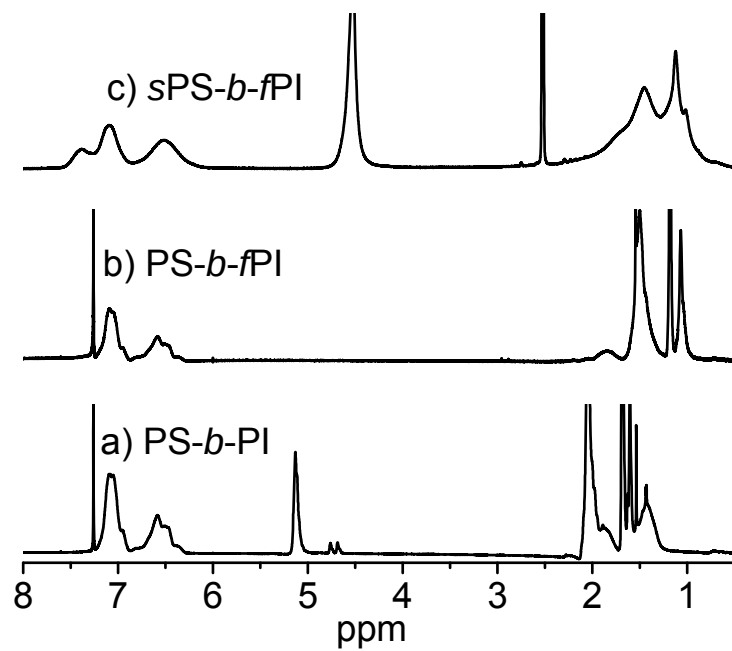


Figure 2.2 ^1H NMR spectra of (a) precursor PS-*b*-PI, in CDCl_3 , (b) fluorinated PS-*b*-PI: PS-*b*-fPI, in CDCl_3 , (c) fluorinated and sulfonated PS-*b*-PI: *s*PS-*b*-fPI, in d_6 -DMSO

same length in both cases) solvating the whole diblock polymer chains once sulfonation started.

In addition to the ratio of acetyl sulfate/St, reaction time is also an important parameter for control of SD. One of the runs in Table 2.3 (Run 4) was monitored during the reaction as a function of time by sampling the reaction solution and quenching it with alcohol. The monitoring data are presented in Figure 2.3. The SD initially increases very rapidly to about 45%, and then gradually levels off to achieve a maximum of about 57%. Thus, a broad range of SD can be obtained by controlling reaction time from 0 to 15 hours.

As one can see in Figure 2.3 that sulfonation reaches a maximum after a period of reaction time even with high ratio of acetyl sulfate and PS, the highest SD that had been reached by this method was around 57%. For higher SD levels, stronger sulfonating reagents are needed. Here we used the complex of triethyl phosphate and SO₃ according to reported procedures.¹³ For two block copolymers PS-*b*-PI (from precursor polymers No. 4 and 5), using excess of TEP/SO₃, full sulfonation and 84.5% SD were achieved for block copolymers with high and low volume fractions of PS, respectively. It was found that sulfonation remains the same after a short reaction time (1 hour), even when increasing reaction temperature from ice bath to room temperature.

For all the materials *s*PS-*b*-PI, ¹HNMR was used for structural characterization and determination of SD. A typical ¹HNMR spectrum of sulfonated PS-*b*-PI (*s*PS-*b*-PI) is shown in Figure 2.2(c). The blunt peaks indicate limited chain mobility in *d*-DMSO due to poor solubility of *s*PS in organic solvent. A new peak, comparing to ¹HNMR of

Table 2.3 Sulfonation of PS-*b*-fPI

Run	Polymer No.	AA ^a /H ₂ SO ₄	[PS] (g/ml)	AS/St ^b	T(°C)	Time(h)	Yield(%)	S.D. (% HNMR)
1	4	2	0.0163	0.62	50	2	88	29.6
2	4	2	0.0163	1.24	50	2	88.6	44.8
3	4	2	0.0135	5	50	2		45.4
4	5	2	0.0272	5	50	43	85.1	57.0
5	5	2	0.0255	0.73	50	2	97.3	38.8
6	5	2	0.0188	0.63	50	2	93.4	13.4

Notes:

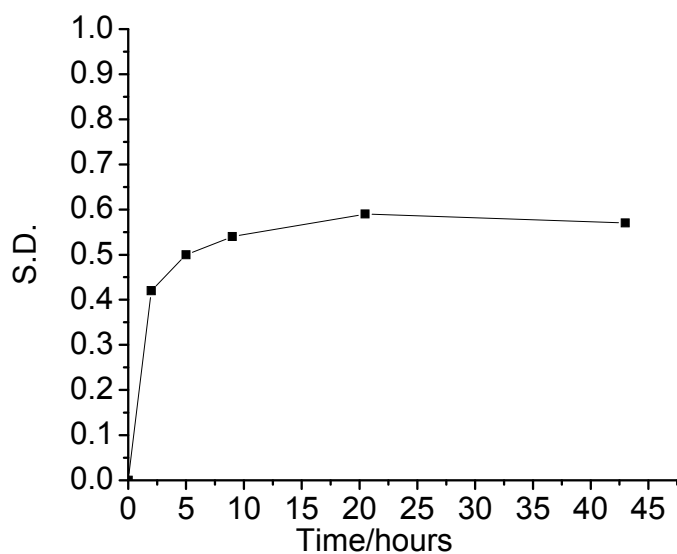
^a, AA: acetic anhydride. ^b, AS: acetyl sulfate.**Figure 2.3** Sulfonation degree (SD) monitored as a function of time in acetyl sulfate method.

Table 2.4 Sulfur content analysis by ^1H NMR and EA (wt%)

Analysis	Sample ^a	SD17.8	SD57.0	SD98.0
^1H -NMR		2.09	6.00	9.09
EA		2.55	6.16	8.22

^a, samples denoted by SD followed by sulfonation degree determined by HNMR

PS-*b*-*f*PI, emerges at 7.4 ppm, and is attributed to meso-protons on the sulfonated aromatic rings; while peaks at 6.5 and 7.1 ppm are assigned to ortho-protons of sulfonated/intact aromatic and para-protons of intact aromatic rings, respectively. By fitting these three peaks with Gaussian function, sulfonation degrees can be obtained according to the areas of each peak.¹⁹ An alternative to obtain SD is to compare areas of peaks between 1.0 and 2.0 ppm, which are attributed to *f*PI and *s*PS backbone proton signals (this can be used as internal reference because those protons don't change before and after sulfonation), and aromatic proton signals before and after sulfonation. The former method was used for SD because it has been well established. Sulfur contents were analyzed by elemental analysis to confirm the accuracy of the ^1H NMR method, which was used for all materials. The results are compared in Table 2.4, where it is seen that a good agreement exists within reasonable experimental errors.

2.5 Conclusion

Well-defined block copolymers PS-*b*-PI, PS-*b*-*f*PI and *s*PS-*b*-*f*PI were synthesized having different compositions. Complete saturation of the PI blocks can be accomplished by optimizing reaction conditions with difluorocarbene to yield well-defined structures. By using the acetyl sulfate sulfonation method and sulfonation using the complex of TEP/SO₃, block copolymers of PS-*b*-*f*PI can be successfully sulfonated, yielding a broad

range of sulfonation degrees. The sulfonation reaction was studied as a function of acetyl sulfate/PS ratio and reaction time. All the materials synthesized were well characterized by ^1H NMR, GPC and EA.

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Chapter 3 Morphology of Charged Block Copolymers of Fluorinated Isoprene and Sulfonated Styrene

Abstract

In this Chapter, two series of block copolymers of sulfonated and fluorinated polystyrene-*b*-polyisoprene were studied with regarding to phase separation behavior in melt. Transmission electronic microscopy and small angle X-ray scattering were used to probe the morphology of these block copolymers composed of charged and neutral blocks with a range of ionic contents. It was found that phase separation was firstly disordered by low sulfonation degree and returned to highly ordered structures for materials from both of the two series. And for the first time, a long range ordered lamellar structure formed for the block copolymers with 100 % sulfonation. The primary domain spacing is a function of sulfonation degree, which decreases as sulfonation degree due to ion condensation when charge contents were low and increased in the range of high sulfonation degree when chain stretching overwhelmed ion condensation.

3.1 Introduction

In the last decade, ionic/charged block copolymers have attracted significant attention from polymer morphologists and researchers in the membrane community. This is due in part, to the fact that ionic block copolymers can be useful as ion conductors for application in green source of energy, for example in proton exchange membrane fuel cell (PEMFC) applications, where these materials have the potential to provide higher proton conductivity than the traditional random ionic copolymers (Nafion[®]). A key reason is the underlying morphologies formed by the block copolymers, which microphase separate into nanometer sized structures to form effective ion (proton) transportation channels. This morphology can be functionally tuned to optimize the proton conductivity as well as the mechanical properties of the materials.¹

Traditionally, manipulation of morphology in neutral/nonionic block copolymers was achieved by utilizing the polymer interaction parameter (χ), volume fraction of each constituent (f), and the overall degree of polymerization (N).² And the morphological behavior of neutral block copolymers (χN locating in low and strong segregation regime) had been thoroughly investigated by experiments and theories.²⁻⁴ The introduction of ionic groups to one (or more) of the blocks can significantly change the behavior of microphase separation typically observed in the classical phase diagram² due to incorporation of additional strong interactions, e.g., columbic interaction/hydrogen bonding into the system.^{5, 6} For example, our previous study showed that minor components of charged block formed the matrix of a long range well ordered hexagonally packed cylinder structures with major neutral component of fluorinated polyisoprene composing the dispersed cylinders.⁵ The traditional phase behavior had been broken

down and “reversed” in this charge block copolymers (Major components should presume matrix of the morphology).⁵⁻⁷ Thus, in the viewpoint of morphology control, these ionic interactions may be effectively utilized as a new means to tune the bulk morphology. However, since early morphological studies of ionic block copolymers by Eisenberg,⁸⁻¹⁰ McGrath and Wilkes,^{11, 12} etc., systematically morphological investigations of charged block copolymers are very limited; most morphological information was obtained by studying physical properties of ionized multiblock thermoplastic elastomers and proton exchange membranes.¹

In this Chapter, we utilized a series of model charged block copolymers of sulfonated polystyrene and fluorinated polyisoprene (*sPS-*b*-fPI*) synthesized basing on the methodologies developed in Chapter 1. These well-defined block copolymers are changed in terms of sulfonation degrees and compositions. Thus their morphological behaviors can be studied in a systematical way by using small angle X-ray scattering and transmission electron microscopy. The results are compared with morphology behavior previously reported for ionic block copolymers.

3.2 Experimental Procedures

3.2.1 Synthesis of *sPS-*b*-fPI* with different SD and compositions: All the experimental details for preparation of these materials refer to Chapter 2 and references^{13, 14, 15}.

3.2.2 Bulk microphase separation: sample preparations.

For morphology studies in bulk, polymers in acid form were dissolved in rigorously dried THF overnight to form polymer solutions of ~ 4 w/v% in glove box. The solutions then were transferred under inert atmosphere to PTFE crucibles in a chamber equipped with N₂ inlet and outlet. THF was very slowly evaporated with bubbling N₂

through this chamber for about 1 week, followed by exhaustive pumping under vacuum for another week to remove solvent residuals.

3.3 Characterization

Number-average molecular weight M_n , polydispersity index M_w/M_n (PDI) of all samples before sulfonation were determined by size exclusion chromatography using a Tosoh EcoSEC instrument which was equipped with two TSKgel Super Multipore HZ-V columns calibrated using standard polystyrenes with M_n from 580 to 7.5×10^6 g/mol. The polymer was eluted in THF at 40 °C at a flow rate of 0.35 ml/min. ^1H NMR spectroscopy was performed on Varian Mercury 300 MHz spectrometer with CDCl_3 and d -DMSO as solvents for precursors PS-*b*-PI, PS-*b*-*f*PI and *s*PS-*b*-*f*PI respectively. Samples for transmission electron microscope (TEM, Hitachi HF-3300 at 330 kv operating voltage) observation were cryo-microtomed at -70 °C and stained by RuO_4 vapor for 1h. The small angle X-ray scattering (SAXS) experiment was recorded on a Molecular Metrology instrument using Cu K α radiation ($\lambda=1.5418$ Å) equipped with a two-dimensional position sensitive proportional detector of circular shape (radius = 2.5 cm). The sample to detector distance was 1.5 m with the q range 0.01 Å^{-1} to 0.15 Å^{-1} . The X-ray operating voltage was 45 kV with the current of 0.66 mA.

3.4 Results and Discussion

3.4.1 Table of Block Copolymers *s*PS-*b*-*f*PI

Anionic polymerization with high vacuum technique was used to prepare well-defined PS-*b*-PI precursors according to standard protocols. The polyisoprene block of precursors was successfully fully fluorinated with *in situ* generated difluorocarbene ($\text{CF}_2\cdot$) by decomposing HFPO at high temperature. The integrity and narrow disperse

nature of all precursors before sulfonation was demonstrated by HNMR and SEC.¹⁵ The volume fractions of PS were 19% and 45% in PS-*b*-fPI precursors; while the molecular weights were varied.

The PS blocks in the copolymers were sulfonated to accomplish a wide range of SD. The sulfonation degree was monitored using HNMR during the sulfonation reaction by sampling the solution. The macromolecular characteristics of block copolymers used in this study are summarized in Table 1. Two series of polymers with different compositions were synthesized with different sulfonation degrees in each series of same precursor (No. 1 and No. 2). The volume fraction of sPS in each series was 0.19–0.24 and 0.45–0.51 dependent on sulfonation degree.

3.4.2 Morphology of sPS-*b*-fPI

Absolutely dry THF (anionic polymerization grade) was used to dissolve polymers in acid form, and the polymer films for bulk morphology study were cast under anhydrous atmosphere of N₂, considering the fact that sulfonate groups are strong electrolytes and the hygroscopic nature of these materials. Previous study showed that traces of water in this polymer solution would cause disordered morphology.⁵ The acid form of sulfonated polystyrene was chosen instead of Na or Cs or other metal salts because of the relatively low viscosity of the polymers in acid form during THF evaporation, which facilitates phase separation. On the other hand, polymers in metal salts own a high dielectric constant and the high viscosity would slow down the process of phase separation (low chain mobility by ionic interaction).¹⁶ Ionic association behavior was also demonstrated in the dissolution process in THF. Block copolymers with Na as counter ions usually take considerably longer time for dissolution in anhydrous THF

Table 3.1 Molecular characteristics of PS-*b*-PI, PS-*b*-*f*PI, *s*PS-*b*-*f*PI

No. /Series	PS- <i>b</i> -PI			PS- <i>b</i> - <i>f</i> PI		<i>s</i> PS- <i>b</i> - <i>f</i> PI ^b
	M_n (SEC)	PDI	f^a	M_n (SEC)	PDI	SD
1	2.1×10^4	1.04	0.45	2.5×10^4	1.08	0
						13.4
						38.8
						47.8
						57.0
						100.0
2	4.6×10^4	1.03	0.19	6.0×10^4	1.05	0
						29.6 ^c
						45.4
						84.5
3	3.1×10^4	1.05	0.19	4.0×10^4	1.02	50.0

^a f , volume fraction of PS in PS-*b*-*f*PI; ^b *s*PS in acid form; ^c *s*PS in Na form.

(Sample No. 2-SD29.6 in Table 3.1) and are even insoluble without addition of water for those with high SD; Moreover, dynamic light scattering showed strong aggregation behavior for polymers in the salt form in THF, while solubility of polymers/aggregation behavior for polymers in the acid form was dependent on sulfonation degree.¹⁵

Small angle X-ray scattering experiments were performed at room temperature to probe the bulk morphology of as-cast films of block copolymers of sulfonated styrene and fluorinated isoprene. The SAXS data for samples in Series No. 1 are shown in Figure 3.1(Left). Samples in series No.1 have sulfonation degrees from 0 (precursor) to 100%, while in this range of sulfonation volume fraction of sPS (f) changes from 0.45 – 0.51 calculated according to the density of sPS, PS and f PI and the compositions determined by HNMR. In Figure 3.1, the precursor of PS- b - f PI with zero sulfonation shows a profile of Bragg scattering peaks ratio of 1:2:3:4, indicating a long-range well-ordered lamellar structure as expected from the volume fraction of PS according to the classical phase diagram.² As SD increased to 13.4 and 38.8, the long-range ordered morphology was lost, but the microphase separation still persisted as observed from the primary scattering at low scattering angle. When SD was further increased to 100%, long range ordered structures gradually developed again. The well-developed phase separation and long range order for 100% full sulfonation was seen in the Bragg scattering peaks at $1q^*$: $2q^*$: $3q^*$: $4q^*$ as lamellae as well as a strong structure demonstration by TEM shown in Figure 3.1 (Right).

The evolution of morphology as a function of sulfonation degree has been a

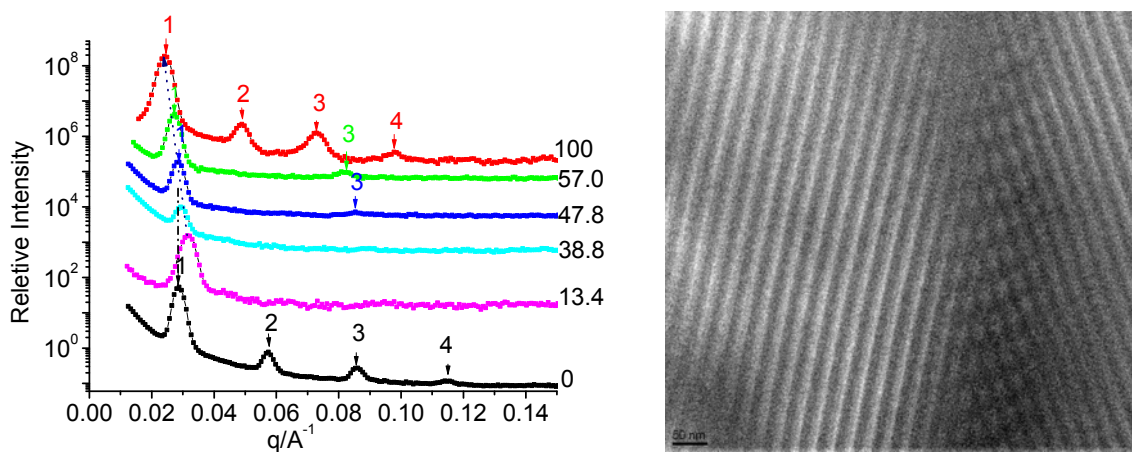


Figure 3.1 Left: SAXS profiles of samples in Series No. 1 as a function of SD shown on the right hand side of curves from 0 – 100%. Numbers on top of arrows shows maximum intensity and the ratios of scattering peaks to primary scattering peaks. Right: TEM of *sPS-b-fPI* with 100% sulfonation degree.

controversial issue. It had been shown that sulfonation could induce a disorder-to-order transition in polystyrene-*b*-poly(methyl methacrylate) (PS-*b*-PMMA) block copolymers with a sulfonation degree below 35% attributed to the increased χ between PS and PMMA arising from the introduction of ionic sulfonate groups.^{17, 18} In sulfonated polystyrene-*b*-poly(ethylene-*alt*-propylene) block copolymers, sulfonation disordered the initially well-ordered lamellar structure of unsulfonated polymers for a wide range SD of 0–43%.¹⁹ This disordering nature was partially in agreement with sulfonated poly([vinylidene difluoride-*co*-hexafluoropropylene]-*b*-styrene diblock copolymers with sulfonation degrees between 22–40%.²⁰ The disordering behavior induced by low sulfonation was reversed when the SD increased. This behavior was shown by small angle neutron scattering data which indicated better ordered structures with increasing SD, while for 100% sulfonation, severely disordered phase separation occurred.²⁰ In our study, the SAXS and TEM data in Figure 3.1 show that low sulfonation degree up to a medium sulfonation degree of about 40%, disordered the well phase separated lamellar structure of precursor. After a threshold of sulfonation between 38.8 and 47.8, further sulfonation helped form long range ordered structures, as clearly seen in the TEM data. We attributed this observation to the introduction of few ionic groups that would distort the phase separation formed from the precursors due to the randomly dispersed aggregation of ionic groups. This would cause the disappearance of Bragg scattering peaks at high angle, while the growing ionic groups would dominate the morphology of *s*PS-*b*-*f*PI in which the role of neutral styrene units in *s*PS block mitigated in phase separation. Interestingly, in a case of block copolymers sulfonated polystyrene-*b*-polymethylbutylene, Balsara^{6, 21} showed lyotropic phase behavior as a function of

sulfonation degree in the range of below about 50%. At any specific sulfonation degree, a well ordered morphology developed.⁶

It is interesting to note that the primary Bragg scattering vector ($d = 2\pi/q^*$) shifted as a function of sulfonation degree as shown in Figure 3.1. It shifted to higher angle (increased from $0.0287 \text{ \AA}^{-1}$, reached $0.032 \text{ \AA}^{-1}$ or greater) then to lower angle as sulfonation degree increased, even lower than the one of the precursors when fully sulfonated (decreased to $0.0242 \text{ \AA}^{-1}$). This indicates that the primary domain spacings decreased first (from 21.9nm to 19.6nm or lower) then increased (to maximum of 25.9 nm). The different observations reported in literature complicate the overall interpretation. All the observations reported exclusively that d , a monotonic function of sulfonation degree, either increases or decreases due to the incorporation of bulky sulfonate groups accounting for the molar volume increase and ion condensation of sulfonate groups, respectively; while in our studies showed two trends. It is clear that the introduction of ionic groups/hydrogen bonding contributes to the ion condensation during the distortion of morphology, which decreases the domain spacing.^{19, 22} The domain spacing begins to expand when the amount of ionic group are enough to increase the interaction parameter between *s*PS and *f*PI blocks to overwhelm the effects from ion condensation, leading to reversing domain shrink. The extremely high χ value causes severe chain stretching due to strong incompatibility between hydrophilic *s*PS and lipophobic *f*PI of fluorine.^{15, 23}

Balsara and coworkers observed a series of phase transitions disorder-order transition and order-order transition as a function of sulfonation degree by studying block copolymers of sulfonated polystyrene and polymethylbutylene with different molecular weights.⁶ The observation we have summarized in this paper^{6, 7} is a general trend: most

lamellae forming precursors form hexagonally packed cylinders (HEX) type structures with the ionic blocks forming the continuous matrix of the morphology for all the materials studied when SD is high. This behavior was also observed in our previous study with the minority block of *s*PS consisting of the matrix of the well ordered HEX structure (Sample No. 3 in Table 3.1).⁵ Thus, it was expected that experimentally with high sulfonation degree a matrix of ionic *s*PS may form in our case. However, instead lamellae were observed as demonstrated by TEM and SAXS. This might be rationalized by the self-assembly process being dominated by *f* and more importantly a “super strong segregation” may contribute. On the other hand, such a long range well-ordered phase separation is surprising because it has never been reported for block copolymers containing fully sulfonated polystyrene or fluorinated components.^{20, 22}

The morphological behavior of samples in series No. 2 in Table 3.1 was also studied by SAXS as shown in Figure 3.2. A well-ordered HEX structure formed as expected from the compositions for the precursor. Only two scattering peaks are present for samples with SD 45.4 and 84.5 indicating the formation of ordered structure but with less long-range order; while for sample with SD29.6 as seen in Figure 3.2, an undistinguished shoulder of the primary scattering peak exists, inferring none well-developed phase separation, this may be attributed to the polymers in Na salt forms. Although this series of block copolymers have different compositions from series No. 1, evolution of morphology and primary domain spacing as a function of sulfonation degree are in consistency. Whether these materials form inverse HEX structures is still under investigation.

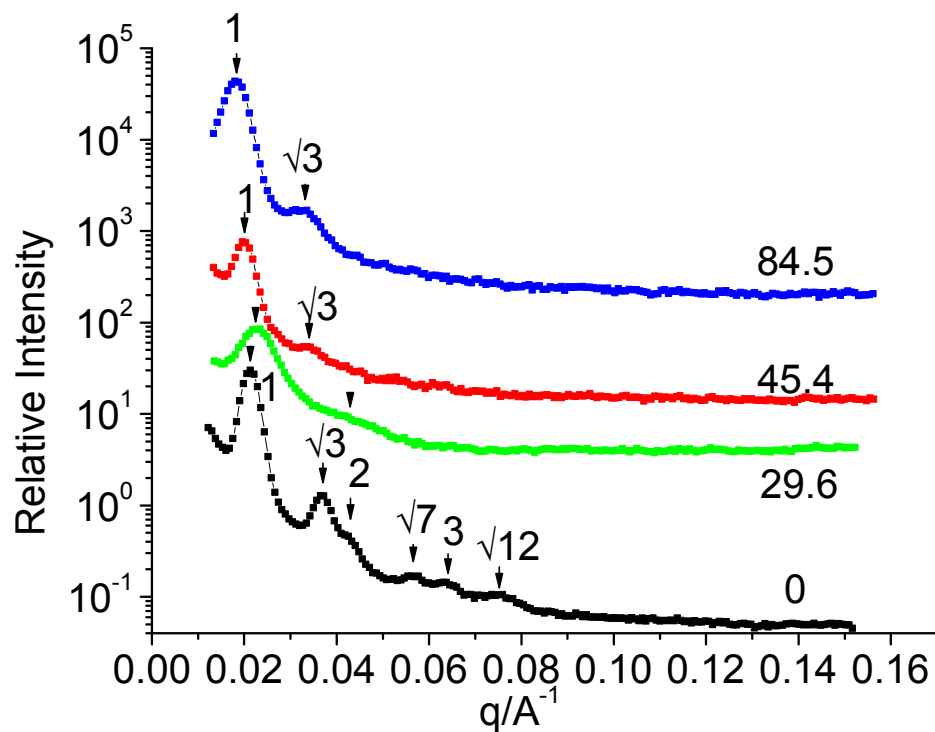


Figure 3.2 SAXS profiles of samples in Series No. 2 as a function of sulfonation degree shown on the right hand side of curves. Numbers on top of arrows shows maximum intensity and the ratios of scattering peaks to primary scattering peaks

3.5 Conclusions

A series of *s*PS-*b*-PI polymers were synthesized with wide range of SD and compositions. Low sulfonation degrees disorder the well phase separated structures of precursors and high sulfonation degrees promote long range ordered structures. The domain spacing is shown to be a function of sulfonation degree and can be explained by considering ion condensation and chain stretching. The inverse morphology and annealing effects are under investigation and will be described in a future publication.

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**Chapter 4 Asymmetrical Self-assembly From
Fluorinated and Sulfonated Block Copolymers in
Aqueous Media**

Abstract

Block copolymers of fluorinated isoprene and partially sulfonated styrene form novel tapered rods and ribbon-like micelles in aqueous media due to a distribution of sulfonation sites and a large Flory-Huggins interaction parameter. A combination of microscopy, light scattering, and simulation demonstrates the presence of these unique nanostructures. This study sheds light on the micellization behavior of amphiphilic block polymers by revealing a new mechanism of self-assembly.

4.1 Background

In a manner analogous to simple surfactant behavior, diblock copolymers in a solvent selective for one block self-organize to form various nanometer-sized aggregates (micelles, vesicles, etc.) in order to stabilize the system by minimizing the free energy. For amphiphilic block copolymers in water, hydrophobic blocks form the core of the micelles while the water-soluble segments form swollen corona. The morphologies of such aggregates are determined by factors including core chain stretching, interfacial energy between the core and the solvent, repulsion between corona chains, volume fraction of each block, and the Flory-Huggins interaction parameter (χ) between core and corona chains.¹ Due to the complex interplay among these parameters, diverse morphologies^{2,3} have been discovered, including classical structures (spheres, vesicles, cylinders),⁴⁻⁶ multicompartment structures,⁷ toroidal micelles,⁸ and helices.⁹ Such self-assembled structures are of interest from a fundamental perspective in nanotechnology development, as well as in applications such as drug delivery.^{4c}

Recently, we reported the bulk morphology¹⁰⁻¹² behavior of model block copolymers of sulfonated polystyrene-*b*-fluorinated polyisoprene (*s*PS-*b*-*f*PI). An “inverse morphology” was observed with the minor phase, 25 vol% *s*PS, forming the matrix and the major *f*PI component forming well-ordered, dispersed hexagonally packed cylinders. This inverse morphology behavior in bulk is due to charge percolation and the high value of χ , reflecting both ion content and fluorine content. Also in the Chapter 3, we systematically studied the morphological behavior of these materials as a function of sulfonation degree and composition as well as annealing

effects. The self-assembly of these materials in aqueous media also reveals novel morphologies, as described in this Chapter.

4.2 Experimental Part

4.2.1 Block copolymers for self-assembly

All the material used in this Chapter were prepared according to procedures described in Chapter 2. Two block copolymers of sulfonated styrene and fluorinated isoprene were utilized for investigation of aqueous self-assembly behavior: No.1-SD38.6 and No.2-SD29.6 from Table 3.1 in Chapter 3. Here we denote this two polymers by using Series 1-SD38.6 and Series 2-29.6.

4.2.2 Self-assembly procedure

Solutions of 0.5 – 1% (w/v) concentration were made using HPLC grade THF. Deionized water was slowly added at 0.3 ml/min by a syringe pump into the polymer solution with vigorous stirring until 50% by volume of water was added. The entire process of addition of water was monitored by shining a laser (lecture use laser point) through the solution in order to observe the Tyndall Effect which is a good indication if colloidal particles were present in the solution, block copolymer aggregates in our case. The Tyndall effect was always found for all of samples investigated, which means that no molecularly dissolved state was achieved over the entire range of solvent composition. After addition of water, the solution was dialyzed against deionized water to remove THF for three days. The concentration of the polymer was calculated from the volume of dialyzed solution and mass of polymers initially added.

4.3 Characterization

Dynamic light scattering (DLS) was used to characterize hydrodynamic diameter (D_h) of the aggregates of the fluorinated and sulfonated polymer solution in THF and water. D_h of the polymers at 25 °C was measured on a PD Expert instrument (Precision Detectors) at a scattering angle of 45° or 95°. The diameters and polydispersity indices (PDIs) were averaged over 10 repetitive measurements. The concentration of the solutions used for DLS was about 0.05–1.2 mg/ml and 8.7 mg/ml (initial concentration for self-assembly before water was added) in water and THF, respectively. It was found that D_h is essentially independent of concentration of polymers in aqueous solution, giving quite close D_h values. All the samples were filtered using a 0.45 μ m Millipore nylon membrane. For the freshly made solution of sample Series 2-SD 29.6, the concentration dependence of D_h was measured with concentrations ranging from 5 μ g/ml – 0.5 mg/ml after aging 4 days. Multiple populations of micelles existed through the whole range of concentrations (Appendix Figure A0), indicating the size measured by DLS is the size of micelles (rather than the size of associated micelles).

Transmission electron microscopy (TEM) was performed using a Hitachi H-800 instrument with 75 Kev voltage. TEM samples were prepared by depositing a droplet (1–4 μ l, 0.5 or 1.2mg/ml) of aqueous solution of polymer on copper grid which was coated by a carbon film, then dried overnight. Atomic force microscopy (AFM) experiments were performed using a Nanoscope IIIa Microscope with Multimode Controller (Veeco Intrument) at ambient temperature and humidity. The tapping mode was employed with an antimony-doped Si tip (radius < 10 nm) at a line scanning frequency of 1 Hz. The set point of amplitude of the cantilever was set to just capture the real morphology of the aggregates and avoid applying excess force to the objects which might lead to squeezing

between scanning target and substrate. After the TEM observation, the same grid was taped to a metal disk substrate, and then was applied for AFM scanning.

4.4 Simulation studies

In order to develop a better understanding of the underlying mechanisms responsible for the morphology, we examined the structural evolution of the charged copolymer systems using Stochastic Molecular Dynamics (MD) simulation in the canonical ensemble (constant NVT). The MD simulations are carried out for a diblock copolymer melt system of chain length 64 with A and B blocks having 32 monomers each. The second block is charged with 28.1% and 46.9% charges respectively. The initial configurations are randomly generated with a number density of monomers $\rho\sigma^3 = 0.5$ (in solution) with equal number of counterions distributed in the system. All the monomers of the system have mass m_i and diameter σ . Polymer chains are modeled following the Kremer-Grest bead spring polymer model in which bonded beads are connected by finitely extensible non-linear elastic (FENE) springs represented by U_{ij}^{FENE} (r_{ij}) = $-0.5\kappa R_0^2 \ln[1 - (r_{ij}/R_0)^2]$, where $R_0 = 1.5\sigma$ is the finite extensibility and $\kappa = 37.5\epsilon/\sigma^2$ is the spring constant. The energetic interaction between any pair of uncharged monomers beads is modeled by a truncated shifted Lennard-Jones potential, U_{ij}^{LJ} (r_{ij}) = $4\epsilon[(\sigma/r_{ij})^{12} - (\sigma/r_{ij})^6 + 1]$. Where ϵ is specific to two different blocks, ϵ_{AA} and ϵ_{BB} are 2.0 and 4.0 respectively with the Lorentz-Berthelot mixing rules applied for cross interactions. For the charged sites, explicit Coulomb interactions have been considered for which Ewald summation techniques are used. The electrostatic interactions are modeled via Coulomb potentials: $U_{ij}^{\text{C}}(r) = q_i q_j / Dr$, where D is the dielectric constant. Temperature is the first energy parameter of the system. We introduce a second energy

parameter, $\xi_B = q^2/(D\epsilon\sigma)$, which is also the strength of Coulomb interaction and inversely proportional to dielectric constant. This interaction strength is directly proportional to the Bjerrum length which is the ratio between electrical energy and kinetic energy of a charged monomer. The motions of the particles are governed by classical Newton-Langevin equation: $m_i d\mathbf{v}_i/dt = -U_i(\mathbf{r}) - \Gamma d\mathbf{r}_i/dt + \mathbf{W}_i(t)$, where U_i is the potential, Γ is the friction coefficient between the chain monomer and background solvent and $\mathbf{W}_i(t)$ represents a Gaussian ‘white noise’ with zero mean acting on each particle. The last two terms couple the system to a heat bath where the friction term acts as a ‘heat sink’ and the noise term acts as a heat source. The advantages of this scheme, is that the natural MD integration time steps are larger, thereby permitting simulation at a longer time scales. On this time scale, only the mean effect of the stochastic forces acting on the system needs to be considered, leading to a first order temperature relaxation which in turn reduces the need of an external thermostat. The dimensionless units are defined as follows, $t^* = t/\sqrt{(m_i\sigma^2/\epsilon)}$, $r^* = r\sigma^3$, $T^* = k_B T/\epsilon$, $U^* = U/k_B T$ and $r^* = r/\sigma$.

For high dielectric constant, snapshots of the simulation are shown in Figure SI 8 at the end of the run. The figures show preferential formation of flat interface demonstrating chain stretching away from the interface caused by increasing interfacial tension between the blocks.

4.5 Results and Discussion

4.5.1 Observation of Self-assemble structures

Well-defined block copolymers, *sPS-b-PI*, were synthesized by anionic polymerization, followed by fluorination and sulfonation, and were characterized according to standard protocols.^{10,13-21} The molecular characteristics of *sPS-b-PI*, as

well as that of the precursors (PS-*b*-PI), are summarized in Table 3.1 in Chapter 3. Two block copolymers were used: Series 1-SD38.8 and Series 2-SD29.6.

Self-assembly of these block copolymers was performed by slowly adding water into a dilute (~0.5% w/v) tetrahydrofuran (THF) solution of polymer until 50 vol% water was reached, followed by dialysis against deionized water. This constitutes a typical “solvent switching” procedure for preparing polymer micelles in aqueous media.²² The morphologies of self-assembled aggregates were examined using transmission electron microscopy (TEM) at concentrations of 0.5~1.2 mg/mL at different aging times. In Figure 4.1, for sample Series 1-SD38.8 (38.8 is the sulfonation degree (SD), percentage of sulfonated styrene in PS block), worm-like nanostructures were observed, which changed from ribbon-shaped (see atomic force microscopy (AFM) analysis below) to tapered structures (see analysis below) as the sample aged from one week to one month. In Figure 4.1(b), mixtures of ribbon-like and tapered-worm like structures (see Appendix for large area and zoom-in TEM images, Figure A1) co-existed with some amounts of short cylinders (or spheres), about two weeks after starting dialysis. The distribution of diameters was quite broad, ranging from 10 to 40 nm, with lengths ranging from 20 nm to several microns. Variations in diameters of the structures obtained by TEM (Figs 4.1a, 4.1b; Appendix) lead us to examine the heights of the nanostructures by AFM for micelles adsorbed on carbon film.

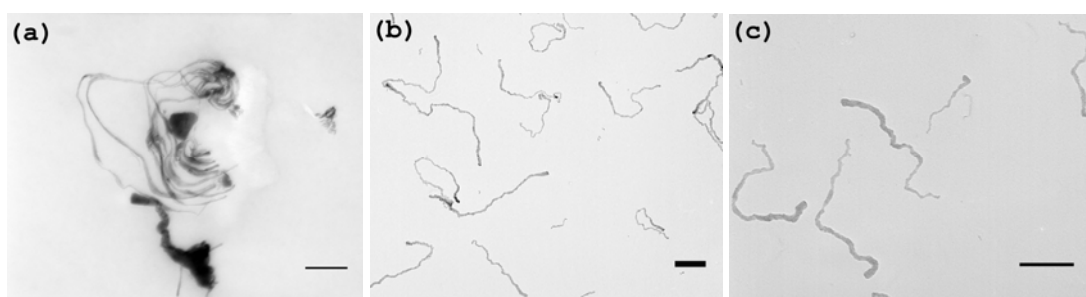


Figure 4.1 TEM images of Series1-SD38.8 in acid form at different aging times: a) 6 days; b), 15 days; c) 27 days after starting dialysis. Scale bar: 250 nm

The results are shown in Figure 4.2 (left-top, height image). Cross-section analysis provides a height of 5–10 nm along the length, while diameters were between about 20–70 nm, in agreement with TEM. It is concluded that novel ribbon-like structures formed during aging, reflecting the unusual self-assembly characteristics of these novel block copolymers (see discussion below), while not entirely excluding the possibility of collapse of the soft core (low T_g , ~ 40 °C)¹⁴ and/or spreading of the cylinder structure due to attraction between *s*PS chains and hydrophilic carbon film. Interestingly, after 27 days from starting dialysis, the structures evolved to a coiled tapered cylinder-like morphology although this structure had already partially developed in Figure 4.1(b) (see Appendix Figure A1 for zoom-in picture) after 15 days as mentioned above. The dimensions of these tapered structure range from 30–40 nm at the large end and 5–15 nm at the small end, while lengths were of the order of 1 μ m. Dynamic light scattering (DLS) demonstrated a distribution of populations with a hydrodynamic diameter (D_h) of 252 nm on average, representing (intensity averaged) 85% of the population, as shown in Figure 4.2 (right). The remaining 12% and 3% populations were attributed to small spherical micelles and huge micelles, respectively.

By comparing D_h of the main population in aqueous media with lengths of those structures showing curled shapes in TEM, Figure 4.1(c), we conclude that the tapered worm-like structures behave as coils in water (soft *f*PI core may contribute to this). DLS indicates that this structure is stable with no significant change in D_h even after aging 10 months

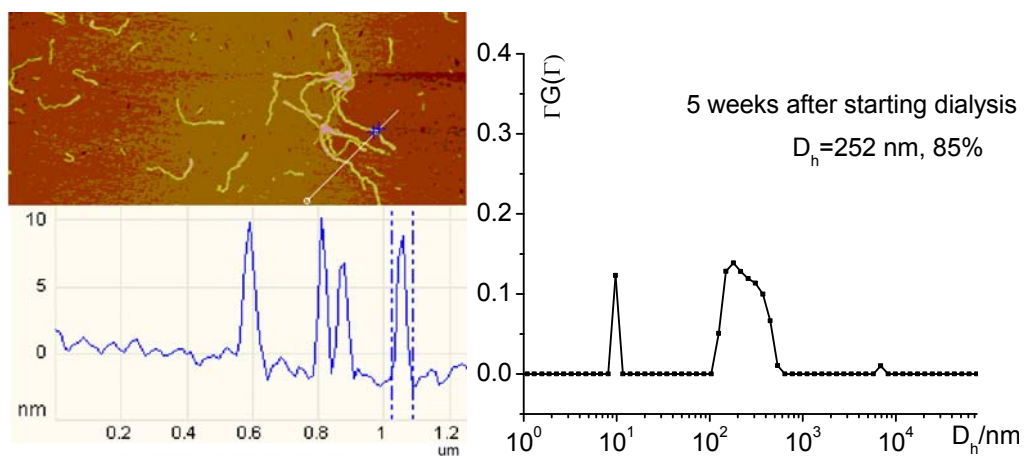


Figure 4.2 Sample Series 1-SD38.8. Left: AFM height image (upper, scale $5 \times 2 \mu\text{m}$) and cross section analysis (lower), the same TEM grid as in Figure 4.1(b); scanning was done over the carbon film of the TEM grid. Right: D_h distribution of micelles in aqueous solution by DLS after 5 weeks.

(see Appendix, Figure A2). Interestingly, the population of spherical micelles decreased significantly as indicated by the decreased content of small size micelles in DLS. DLS results strongly suggest that the unique structures observed in TEM and AFM also exist in aqueous media.

The tapered behavior as described above was also observed when self-assembly of sample Series 2-SD29.6 was conducted. As shown in Figure 4.3, it has a small end of diameter ranging around 20–25 nm, while the large end exhibits diameters around 45–80 nm, after this sample aged for 25 days (Figure 4.3c). As seen in Figure 4.3(a-c), we also observed aging effects for this sample. The morphology evolved from spheres (Figure 4.3a) to tapered rod-like micelles (Figure 4.3b) after aging 4 and 13 days, respectively. At early times, mixed morphologies were observed: spheres, large compound micelles and rigid long fibers (Figure 4.3a). As the sample aged, tapered rods developed (Figure 4.3b and 4.3c) and further evolved to more complicated structures (aged for 60 days), which consist of hairy worms, smooth rigid “fibers”, and the same extended tapered rod morphology as discussed above (Appendix, Figure A3), for which all of the dimensions are of the same order as those observed at early aging times. After 3–4 months, all samples in Series 2/No.2 in Table 3.1 showed precipitates in solution as observed with the naked eye, which shows that the stability of the structures was eventually lost.

DLS confirmed this aging effect with a freshly made solution of Series 2-SD29.6 sample, as shown in Figure 4.3(d). At early aging time (4 days), two populations were observed with the main one located around 28 nm (Figure 4.3d-(i)) which is attributed to spherical micelles as seen in Figure 4.3(a)(diameter of spheres,

27 nm). The second population (D_h around 230 nm) reflects compound micelles and some rigid fiber aggregates. After 7 more days of aging, a drastic increase of intensity at low scattering angle (45°), with appearance of multiple populations at large D_h , suggests growth of large particles at the cost of small spheres (Figure 4.3d-(ii, iii)). Micelles at high D_h are attributed to worm-like structures seen in TEM (Figure 4.3b, 4.3c). The intensity-intensity time correlation functions (see Appendix, Figure A4) clearly show that slow modes increase as a function of time, indicating the growth of large micelles.

The Series 2-SD29.6 sample exhibited similar morphologies (Figure 4.3c), although the micelles appeared to be stiffer than those from Series 1-SD38.8 (Figure 4.1c), as the former appears more stretched. This may be attributed to the higher SD softening the assembled structure due to increased solubility of corona chains in water; low core chain molecular weight may also contribute to this flexibility. Simulation analysis (below) gives similar results for chain conformations.

4.5.2 Mechanism of Self-assembly

In interpreting these results, one should bear in mind that the sulfonation is not uniformly distributed across the *s*PS chains and the SD obtained by ^1H -NMR is an average over the entire population of molecules present. Indeed, the final *s*PS-*b*-*f*PI is a diblock terpolymer composed of a well defined block of *f*PI but a second block consisting of a “random” copolymer of *s*PS and PS^{21,28}. *Some individual PS blocks were sulfonated to higher levels, others to lower levels.*

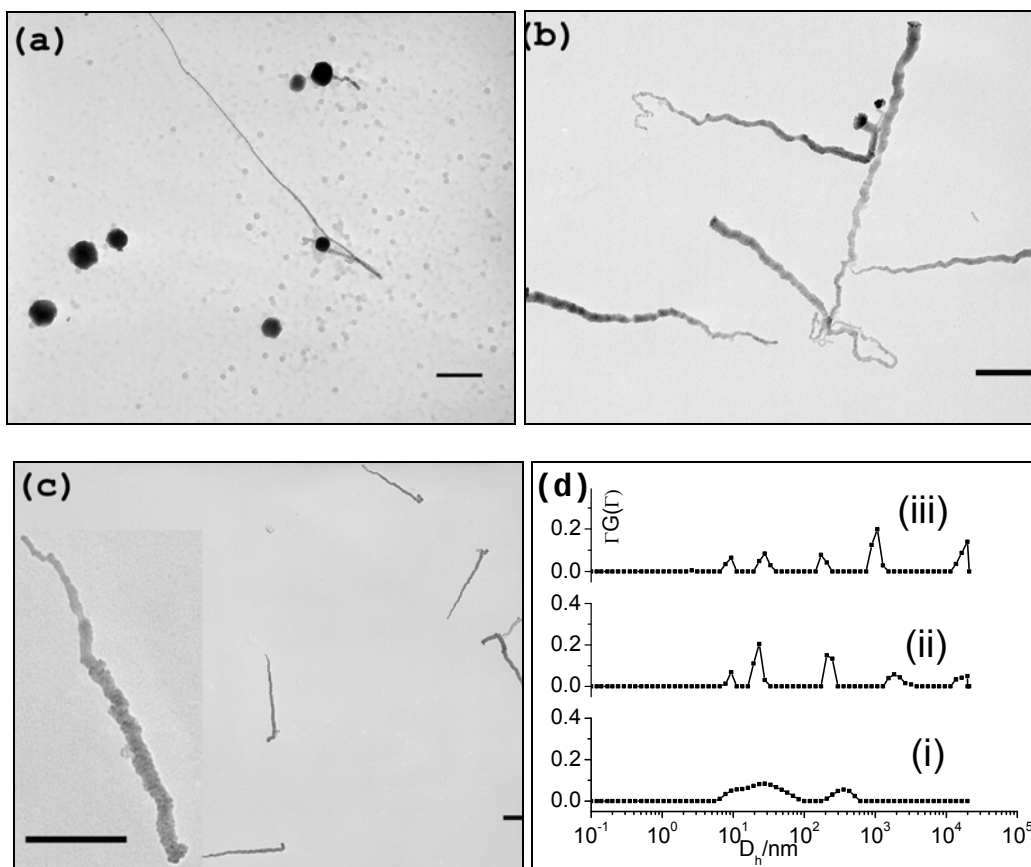


Figure 4.3 Aging effects of Sample Series 2-SD 29.6 in Na form. a-c: TEM images at a), 4 days; b), 13 days; c) 25 days, scale bar 250 nm; d: D_h distribution by DLS at 45° scattering angle with a freshly made solution at 0.5mg/ml: (i), 4 days; (ii), 11days, (iii), 35 days.

This chemical heterogeneity may affect the nature of morphologies formed in aqueous media, and account for the unusual self-assembly behavior resulting in complex morphologies. The unique tapered shape observed for many of the assemblies thus reflect four-party interactions among H₂O, sPS, PS, and fPI. To the best of our knowledge, this type of tapered self-assembled structure has not been previously observed.

To fully understand the tapered morphologies observed in the experiments requires consideration of the detailed mechanism for self-assembly of amphiphilic diblock copolymers (partially charged PS and highly hydrophobic fPI in this case) and the role that interfacial curvature and chain stretching play in the assembly process.^{23,24} The interfacial curvature is determined by volume fraction, conformational differences of corona and core components, and the most important factor here, interfacial energy. Increasing interfacial energy would drive chains to stretch away from the interface with preferential formation of flat interface, finally leading to domain size expansion.²⁴ As a result, the extent that the chains stretch depends on interfacial tension, e.g. interaction between corona and core, H₂O/sPS and fPI in our case. Therefore, *we propose that the tapering behavior is a result of fPI chains stretching to various extension levels along the axis of the tapered assembly* by considering the fact that interfacial tension is a function of sulfonation degree of polystyrene block. In other words, SD within the tapered assembly varies systematically along the tapered rods. As discussed above, the sulfonation reactions give an array of SD within a single sample. Matsen and Bates²⁴ showed that domain spacing increased monotonically with segregation (χN) of two blocks, which supports

the chain stretching proposal (this is also corroborated by SAXS data on bulk morphology for a series of polymers with different SD, which will be detailed in a future paper). Pochan et al.²⁵ reported that a higher interfacial energy for a triblock copolymer containing poly(pentafluorostyrene) (PPFS) caused intra-micellar phase separation and, more importantly, the undulation of cylindrical assembly due to more stretching from the PPFS block. Alternatively, in our case, the substantial stretching of core chains at the large end is caused by strong stretching of sulfonated PS blocks with high SD (strong ionic repulsions) due to reduction of interfacial cross section of the polymer chains. On the other hand, the low T_g of *f*PI (~ 40 °C^{14,16}) imparts core chain mobility to self-organize in a way that SD “tapers” along the axis of the assembly due to intra-micellar segregation of sulfonated PS chains with different SD as indicated above. It also has been reported that octopus micelle formation, as a state of intramicellar segregation, is driven by a bimodal distribution of poly(ethylene oxide) (PEO) corona blocks in an aqueous solution of two diblock copolymers with a single core molecular weight,²⁶ as indicated by Halperin for a binary polymeric micelle system.²⁷ Thus, *we hypothesize that in our case the intra-micelle segregation is driven by a distribution of sulfonation degrees* as PS and *s*PS (sulfonated PS with different SD can be considered as different components) would undergo microphase separation, which is a further support of Halperin’s theory, although his theory was developed for nonionic polymers.²⁷ Another argument for the formation of uneven diameters at the two ends of the rods is based on the consideration that non-sulfonated PS would be collapsed on the core of *f*PI since PS and *f*PI would undergo phase separation due to unfavorable interaction. Less sulfonated PS would reside in the

core, with low SD polymer chains forming a thicker layer of PS on the core and PS in high SD polymer chains forming a thinner layer on the core. We believe that the first argument is the most likely path due to the following facts: *first*, as indicated above, the strongly unfavorable interaction between *s*PS, PS and *f*PI would form a core-shell-corona structure due to intra-micelle phase separation; we did not observe the core-shell structure in TEM. *Secondly*, it would be extremely difficult for non-sulfonated PS to fold back to the *f*PI core surface to form a looped *s*PS structure if the PS segments adjacent to *f*PI are sulfonated, due to doubly unfavorable interactions between *s*PS and PS as well as PS and *f*PI. A pearl-necklace morphology was proposed for sulfonated homo-PS in water with *s*PS segments covering the pearls,²⁸ we believe that the sulfonated PS block in our case self-organizes in a similar way. *Thirdly*, and most importantly, the tapering behavior is driven by a distribution of sulfonation degrees. If *f*PI cores were covered by PS shells, there would be no driving force to form tapered rods. From our TEM results, a precise interpretation of the aging process for sample Series2-SD29.6 from spherical micelles to tapered worm-like structure is not possible. We suggest that a fusion process whereby spheres of different sizes form cylinders²⁹ pre-assemble the tapered structure. After the fusion, further slow intra-micelle segregation builds the final structure, as discussed above.

To augment the understanding of tapered morphologies in terms of chain stretching, a standard Kremer-Grest bead spring model simulation was carried out to investigate the chain conformations of a model charged diblock copolymer with explicit Coulomb interactions. These simulations were performed to understand the chain stretching at the molecular level, not to understand the large scale

morphologies. The diblock consists of 50-50 charged-uncharged blocks with 28.1% and 46.9% charges on the randomly charged block. The chain conformations for the two different degrees of ionizations are shown in Figure 4.4. At this high dielectric constant the chain stretching can be observed for both cases. The chain with higher charges shows longer stretching of both blocks (due to strong electrostatic interactions) giving rise to softening of the structure. Therefore, the lower the charge states, the stiffer the structures would be (Figure 4.1c and Figure 4.3c). The interfacial tension can be directly correlated to the chain stretching mechanism that is clearly reflected on the formation of flat interface caused by strong incompatibility of the two blocks. (Appendix, Figure A5).

Concerning effects of sulfonation degree on self-assembly of block copolymers, Balsara et al. recently observed coexistence of two morphologies, HPL and LAM, in a single sample of sulfonated poly(styrene)-*b*-poly(methylbutylene)(sPS-*b*-PMB).³⁰ They attributed this coexistence to a distribution of sulfonation exactly as we discussed above, which also leads to different primary domain spacing (characteristic scattering peak, q^*) in each phase. Different sulfonation degrees would cause polymer chains to behave differently due to the large χ between sulfonated and non-sulfonated monomers,³⁰ which in our case is more severe

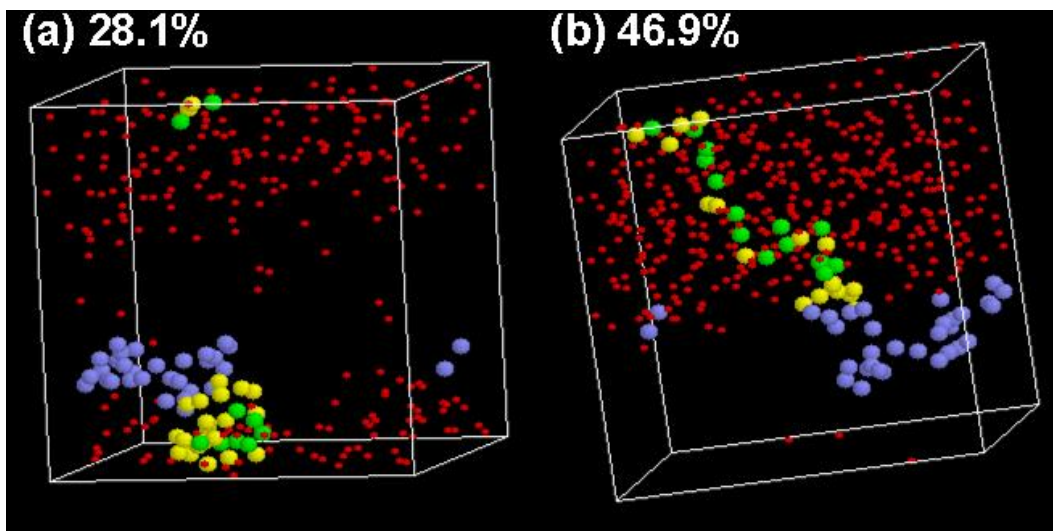


Figure 4.4 Snapshot of the central simulation cell at the end of the run. Blue dots are uncharged block of the chain, yellow dots are the uncharged monomers of the charged block and the green dots are the charged monomers of the charged block. Red dots are counterions. Only one chain is shown here to highlight the exclusive chain conformation, but all the counterions are shown. Due to periodic boundary condition, there are some scattered independent monomers that can be seen at the box edge.

because of fluorination^{30d} ($\chi_{sSt-fl} > \chi_{sSt-St} > 5.6 - 25$, $\chi_{sSt-MB} = 6.54$)³⁰. This supports the idea that polymer chains with different SD tend to phase separate into domains composed of chains that have similar SD, leading to different domain spacing, e.g. diameter of the worm-like structure in our case.

The observed ribbon-like micelles may also reflect phase separation of our *sPS-b-fPI* polymers in the super-strong segregation regime (SSSR), in which the interfacial energy overwhelms entropic penalties from chain stretching, and thus flat interfaces form. It was predicted that aggregates with flat interfaces, such as disk-like micelles, are stable in the SSSR,^{30d,31} and they were observed experimentally by Pochan³² and Lodge.³³ To the best of our knowledge, *ribbon-like self-assembly of amphiphilic diblock copolymers in aqueous media has not previously been reported*. Finally, nonergodicity of the system may affect the aging process leading to multiple morphologies (Figure A3).^{26,34,35} The high hydrophobicity of *fPI* prevents inter-micelle chain exchange, which leads to a non-equilibrium state in the global system; local minimization of free energy *via* similar assembling paths is still accessible by intra-micelle self-organization of chain packing and adjustment of conformation. With regard to the equilibrium state and kinetics of the system³⁶ (Series2-SD29.6), we speculate that the structures evolved during aging by slow dynamics, thus experimentally observable due to the “kinetically trapped state”.

The structures of these block copolymers in THF (good solvent for PS and *fPI*, non-solvent for *sPS*) are quite interesting as shown in Table A1 (Appendix). For series No.1 samples, DLS showed that the sample with SD13.4 gave a 91% population of unimers with D_h of 5.95 nm, along with 9% of aggregates of size from

14.9 to 148 nm. Series 1-SD38.8 showed two populations with 53.3% of unimers (D_h 7.52 nm) and 46.7% of aggregates (D_h 150.6 nm); while Series 1-SD98 exhibited one narrow population of stable aggregates with D_h 20.6 nm. The aggregation is attributed to strong ionic interactions and hydrogen bonding between sulfonic acid groups, while *f*PI serves as corona chains in its good solvent THF. Samples in sodium form in series No. 2 exhibited a more complicated aggregation process during water addition. A dispersion of polymer in pure THF is formed, followed by dissolution then cloudy precipitation, and finally a clear transparent solution as water was gradually added. We believe that this process reflects water-induced inversion of polymer aggregates. Ionic aggregation occurs when polymer is dissolved in a low dielectric solvent like THF with low water content. On addition of water, ionic interaction of sulfonate groups is broken and thus *s*PS dissolves, while this high polar solvent mixture become a non-solvent for *f*PI, which is forced to segregate to form the core of aggregates with corona composed of sulfonated PS¹¹. *s*PS migrates from inside (core) to outside (shell) of the aggregates when changing from low to high dielectric constant solvent. During the inversion process, precipitation takes place due to instability of the system (interface of the micelles changed) and the system behaves as a normal diblock in higher dielectric constant media¹¹. It is quite interesting to compare this to behavior of classical amphiphilic block copolymers, polystyrene-*b*-polyacrylic acid/polyethylene oxide, for example, which exhibits reversibility of micellar structure dependent on water concentration³⁷, and will be further studied. This inversion process could be useful, because it provides an approach to encapsulate both hydrophobic and hydrophobic guests in targeted delivery.

4.6 Conclusions

In summary, model block copolymers of *s*PS-*b*-fPI show unique self-assembly behavior, forming novel tapered rods and ribbon-like micelles. It is believed that a distribution of sulfonation degrees and residing in the SSSR drive the self-assembly of these materials in unique ways, accounting for axial dimensional changes and interfacial flatness. Further experimental work is required to verify the hypothesis of intra-micelle segregation driven by distribution of sulfonation degree, such as elemental mapping of sulfur and oxygen by energy-filtered transmission electron microscopy to explore the distribution of elements along the tapered structures. The concept of spatial distribution of sulfonation degrees across asymmetrical micelles, opens up a new approach to tuning self-assembly of block copolymers. The sulfonation degree strongly impacts the self-assembly of the polymers in THF, and the inversion of aggregates as water is gradually added offers potential for applications in cargo delivery.

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**Chapter 5 Synthesis and Self-assembly of
Polystyrene-*b*-Sulfonated Poly(1,3-cyclohexadiene)
Having Controlled Microstructures**

Abstract

Well-defined diblock copolymers of styrene and 1,3-cyclohexadiene, with the latter blocks having controlled microstructures, were synthesized by anionic polymerization and post-polymerization chemistry. Sulfonation reactions were selectively carried out on the PCHD blocks by use of SO_3 /dioxane as the sulfonating reagent at low temperature. ^1H NMR and elemental analysis (EA) were used to obtain the sulfonation degrees, which were similar for all the block copolymers under the chosen reaction conditions. A combination of ^1H NMR and EA demonstrated that addition products of SO_3 to the double bond of PCHD were obtained. These strong electrolyte amphiphilic diblock copolymers self-assembled into micellar structures in aqueous media. In particular, a sample with a hydrophilic composition of ~ 70 wt% formed a vesicular morphology; while in this composition range, spherical structures are usually formed. Transmission electron microscopy (TEM), cryo-TEM, and static and dynamic light scattering techniques were employed to characterize these aggregates. The mechanism of vesicle formation and microstructure effects on solution behavior of these block copolymers is discussed in detail.

5.1 Introduction

As is well known, amphiphilic block copolymers mimic the behavior of surfactants in aqueous solution, forming self-assembled structures of nanometer size, for example, spheres, rods and vesicles, with hydrophobic blocks forming the core of micelles and hydrophilic segments forming swollen corona. The phenomenon of segregation of two chemically different segments that are covalently linked into individual zones is triggered by the incompatibility between the aqueous environment and the hydrophobic block, so that the free energy of the system is minimized and the formed micelles can be stabilized, avoiding macro-phase separation. For the purpose of minimizing free energy of the system, different morphologies of micelles, reflecting different interface curvatures, can form, which is determined by a variety of factors, *e.g.* chain length of each block, volume fraction, the Flory-Huggins interaction parameter, as well as environmental effects (pH, ionic strength, temperature, additives).¹ Extensive manipulation of these variables has been performed to realize different morphologies, including multicompartment structures, toroidal micelles, helices, tapered- and ribbon-like micelles, in addition to the traditional nanostructures mentioned above.² The potential application of nanostructures in nanotechnology development and medical applications such as drug delivery have focused substantial attention and significance on the investigation of self-assembly of amphiphilic block copolymers.³

The majority of self-assembly studies^{1, 4, 5} have focused on amphiphilic di- and tri-block copolymers, where the backbones of hydrophilic blocks are saturated before or after bestowing hydrophobicity, for example, sulfonated polystyrene,⁶⁻⁹ poly(ethylene oxide),¹⁰⁻¹² poly(acrylic acid),¹³⁻¹⁶ polymers based on amino-type monomer¹⁷⁻²⁰ and so on.

However, strong electrolyte block copolymers derived from polydienes, *e.g.* sulfonated polydienes, have been rarely studied due to synthetic difficulties in their controlled syntheses.²¹⁻²³ Apart from the synthetic aspects, the range of morphologies formed by strong electrolyte block copolymers have thus far been quite limited, mainly spherical and cylindrical micelles;^{6, 8, 24, 25} Recently we discovered ribbon-like and tapered cylinders from charged and fluorinated block copolymers, taking the advantage of the high interaction parameter between the blocks.⁶ The charged nature of strong electrolytes gives rise to the possibility that the segregation between blocks may reside in the super strong segregation regime.^{26, 27} Neutral amphiphilic block copolymers or block copolymers containing weak electrolyte blocks such as poly(acrylic acid) exhibit a myriad of morphologies due to the tunability of charge effects by, for example, adjusting the pH of their solutions.²

Poly(1,3-cyclohexadiene) is a polydiene that shows unique properties such as thermal stability (high glass transition temperature T_g)^{28, 29} due to the six-membered ring in the polymer backbone, which can be hydrogenated to poly(cyclohexylene) and aromatized to poly(phenylene).^{30, 31} The flexibility and solubility differences of PCHD chains having different microstructures have an impact on the solution behavior of such block copolymers.³² Incorporation of strong ionic groups on the PCHD backbone imparts a hydrophilic nature, thus amphiphilic block copolymers composed of functionalized PCHD may exhibit some unusual properties that are characteristics of chain conformation/microstructures (ratio of 1,4-/1,2- units). Polymer chain microstructure is an important factor that affects micelle formation and has never, to our knowledge, been studied in the past. On the other hand, the J-aggregation formation of chromophores in

sulfonated PCHD by self-assembly revealed interesting photophysical properties for organic light-emitting diode applications.³³ In this paper, we report the synthesis and study of self-assembly in water of amphiphilic block copolymers of styrene and sulfonated cyclohexadiene with different 1,4-/1,2-microstructures, as well as with different block lengths, based on the methods previously developed, affording well-controlled sulfonated polydienes.³⁴

5.2 Experimental Part

5.2.1 Materials

Poly(styrene)-*b*-poly(1,3-cyclohexadiene) (PS-*b*-PCHD, PCHD: molar ratio 1,4-/1,2- microstructure = 90/10, 70/30, 50/50) block copolymers were synthesized according to standard protocol via anionic polymerization using high vacuum techniques.³⁵ The different microstructures of PCHD were realized by using different additives during polymerization of CHD, e.g. 1,4-diazabicyclo[2.2.2]octane (DABCO, Aldrich, 98%), *N,N,N',N'*-tetramethylethylenediamine (TMEDA, Aldrich, >99%) and 1,2-dimethoxyethane (DME, Acros, >99%).²⁹ Anhydrous 1,4-dioxane (Acros, 99.0%), dichloromethane (Acros, 99.9%, extra dry), chloroform (Aldrich, $\geq 99\%$, anhydrous) and sulfur trioxide (SO₃, Aldrich, 99%) were used as received. Dimethyl sulfoxide-*d*₆ (*d*-DMSO, Acros) was used as the solvent for ¹HNMR characterization.

5.2.2 Synthesis of sulfonated poly(1,3-cyclohexadiene)-*b*-polystyrene (PS-*b*-sPCHD)

In a typical reaction, two round-bottom, two-necked flasks with stirring bars (dried at 200 °C) were set up separately under nitrogen flow. A small flask of 100 ml equipped with a dropping funnel was charged with 1.3 mL of dioxane and 15 mL of dichloromethane and placed in an ice bath. To the dropping funnel, 0.25 mL of sulfur

trioxide and 6 mL of dichloromethane were added, followed by slowly adding the SO₃ solution into dioxane which was thermostated around 0~5 °C. The complex of SO₃/dioxane was stirred about for 30 minutes in an ice bath before it was mixed with PS-*b*-PCHD (0.5 g) in CHCl₃ (20 ml) in the second flask, cooled by an ice bath. All the operations were handled under N₂. The mixture was stirred for about 4 hours before it was quenched by addition of aqueous NaOH solution (0.54g of NaOH, 5 wt%). The chlorinated solvents were exhaustively removed by rotary evaporation at 50 °C.

5.2.3 Purification of PS-*b*-sPCHD

After removing chlorinated solvents, the mixture of precipitated sulfonated polymer, water, and salt were made a transparent homogenous solution by adding tetrahydrofuran (THF) and water (v/v water/THF ~1/2 – 1/1, ~ wt 2 % polymer concentration). This solution was transferred into a dialysis bag (MWCO 3500 g/mol), followed by dialysis against deionized water for 3 days to remove salt and THF. The polymers were recovered by freeze-drying as yellowish solids. Yields: 0.68g, 80%.

5.2.4 Micelle formation in aqueous media and concentration determination

For all PS-*b*-sPCHD samples, about 40 mg of each was dissolved in DMSO to make a solution of about 1% (w/v) in vials of 20 ml. This solution was stirred overnight to insure complete dissolution. Deionized water was slowly added into the DMSO solution, with vigorous stirring, at a rate of 6.8ml/h using a syringe pump until 50% (v/v) of water was reached. This solution was stirred for 48 hours before it was dialyzed against deionized water for 3 days (MWCO 3500 g/mol) with regular water changes.

After dialysis, a major portion of the solution (about 15 grams out of about 20 grams) was precisely weighed and then freeze-dried to determine the concentration of the

polymer solution. Three of six sample solutions (No.1, 3, 5, Table 5.1) were diluted to obtain a total of five concentrations (c_1 - c_5 , c_1 mother solution as the highest) for characterization by static and dynamic light scattering (SLS and DLS). The other three samples (No.2, 4, 6 Table 5.1) were characterized using DLS. Transmission electron microscopy (TEM) was used to observe morphological structures of all samples.

5.3 Characterization

Size exclusion chromatography (SEC). Number-average molecular weight M_n and polydispersity index M_w/M_n (PDI) of all samples before sulfonation were determined by SEC using a Tosoh EcoSEC instrument which was equipped with two TSKgel Super Multipore HZ-V columns, calibrated using standard polystyrenes with M_n from 580 to 7.5×10^6 g/mol. The polymer was eluted in THF at 40 °C at a flow rate of 0.35 ml/min.

^1H NMR spectroscopy was performed on Varian Mercury 300 MHz spectrometer with CDCl_3 and d -DMSO as solvents for all precursors PS-*b*-PCHD and sulfonated PCHD-*b*-PS respectively. Elemental analysis (EA) was conducted in Galbraith Laboratories Inc. for sulfur contents.

Static and Dynamic Light Scattering. Dynamic (DLS) and static (SLS) light scattering experiments were performed using an ALV/DLS/SLS-5022F spectrometer equipped with an ALV-5000 multiple tau digital correlator and a He-Ne gas laser ($\lambda_0=632.8$ nm). The intensity of the scattered light was calibrated with pure toluene. All solutions were filtered through a Millipore membrane with pore size of 0.45 μm . The scattered light was collected for each solution and for the pure solvent at scattering angles θ ranging from 20° to 146° for a duration of 60 second.

The excess Rayleigh ratio ΔR_θ of each solution over that of the solvent was calculated from the scattered intensities of the solution and the solvent. The apparent molecular weight and radius of gyration (R_g) were extracted from Zimm Plots. The autocorrelation functions were analyzed by using the routine CONTIN assuming the superposition of exponentials for the distributions of relaxation times. The diffusion coefficients were obtained from the decay rate and the scattering wave vector and the apparent hydrodynamic radius R_h were computed using Stokes-Einstein equation.

Transmission Electron Microscopy (TEM) imaging was performed on a Tecnai 12 microscope operating at an accelerating voltage of 120 kV. TEM samples were prepared by applying a drop of polymer solution onto a carbon coated copper TEM grid and allowing the solvents to evaporate under ambient conditions or by blotting to remove excess of solution with edge of a filter paper. For cryo-TEM, a small droplet of the solution was placed on a holey carbon film supported on a TEM copper grid within a Vitrobot vitrification system (FEI Inc.). The specimen was blotted and plunged into a liquid ethane reservoir cooled by liquid nitrogen. The vitrified samples were transferred to a Gatan 626 cryo-holder and cryo-transfer stage cooled by liquid nitrogen. During observation of the vitrified samples, the cryo-holder temperature was maintained below 170 °C to prevent sublimation of vitreous water. All the images were recorded digitally with a Gatan CCD camera.

5.4 Results and Discussion

5.4.1 Sulfonation of PS-*b*-PCHD: PS-*b*-sPCHD

The precursors PS-*b*-PCHD having different compositions and microstructures were synthesized by anionic polymerization using different additives during polymerization of CHD.^{28, 29} In some cases, traces of homopolystyrene accidentally terminated by impurities during the addition of additives and/or the second monomer were removed by fractionation using solvent/non-solvent techniques. All SECs of these materials showed unimodal narrow distributions, although the block copolymers with higher contents of 1,2-microstructure exhibited broadened molecular weight distributions with tailing at low molecular weight due to the limited solubility of 1,2-PCHD in benzene at room temperature and due to side reactions taking place during polymerization.²⁹ The molecular characteristics of PS-*b*-PCHD are summarized in Table 5.1. The targeted molecular weight and compositions agree well with the reaction stoichiometries as demonstrated by SEC and ¹HNMR.

In Table 5.1, the six samples have polystyrene chains of about the same length (around 4×10^3 g/mol). Among these materials there are two groups of compositions having ~30 wt% (Nos. 1, 3, 5) and ~20 wt% (Nos. 2, 4, 6) of PS, while in each group PCHD has different microstructures: molar ratios of 1,4-/1,2-CHD of 90/10, 50/50, and 70/30. With polymers having these molecular characteristics, the effects of microstructure and composition on self-assembly behavior can be evaluated.

The sulfonation of polydiene segments in block copolymers of styrene and cyclohexadiene was accomplished by using the complex of 1,4-dioxane /SO₃ (2.5/1, mol/mol) which had been well documented to sulfonate polydienes in a well-controlled

Table 5.1 Molecular Characteristics of PS-*b*-PCHD and PS-*b*-sPCHD

No	PS(SEC)		PS- <i>b</i> -PCHD(SEC)		1,4- /1,2- CHD	St/CHD (m/m, St %) ^a	Sf. Dg. ^b /S % ^c (HNMR)	S % ^d (EA)
	M_n /g/mol	PDI	M_n /g/mol	PDI				
1	3.8×10^3	1.08	1.4×10^4	1.07	90/10	1/2.98, 30.4	74.2/11.6	9.14
2			1.8×10^4	1.17		1/5.26, 19.8	64.6/11.7	10.1
3	4.5×10^3	1.07	1.5×10^4	1.19	50/50	1/2.34, 35.7	62.4/10.0	8.24
4			2.1×10^4	1.24		1/5.45, 19.3	68.8/12.1	8.62
5	4.1×10^3	1.07	1.5×10^4	1.10	70/30	1/2.80, 31.7	65.7/10.7	8.62
6			2.1×10^4	1.17		1/4.75, 21.5	60.2/11.1	9.22

^a, Molar ratio of repeating units and weight percentage of styrene in PS-*b*-PCHD determined by ¹HNMR

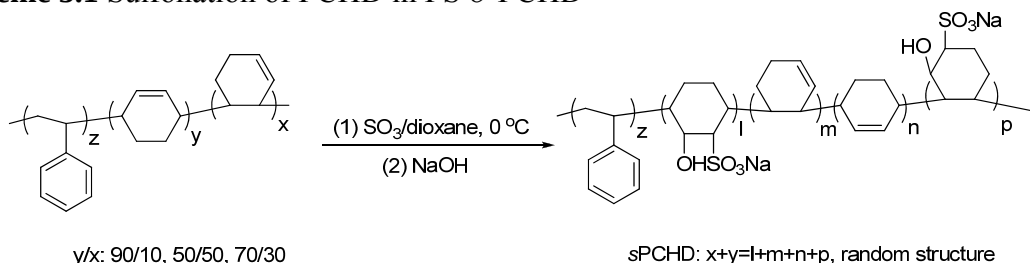
^b, Sulfonation degree (Sf. Dg.): the molar percentage of sulfonated CHD repeating units in PCHD blocks, determined by ¹HNMR assuming sulfonation was only yielded from addition products.

^c, Weight percentage of element sulfur in block copolymers calculated from sulfonation degree.

^d, Weight percentage of element sulfur in block copolymers measured by elemental analysis.

manner while keeping polystyrene intact at low temperatures.^{21, 22, 36} The sulfonation reaction of polydienes can result in addition and/or substitution products, resulting in saturated carbon-carbon double bonds or retaining double bonds, respectively.²¹ In our reactions, the addition product is obtained, as discussed below (see Scheme 5.1).

Scheme 5.1 Sulfonation of PCHD in PS-*b*-PCHD



Although a 1.2/1 molar ratio of SO_3 and CHD repeating units was used for all the reactions, complete sulfonation of PCHD was not achieved. As shown in ^1H NMR spectra (Figure 5.1), the intensity of double bond protons (5.0-6.0 ppm) is greatly reduced, but there still exist signals of vinyl protons after sulfonation, indicating the presence of residual unsulfonated CHD repeating units. By comparing the area of the vinyl peaks in ^1H NMR to the area of the aromatic peaks of PS before and after sulfonation, the sulfonation degree can be calculated (with the assumption that addition products are the only sulfonated structure in PCHD chains), which is shown in Table 5.1. The actual sulfonation will be higher than the calculated value from ^1H NMR data if some substitution products are obtained. However, the weight percentage of element sulfur obtained by elemental analysis (S %, Table 5.1) is in close agreement to the ^1H NMR value (or even lower but within experimental error), which strongly suggests that the addition products are the exclusive product of sulfonation of PCHD under our chosen conditions. It is interesting to note that although the PS-*b*-PCHD precursors have

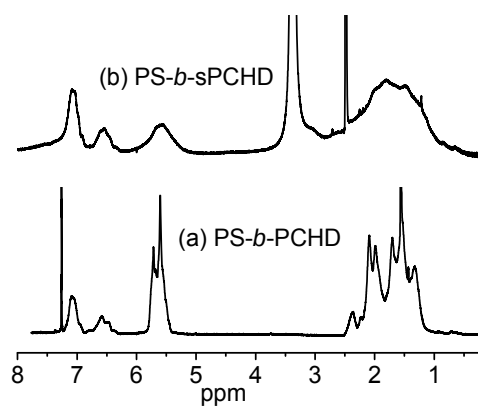


Figure 5.1 ^1H NMR spectra of (a) precursor PS-*b*-PCHD in CDCl_3 and (b) PS-*b*-*s*PCHD in *d*-DMSO. Sample from No. 6 in Table 5.1.

different microstructures in the PCHD blocks, the sulfonation degree stays essentially the same under these sulfonation conditions. This suggests the sulfonation is not affected by PCHD microstructure and thus that the PCHD blocks were sulfonated randomly, which makes the comparison of self-assembly only focused on the effects of microstructure and chain length. From the ^1H NMR spectra (Figure 5.1), it is also seen that PS generally remained intact, although in some cases traces of sulfonation of polystyrene were also observed. These very lightly sulfonated PS blocks remain hydrophobic.

5.4.2 Self-assembly studies

Due to the limited solubility of amphiphilic block copolymers of styrene and sulfonated CHD in water, the polymers were first dissolved in a high dielectric constant solvent, DMSO, although it took overnight to form an optically clear solution. The self-assembly of these block copolymers in water was achieved by slowly adding water into the DMSO solution, followed by dialysis against deionized water. The self-assembly was facilitated by stirring the polymer solution in a mixture solvent of water/DMSO (v/v, 50/50) imparting the polymer chains with sufficient mobility and time to respond to the addition of water. The aggregation of polymers upon water addition was initially evidenced by the Tyndall effect by shining a “lecture laser” through the solution. The formed micelles of PS-*b*-sPCHD in aqueous system after dialysis were investigated in details by a combination of transmission electron microscopy and light scattering.

5.4.2.1 TEM observation

The morphologies of micelles were examined by regular- or cryo-transmission electron microscopy (TEM and cryo-TEM). During the experimental process, the strong electrolyte nature of the materials brought about difficulties in TEM observation by

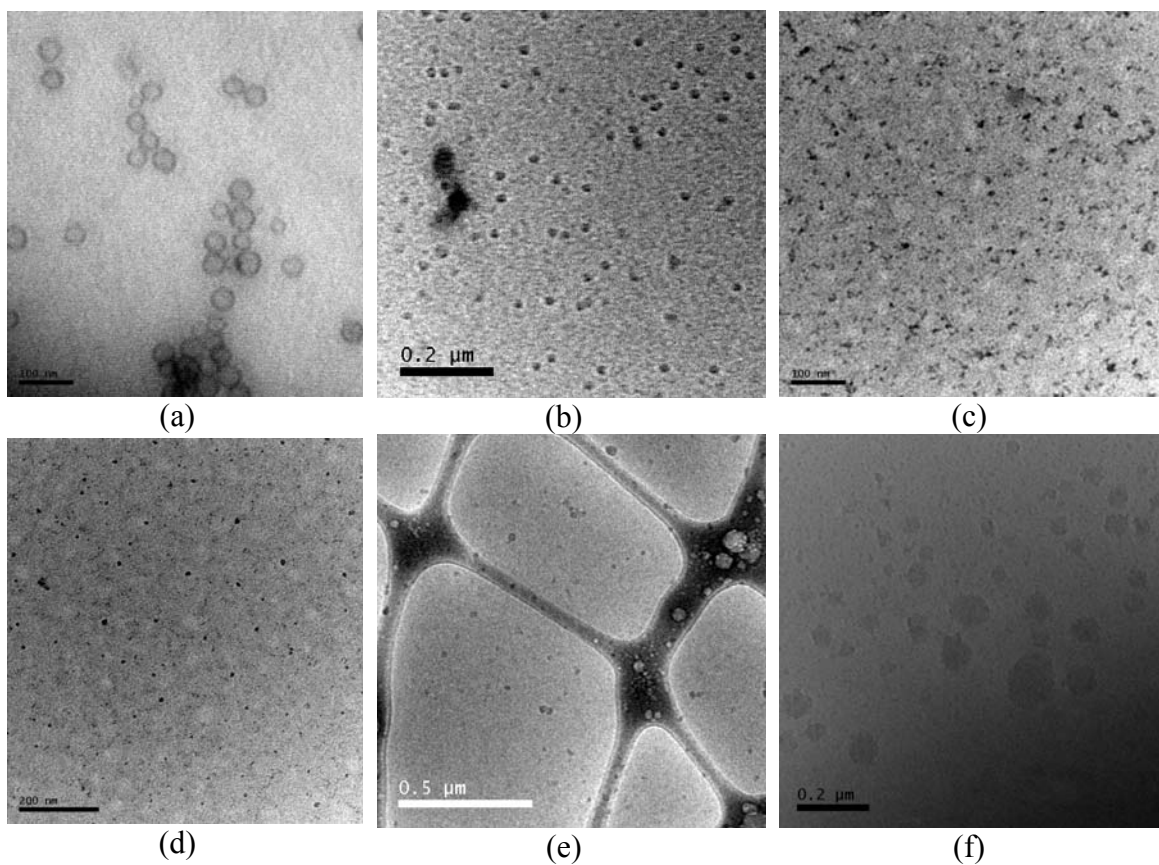


Figure 5.2 TEM images of aqueous self-assemblies formed by the six samples in Table 5.1. (a) Sample No.1, regular TEM. (b) Sample No. 2, regular TEM. (c) Sample No. 3, regular TEM. (d) Sample No. 4, regular TEM. (e) Sample No. 5, cryo-TEM (f) Sample No. 6, cryo-TEM.

coiling the carbon film or yielding low contrast for cryo-TEM. Typical observation for each sample is presented in Figure 5.2. Generally, it was seen that spherical shaped micelles formed, either hollow (vesicles) or solid (spheres). For sample No.1, we observed a vesicle morphology with diameter (d) around 40 nm and wall thickness of about 9 nm (Table 5.1). This behavior is quite unusual based on two general trends: (1), this particular composition of ~70 wt % of hydrophilic components usually forms spheres. (2) the size of the vesicles (d) is around 30-40 nm, much smaller than commonly observed for polymer vesicles (usually $> \sim 100$ nm).¹⁶ The formation of vesicular micelles will be further demonstrated by light scattering as discussed in the next section. Their formation is rationalized by taking into account the solubility characteristics (interaction between corona and water) of *s*PCHD chains in water. For the other samples, due to the differences in chain length, and solubility leading to different contrast in cryo-TEM, spheres were observed having various sizes as evidenced by TEM and cryo-TEM. In particular, sample no. 5 (Figure 5.2(e)) forms a spherical structure in water with diameter around 18 nm, which is the size of PS core; the corona consisting of *s*PCHD is invisible due to low contrast. Interestingly, the core size coincides with the vesicle wall thickness (9 nm, Figure 5.2(a)) by a factor of 2. This is quite reasonable because both of the diblock copolymers almost share the same length of hydrophobic PS chains (Table 5.1) which forms the walls of vesicles and cores of the spheres. However, the nanostructure observed for sample 6 is quite different from that of sample 5 in terms of size, diameter of which is estimated to be an average of 68 nm, comparable to 101 nm D_H determined by DLS at c_1 (Figure 5.4(a), see below). The fluffy structure for sample 6 shown in cryo-TEM in Figure 5.2(f) is attributed to “visible” corona in cryo-TEM. In Figure 5.2, it is seen that

different aggregates were formed in terms of size, morphology and contrast in cryo-TEM which may be attributed to PCHD microstructure effects and compositions of block copolymers.

5.4.2.2 *Light Scattering*

In order to probe morphology of micelles formed in aqueous solution and confirm the structures that were observed by TEM, light scattering was performed to achieve insight into their morphologies in water. Cryo-TEM is a robust technique to describe the real structure in the solution state, however, as mentioned above, difficulties were encountered for some of the samples due to the limited contrast. Thus, a combination of light scattering and TEM provide complementary insight into the nature of the micelles. In this section, DLS and SLS results are presented and discussed.

Previous studies showed that within a similar range of PS contents in block copolymers of PS-*b*-sPCHD, critical micelle concentrations (CMC) were on the order of 0.15 mg/ml.³³ The polymer solutions in this work were prepared above this concentration for SLS experiments, although CMC is a function of multiple parameters *e.g.* hydrophilic contents, chain length and sulfonation degree of PCHD. It was found that the concentrations decreased after dialysis. This might be due to loss of unimers (unassociated polymer chains) or small aggregates during dialysis process. Thus re-measuring polymer concentrations after dialysis becomes necessary for SLS experiments. SLS and DLS data were collected simultaneously for samples No. 1, 3 and 5 as a function of scattering angle and concentration, thus allowing a range of parameters of interest to be determined: hydrodynamic radius (R_H), radius of gyration (R_g), second virial coefficient (A_2), and weight-average molecular weight. For samples No. 2, 4, and 6, only

DLS was carried out to measure the angular dependence of R_H at concentrations comparable to that of most concentrated solutions used for SLS.

A. Dynamic light scattering:

Angular dependence of hydrodynamic size was examined for all the samples by plotting apparent diffusion coefficient (D) as a function of q^2 (q , scattering vector) at concentration c , and $D(c)$ at zero angle is obtained by extrapolate $D(c, q)$ to $q^2 = 0$ as following:

$$D(c) = \lim_{q^2 \rightarrow 0} \frac{\Gamma}{q^2}$$

in which Γ is the first cumulant. All the samples at the highest concentration (c_1) gave D values essentially independent of scattering angle, with a slight positive slope possibly attributing to polydispersity, which is in agreement with a spherical morphology as shown in TEM observations. A typical plot is shown in Figure 5.3(a) (Sample No.5 in Table 5.1). The linearity and near zero slope of this plot indicates an exclusive translational motion of particles in the solution, suggesting a spherical morphology, which is consistent with the cryo-TEM results, as shown in Figure 5.2(e). However, the hydrodynamic radius obtained by extrapolating D to zero angle and calculated from the Stokes-Einstein equation is 45.4 nm, which is significantly greater than the value of 9 nm estimated from TEM. This may be due to cryo-TEM only observing the collapsed PS core because of low contrast between corona and background, while the size of the micelles in water reflect contributions from both the hydrophilic polymer segments (sPCHD) (swollen corona) and the core. Shown in Figure 5.3(b) is a typical distribution of R_H at the highest concentration (c_1) for the same sample at detector of 96° . The majority of the population shows an intensity averaged 97% of spherical aggregates with

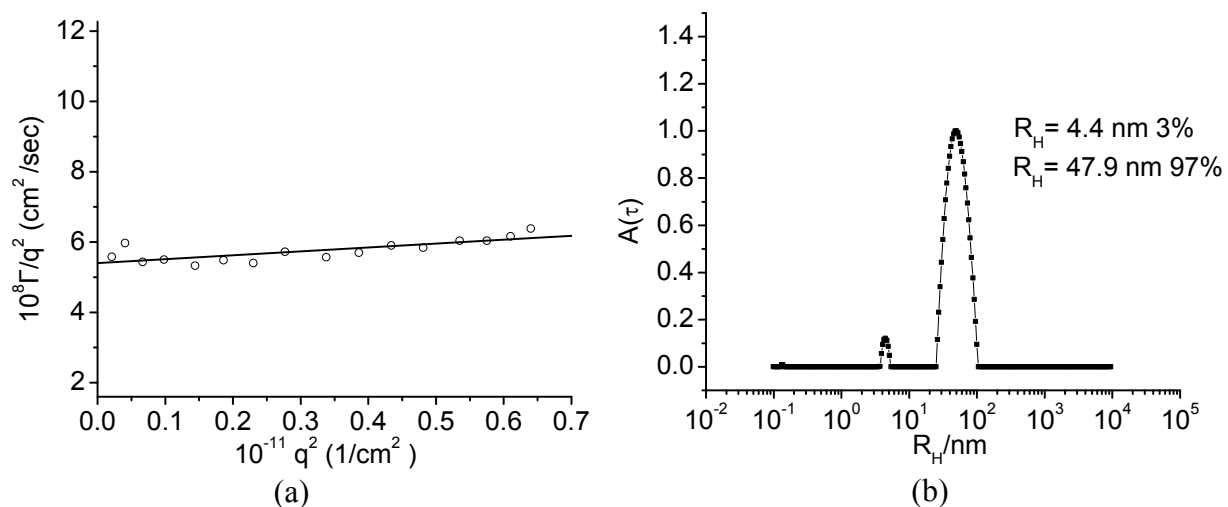


Figure 5.3 Dynamic light scattering data at concentration $c_1=1.474$ mg/ml for sample No. 5 in Table 5.1. (a) Angular dependence of diffusion coefficient $D = \Gamma/q^2 \sim q^2$; (b) Hydrodynamic radius distribution at scattering angle 96° .

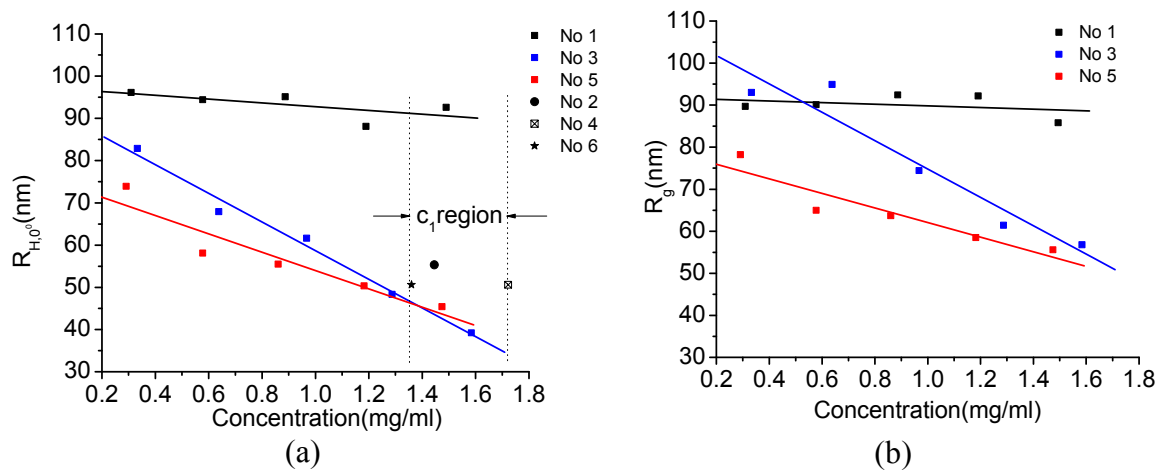


Figure 5.4 Concentration dependence of (a) R_H measured by dynamic light scattering extrapolated to zero angle along with values for samples No. 2, 4, 6 at highest concentrations (c_1 region); (b) R_g measured by static light scattering. Lines are linear fitting for samples 1, 3, 5.

$R_H = 47.9$ nm which is very close to the R_H at zero angle discussed above in consistent with the angular independence. The remaining 3% of small aggregates ($R_H = 4.4$ nm) is attributed to unimer or small aggregates (dimer, trimer) contributing to the decrease of concentration after dialysis.

The concentration dependence of R_H for samples No. 1, 3, 5 in Table 5.1 was investigated by DLS as shown in Figure 5.4(a) including R_{HS} for samples No. 2, 4, 6 at highest concentrations (c_1 region). It can be seen that sample 1 has the maximum size among samples 1, 3, 5, at any concentration, with an R_H around 95 nm, although they are composed of similar molecular weights of diblock copolymers with similar compositions and sulfonation degrees. This value is also larger than those observed for samples No. 2, 4, 6, as observed from the R_H at highest concentrations of c_1 region (Figure 5.4(a)), although the latter three samples have much longer chain lengths (Table 5.1). The reason for this difference is the formation of a vesicular morphology for sample 1, while the others form solid spherical structures as shown in Figure 5.2, and confirmed by more detailed analysis of sample 1 as discussed below. It is understandable that the size determined by DLS will be much larger than that from TEM (Figure 5.2(a)) due to the drying process during sample preparation for TEM. It is also observed in Figure 5.4(a) that samples No. 3 and 5 had a much stronger concentration dependence of hydrodynamic size than that of sample No. 1; while sample No. 1 has no concentration dependence and the slight negative slope is attributed to corona expansion. At five concentrations studied for samples No. 1, 3, and 5, all samples show no angular dependence of diffusion coefficient. Thus, no morphological changes are expected during dilution for light scattering. Generally, the sizes of sample 2 and 4 are greater than

sample 3, 5 due to a longer hydrophilic *s*PCHD chain segment in a similar range of concentration, for example in c_1 region (Figure 5.4(a)); while the size of samples 3 and 4 with 50% of 1,4-microstructure is respectively larger than that of samples 5 and 6 with 70% of 1,4-microstructure, clearly indicating the effects of microstructure of PCHD the “kinks” play an important role in determining the size of the assemblies even possibly affecting the morphology (Figure 5.2(a) and (e)).

B. Static light scattering

In order to obtain more detailed morphological information regarding the self-assembled structures in solution, static light scattering (SLS) was performed to measure radius of gyration (R_g) and aggregation number for samples 1, 3, and 5. In addition, the 2nd virial coefficient (A_2) characterizing the thermodynamic interactions between the solvent (water) and the micelles can be evaluated from the concentration dependence of the scattering intensity.

The data from SLS were obtained via Zimm plots, and a typical example (sample No. 5) is shown in Figure 5.5. The size characteristics of samples 1, 3, 5, *e.g.* R_g , micellar molecular weight, unimer molecular weight, micellar aggregation number along with A_2 are summarized in Table 5.2. Other than those in the Table, apparent R_g s at 5 different concentrations for each sample (No.1, 3, 5) were also obtained during processing SLS data, which are plotted against concentrations in Figure 5.4(b). It clearly shows the same concentration dependence of dimension as in Figure 5.4(a), *e.g.* a constant size for sample No. 1, a negative slope for fitting of $R_g \sim$ concentration and microstructure effects that sample No. 3 has greater size than sample No. 5 in any concentration region, all of which are in good agreement with DLS results.

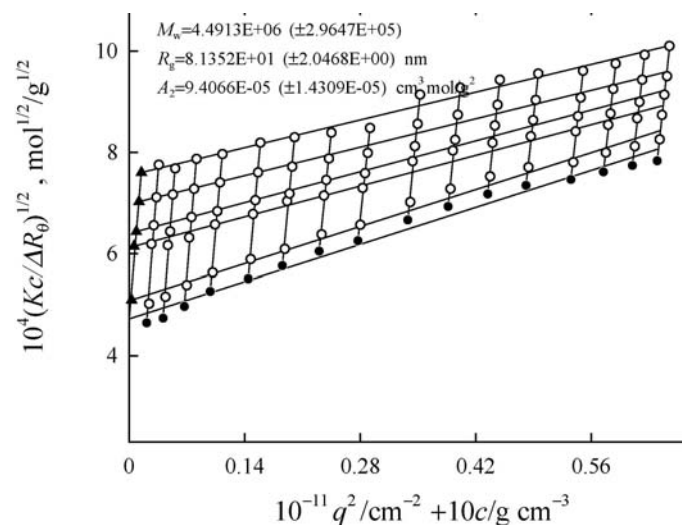


Figure 5.5 Zimm plot from static light scattering experiment for sample No.5 in water at 25 °C.

According to the molecular weight of precursor PS-*b*-PCHD and taking into account sulfonation degree (incorporation of groups –SO₃Na and -OH), the average mass of each sulfonated polymer chain (PS-*b*-sPCHD) (unimer molecular weight) can be calculated as listed in Table 5.2. Aggregation number (number of polymer chains in each micelle) can thus be obtained by dividing the apparent molecular weight of micelles (micelle molecular weight) determined by SLS by the unimer molecular weight. Vesicles of sample 1 exhibit almost twice the aggregation numbers of sample 3 and 5, while the latter two have about 200-300 polymer chains in their spherical micelles, as shown in Table 5.2. It is worthwhile to calculate the polystyrene core size of the spheres formed in sample 3 and 5 by using these aggregation numbers. By substituting molecular weight of PS (MW_{PS}) (Table 5.1) and its density (*d*) in solid/melt state along with aggregation number (Agg. No.) into the equation below:

$$\frac{\text{Agg.No.} \times MW_{PS}}{N_A} \times \frac{1}{d} = \frac{4}{3} \pi R_{core}^3$$

in which N_A is the Avogadro constant, R_{core} , radius of PS core of sphere micelles can be attained. The values for sample 3 and 5 are 7.8 nm and 6.9 nm, respectively as shown in Table 5.2. As we have discussed above in TEM section, the core size by cryo-TEM for sample 5 is ~9 nm in radius which agrees very well with the calculated value $R_{core} = 6.9$ nm by SLS. This strongly suggests only the core of the micelles is visible in cryo-TEM.

Table 5.2 Micelle information by static light scattering experiment in water at 25 °C

Sample No.	$R_{g,0}$ ^a (nm)	Micelle MW (10 ⁶ ×g/mol)	Unimer MW ^b (10 ⁴ ×g/mol)	Agg. No. ^c	R_{core} ^d (nm)	A_2 (10 ⁻⁴ cm ³ mol/g ²)
1	92.7	11.5	2.22	519	N/A	0.3
3	120.9	5.7	2.02	282	7.8	1.7
5	81.4	4.49	2.16	208	6.9	0.9

^a, R_g obtained by Zimm plot by extrapolating to zero concentrations; ^b, molecular weight of single PS-*b*-sPCHD chain; ^c, Apparent aggregation number of micelles: micelle MW/unimer MW; ^d, radius of PS core in micelles: calculated from density of PS (1.05 g/cm³) and Agg. No.

5.4.2.3 Combination of SLS and DLS and TEM: formation of vesicles

As mentioned above, for 70% compositions of hydrophilic components, the amphiphilic diblock copolymers usually form spherical structures.¹⁶ The key issues here are to confirm the vesicular morphology for sample No.1 and to establish the reason for its formation.

We used combined DLS and SLS to probe the solution morphology of sample No.1 along with the visible evidence obtained from TEM. Direct structural insight may be gained by examining the ratio R_g/R_H , which is theoretically 0.774 for hard spheres and unity for vesicles. The R_g/R_H values for the micelles are plotted against concentration in Figure 5.6. For sample 1, the values of R_g/R_H scatter around unity independent of concentration, which is a strong evidence for the hollow sphere structures, confirming the morphology observed in TEM (Figure 5.2(a)). The reasons for vesicle formation for sample 1 may be attributed to (1) a severe chain stretching from *s*PCHD due to the electrostatic repulsion from the charge characteristics, leading to a flat interface between corona and hydrophobic PS wall (decrease of interfacial curvature);⁶ (2) a weak hydrophilicity of corona chains which cannot stabilize the spherical morphology. The second reason can be inferred from the 2nd virial coefficient (A_2) which indicates the quality of solvent (water). In Table 5.2 are listed A_2 values for sample 1, 3, 5, which are $A_{2,1}=0.3 < A_{2,5}=0.9 < A_{2,3}=1.7$ ($10^{-4}\text{cm}^3\text{mol/g}^2$). The A_2 values for hydrophilic polymers in water as a good solvent are of the order of $10^{-4}\text{cm}^3\text{mol/g}^2$,^{37, 38} while A_2 values on the order of $\sim 10^{-5}\text{cm}^3\text{mol/g}^2$ or below are quite small and may be considered as close to theta solvents.³⁸ As one can see here, A_2 for sample 1 is quite small, perhaps too small to stabilize spherical structures. A_2 values for samples

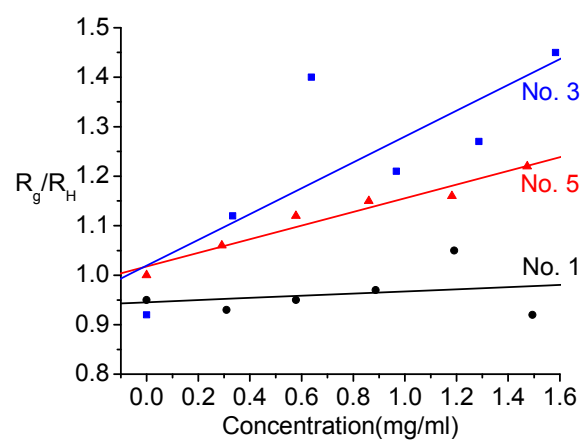


Figure 5.6 R_g/R_H as a function of concentration, a combination of dynamic and static light scattering. Lines are linear fitting to the scattered data for samples No. 1, 3, 5 in Table 5.1.

3 and 5 are larger (in the good solvent range) and are thus able to form stable spherical morphologies. The order of values of A_2 for samples No. 1, 3, 5 shown above coincides with the contents of 1,2-microstructures of PCHD, e.g. No.1 < No.5 < No.3, which reflects the effects of microstructure on solubility of sPCHD in water, and affects the morphology of micelles, as demonstrated by TEM and light scattering.

On the other hand, R_g/R_H values for samples 3 and 5 are a function of concentration and approach unity when concentration decreases to zero. Since the angular dependence of size was not observed for all the samples, morphological evolution with dilution is not anticipated. The interpretation for the behavior of R_g/R_H , e.g. decrease of R_g/R_H from about 1.2~1.4 to 1.0, is that the aggregation number of the star-like micelles decreased (e.g. number of arms decreases in a star structure) as concentration increased (calculated from SLS data, not shown), which causes R_g/R_H to increase.^{39, 40} The decrease of R_g/R_H probably reflects that a hyperbranched cluster (in a single micelle) become star-like micelle (tiny core and long corona chains) as the solution is diluted. The hyperbranched structure is from the J-aggregation behavior as it had been previously reported.³³

5.5 Conclusion

Strong electrolyte-containing diblock copolymers of styrene and sulfonated cyclohexadiene, with different microstructures in the PCHD block, were successfully synthesized with ~ 65% sulfonation degree (SD). ¹HNMR and elemental analysis showed that the sulfonation chemistry had no selectivity toward the different microstructures of PCHD and could be controlled to yield the same SD in diblock copolymers containing PCHD blocks having different microstructures. Transmission electron microscope (TEM),

cryo-TEM and static and dynamic light scattering demonstrated that all samples self-assembled into micelles, in which a sample having about 70 wt% of the sulfonated PCHD block and the highest 1,4-microstructure of PCHD formed vesicles, while all other micelles were spherical aggregates. The electrostatic repulsion in charged PCHD chains and the poor thermodynamic interactions between corona chains and water contribute to the vesicle formation. In this study, it was shown that hydrophilicity of sPCHD increases with 1,2-microstructure in PCHD block as indicated by A_2 . Thus, we have demonstrated microstructure effects on solution properties and self-assembly behavior of amphiphilic block copolymers for the first time.

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**Chapter 6 Well-defined PI-b-PAA/PS-b-PI-b-PAA
Block Copolymers and Hierarchical Structures
within Different Micellar Morphologies in
Aqueous Self-assembly**

Abstract

Well-defined acid based block copolymers containing polyisoprene are reported. The challenge of keeping an integrity of polydiene when producing polyacid had been addressed in this communication by controlling delicate reaction conditions in terms of reaction time and reactant ratio. A general purification method-column chromatography was also presented taking advantage of the different polarity of each block. The PS-*b*-PI-*b*-PAA triblock terpolymers form multicompartmental structures via aqueous self-assembly. Our work reveals the morphological consequences of unique interplay between global and local self-assembly.

6.1 Introduction

Amphiphilic block copolymers hold great potential for applications such as in biomedicines and nanotechnology fields owing to their surfactant behavior, e.g. self-assembly into micelles in a selective solvent.¹ They can exhibit an array of morphologies dictated by multiple interactions among the hydrophilic, hydrophobic segments and solvent (typical water).^{1, 2} Although much progress has been made in the past two decades,²⁻⁵ it is still challenging to predict structure-property relationships for block copolymer based micelles, which is critical to utilizing their characteristic properties.

In utilization of amphiphilic block copolymer micelles, for example as delivery vehicle/carrier, the core of the micelle serves as a micro-environment for the incorporation of lipophilic ingredients, while the corona stabilizes this hydrophobic core. The hard cores (mostly polystyrene (PS), high T_g) of the carriers make the incorporation of lipophilic drugs difficult, since they “freeze” the micro-environment of micelles. Therefore, it is advantageous to develop amphiphilic block copolymers forming soft-core consisting of blocks of low T_g . In addition, soft-core materials could promote dissipation of fracture energy upon deformation, lubrication between the different domains, and potentially even allow sacrificial bonding interactions when use micelles as model colloids.⁶

Furthermore, strategic development of “self-assembly engineering” requires a variety of immiscible hydrophobic components to create multiple compartments in the micellar core.^{7, 8} The majority of studies to date have focused on glassy materials such as polystyrene as the hydrophobic constituents in block copolymer based micelles. Poly(acrylic acid) (PAA) as stimuli-responsive corona blocks mostly conjugated with PS

have been well studied.^{9, 10} In contrast, PAA-based diblock copolymers containing polydienes (polyisoprene (PI) or polybutadiene (PBD)) which can self-assemble to create micelles having hydrophobic soft cores are rarely studied, mainly due to challenges in material synthesis and purification.¹¹⁻¹⁴ Well-defined polydienes are most readily obtained using anionic polymerization, but this method is not applicable for acrylic acid. Synthetic difficulties in creating well-defined PI-*b*-PAA and PBD-*b*-PAA block copolymers center around the intrinsically unstable nature of polydienes under strongly acidic conditions (40% loss of double bonds was reported when HCl catalyzed hydrolysis was carried out¹⁵), which are necessary for hydrolysis of poly(*tert*-butyl acrylate) (PtBuA), the commonly used precursor to PAA. No solid evidence for the structural integrity of the polydiene components of block copolymers with PtBuA (or PtBuMA) after hydrolysis to PAA has ever been archived.¹¹⁻¹⁵ It is critical to ascertain that the double bonds of the polydiene remain intact after the ester hydrolysis, since retention of the residual double bonds of the polydienes in the self-assembled structures provide further opportunities for functionalization and chemical modification. For example, crosslinking can be carried out in order to stabilize the as-formed structures or sacrificial degradation (e.g. by ozonolysis) can be employed in order to create cavities for bioactive reagent encapsulation.^{15, 16}

6.2 Experimental Part

6.2.1 Block copolymer synthesis:

Diblock copolymers of isoprene and *tert*-butyl acrylate (PI-*b*-PtBuA) and triblock copolymers of styrene, isoprene and *tert*-butyl acrylate (PS-*b*-PI-*b*-PtBuA) were synthesized by sequential anionic polymerization using high vacuum techniques

according to standard protocol.¹⁷ The detailed procedures for preparation of all-glass apparatuses and purification of monomers, solvents and additives are described in the literature.^{17, 18}

6.2.2 Purification

Purification/fractionation was needed to remove homopolymers PI and PS, and PS-*b*-PI contaminants from PI-*b*-PtBuA and PS-*b*-PI-PtBuA, respectively. Flash column chromatography was used to purify diblock and triblock copolymers. A typical procedure is described as follows.

0.89 g of PI-*b*-PtBuA was dissolved in a mixture solvent of toluene and hexane (10ml/5ml). A glass tube of 3.7 cm in diameter and 10 cm in length was reduced in size at one end to be 0.5 cm in diameter. A cotton ball was used to tightly seal the small size outlet of this column from the inside. The 8 cm column was filled with aluminium oxide particles (neutral, activated, ~150 mesh). The polymer solution prepared above was loaded onto this column and stayed for 20-30 min before elution with about 200ml of toluene. The first 2 ml of eluents of toluene were collected for gel permeation chromatography (GPC) analysis. Another ~ 150 ml of hexanes were used to flush the column after toluene. Finally, about 200 ml tetrahydrofuran (THF) was used to elute all the diblock or triblock copolymers out of the column, and they were collected. The polymers were recovered by concentrating the THF solution with rotary evaporation and were then dried in a vacuum oven at 50 °C for 3 days. Diblocks, yield: 0.87g; while in triblock copolymer case, polymers were recovered by precipitation into large excess of water/toluene (20/80, v/v) after THF solution was concentrated.

6.2.3 Hydrolysis

A typical process of hydrolysis is as following:

0.5 g of PI-*b*-PtBuA and 17 ml of dioxane were placed in a 50 ml flask which was equipped with a N₂ inlet, condenser with N₂ outlet and a magnetic stirring bar. The flask was immersed into an oil bath thermostatted at 85 °C with N₂ slowly flowing through the flask. After the polymers were dissolved, 1.35 g of HCl (6-fold excess relative to *t*BuA repeating units) aqueous solution (37 wt%) was charged into the solution. This reaction solution was monitored by ¹HNMR by sampling the solution until hydrolysis was complete as indicated by the disappearance of the *tert*-butyl peak at 1.44 ppm in ¹HNMR spectrum (Figure 6.1). Afterwards, the solution was cooled by tap water under N₂, followed by transferring into a dialysis bag (MW cutoff: 3500g/mol) to remove residual HCl, *tert*-butyl alcohol and solvent. After dialysis against deionized water for 3 days, polymers were recovered by freeze drying. Yield: 0.335g, 90.1%.

The purification and hydrolysis for triblock copolymers were the same as for PI-*b*-PtBuA.

6.2.4 Self-assembly of triblock copolymers of styrene, isoprene and acrylic acid

5 mg of PS-*b*-PI-*b*-PAA was dissolved in 0.5g of THF, and then 0.5 of H₂O was slowly added into this solution. This solution was allowed to stand overnight, followed by addition of 0.5 g of water and again allowed to stand overnight. Before preparing samples for TEM imaging, another 0.5 g of water was added into the micellar solution.

6.3 Characterization

Number-average molecular weight M_n , polydispersity index (PDI) of all samples before hydrolysis were determined by size exclusion chromatography using a Tosoh

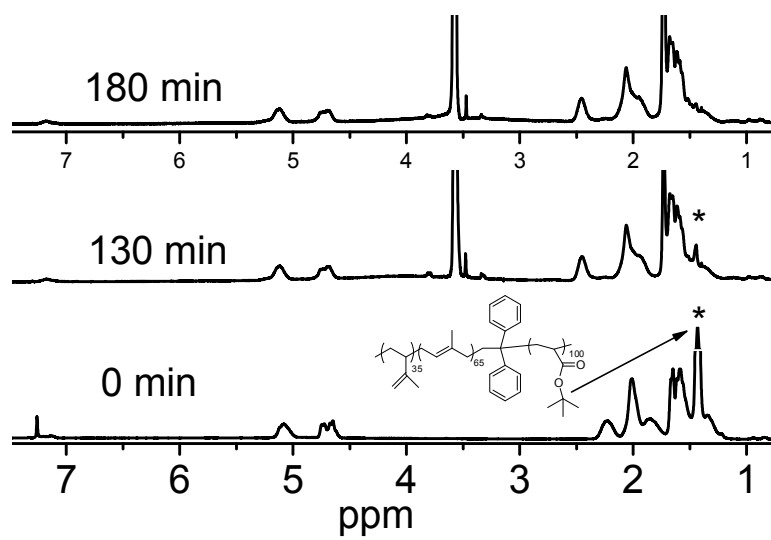


Figure 6.1 ^1H -NMR monitors hydrolysis process of PI-*b*-PtBuA at 0, 130, 180 min. In *d*-THF.

EcoSEC instrument which was equipped with two TSKgel Super Multipore HZ-V columns and calibrated using standard polystyrenes with M_n from 580 to 7.5×10^6 g/mol. The polymer was eluted in THF at 40 °C at a flow rate of 0.35 ml/min.

^1H -NMR spectroscopy was performed on Varian Mercury 300 MHz spectrometer with *d*-THF as solvent.

Transmission electron microscopy (TEM) was performed using a Hitachi HF-3300 instrument operating at voltage 330 Kev. TEM samples were prepared by depositing a droplet (1-4 μl) of self-assembled solution of polymer on copper grid coated by a carbon film. The excess water was blotted by using an edge of filter paper. The TEM samples were exposed to the vapor of an OsO_4 aqueous solution (2 wt%) for 2 hours.

6.4 Results and Discussion

6.4.1 Synthesis and Purification of PS-*b*-PI/PS-*b*-PI-*PtBuA*

Here, we report the synthesis of well-defined block copolymers of isoprene and acrylic acid with the carbon-carbon double bonds in the PI block remaining intact after hydrolysis of *PtBuA*. We also describe a simple and scalable purification method for PI-*b-PtBuA* containing PI homopolymers, where traditional solvent/nonsolvent fractionation fails. We then extend this method to the synthesis of PS-*b*-PI-*b*-PAA triblock terpolymers. Preliminary morphological studies in water reveal distinct multicompartment phase separation in the core of various micelles of same triblock molecules.

Well-defined precursors PI-*b-PtBuA* and PS-*b*-PI-*b-PtBuA* were synthesized by anionic polymerization employing high vacuum techniques and sequential polymerization of styrene (in the case of triblock terpolymers), isoprene and *tert*-butylacrylate, according to standard protocol.¹⁷ The microstructure of PI can be

controlled by adding an appropriate dose of tetrahydrofuran (THF) as an additive with hexanes as the primary solvent. A solvent switching process was necessary to remove hexanes and introduce pure THF for polymerization of *tert*-butylacrylate at low temperature. Due to multiple steps of introducing additives and reagents, including solvent switching, addition of 1,1-diphenylethylene, LiCl and monomers, some PI anions may be terminated due to traces of impurities introduced during these processes. Purification/fractionation may or may not be needed to remove residual PI homopolymers. A gel permeation chromatogram (GPC) of as-synthesized PI and PI-*b*-PtBuA is shown in Figure 6.2(a). A shift to lower retention time (R_t) demonstrates the successful growth of the PtBuA block, while the minor peak in the diblock chromatogram is assigned to PI homopolymer (Figure 6.2(a)).

Great challenges were encountered in the purification of diblock copolymers containing PI contaminants. Classical solvent/non-solvent fractionation and Soxhlet extraction, often used to purify block copolymers,¹⁹⁻²¹ failed to remove PI. This is due to the intrinsic solubility characteristics of polyisoprene and poly(*tert*-butyl acrylate), which causes amphiphilic behavior such that any common solvent selective for one of the blocks is a non-solvent for the other one, leading to micelles forming in the fractionation medium. While water is the only common non-solvent for both PI and PtBuA, in a trial of THF/water as solvent/non-solvent, macrophase separation slowly occurred (>2 weeks) to form droplets of one phase suspended in another, which makes separation impossible. Upon considering the difference in polarity of polyisoprene and poly(*tert*-butyl acrylate) and their molecular interactions with aluminium oxide (neutral, activated, ~150 mesh), a column separation using toluene as eluent gave highly efficient purification in high yields

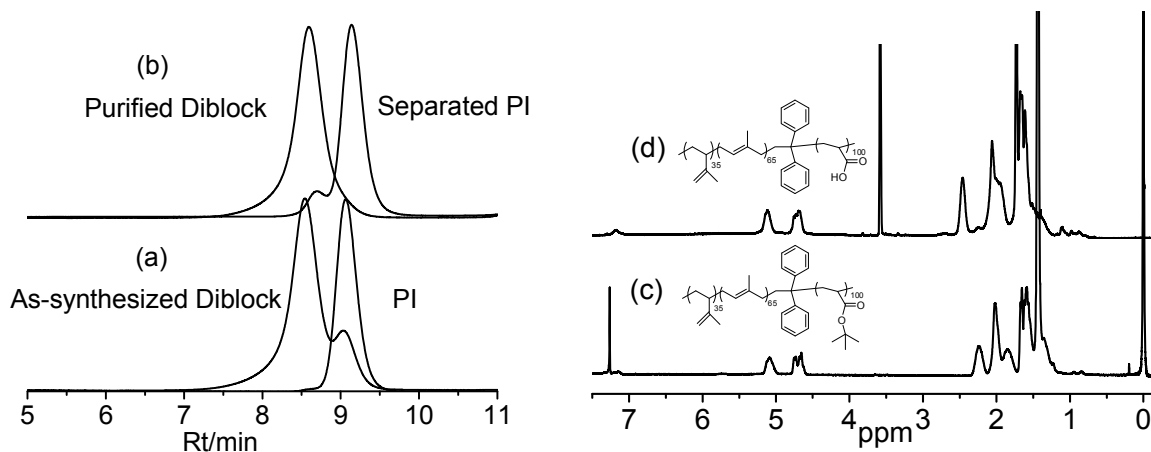


Figure 6.2. Left: GPC traces of PI and diblock copolymers (a) as-synthesized diblocks (PI-*b*-PtBuA) and polyisoprene (PI) which was sampled before addition of *t*BuA monomer in the polymerization; (b) Purified diblock copolymers and PI separated by neutral alumina column. Right: (c) ^1H -NMR of purified PI-*b*-PtBuA in CDCl_3 ; (d) ^1H -NMR of PI-*b*-PAA in *d*-THF.

as demonstrated by Figure 6.2(b). The purified diblocks are free of PI homopolymer, and the yield of diblocks was quantitative. The high purity and high yield can be inferred from Figure 6.2(b), where purified diblock shows a symmetrical peak and the minor peak in the as-synthesized diblock is completely gone after purification. The separated PI chromatogram corresponds to PI sampled from the anionic polymerization and terminated with methanol before addition of the second monomer. The minor peak in the GPC trace of the isolated PI at shorter R_t is PI-*b*-PtBuA with low PtBuA contents which was eluted with PI due to its low adsorption to the column. The purification of triblock copolymers was accomplished in the same way, where trace levels of PS homopolymer and PS-*b*-PI contaminants were easily removed in one step.

6.4.2 Hydrolysis of PtBuA in Block Copolymers

Although there are several reports regarding synthesis of block copolymers of butadiene (and one report on isoprene¹⁵) and acrylic acid,¹¹⁻¹⁴ no detailed ¹H-NMR characterization has been presented before and after the production of the acrylic acid (or methacrylic acid) block. The limited data suggest difficulties in obtaining a well-defined polyacid block while retaining a well-defined polydiene block because of the instability of the diene double bonds in strong acid environment and at high temperatures, which are usually utilized during hydrolysis of *tert*-butyl esters.¹⁵

We executed multiple attempts to cleave the *tert*-butyl groups of PtBuA under acid and base conditions without jeopardizing the double bonds of PI. All attempts were monitored by ¹H-NMR as a function of time. Hydrolysis by methanol/NaOH or KOH/dioxane/methanol, with or without 18-crown-6, took place extremely slowly.²² A system of *t*BuOK/THF/H₂O (H₂O in stoichiometric amounts) can be efficiently used to

cleave *tert*-butyl group within 1 week at room temperature,^{23, 24} however, macrophase separation occurred during purification by dialysis with precipitation of PI due to unknown reasons in the hydrolysis process (¹H-NMR showed PI profiles of precipitants). Trifluoroacetic acid and iodotrimethylsilane²⁵ can rapidly cleave the *tert*-butyl group under mild reaction conditions at room temperature over ~1–2 hours, but the double bonds of PI were significantly decreased due to the addition reaction between reagents.²⁶ All these methods complicate the di/tri- block copolymers by compromising the well-defined structure of PI.

Usually, refluxing HCl/dioxane/polymer solution is applied for hydrolysis of *PtBuA*, but it is known that hydrochlorination happens under this condition.¹⁵ However, by carefully controlling the molar ratio of HCl to *tBuA* (mol/mol, 4–6), reaction time (2–3 h), polymer concentration (~2 %, w/v, of *PtBuA*) we obtained PI-*b*-PAA with complete hydrolysis, and with the PI blocks retaining all their double bonds (no changes in ¹H-NMR). As shown in Figure 6.2 (c)(d), the characteristic peak of the *tert*-butyl group at 1.44 ppm disappeared after hydrolysis. Integration of vinyl peaks of PI relative to backbone methine proton ($-CH-$) of *PtBuA* (at 2.24ppm in CDCl₃)/PAA (at 2.50ppm in *d*-THF) proves that the double bonds of PI remain intact within the error of ¹H-NMR detection. The same strategy was applied to hydrolyze *PtBuA* in triblock copolymers of PS-*b*-PI-*b*-*PtBuA*, and ¹H-NMR showed the same peak ratios of aromatic, vinyl, methine protons of PS, PI, *PtBuA*/PAA respectively, before and after hydrolysis and purification. The molecular characteristics of precursors and hydrolyzed products are summarized in Table 6.1. Molecular weights of each block were determined by GPC equipped with light scattering detectors and by ¹H-NMR through calculation of component ratios.

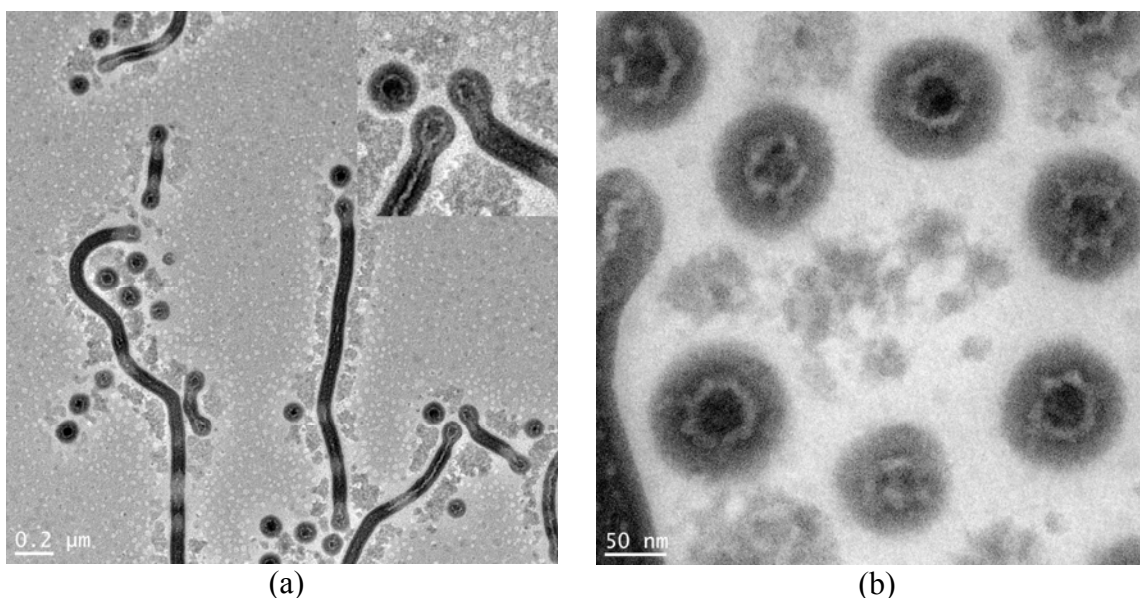
Table 6.1 Molecular Characteristics of Diblock and Triblock Copolymers^a

No.	PI			PI- <i>b</i> -PtBuA		I/ <i>t</i> BuA ^b	I/AA ^b
	<i>M</i> _{n,LLS}	<i>M</i> _{n,SEC}	PDI	<i>M</i> _n	PDI	(<i>t</i> -butyl ^c , -CH ^{-d})	(-CH ^{-d})
1	8.6×10 ³	1.2×10 ⁴	1.04	2.4×10 ⁴	1.15	0.95/1, 0.98/1	N/A
2				1.9×10 ⁴	1.09	1.36/1, 1.43/1	N/A
3	1.2×10 ⁴	1.6×10 ⁴	1.05	2.3×10 ⁴	1.19	1.58/1, 1.50/1	1.45/1
4				2.2×10 ⁴	1.12	1.99/1, 1.75/1	1.99/1
5	7.0×10 ³	9.6×10 ³	1.06	1.9×10 ⁴	1.28	0.95/1, 1.01/1	0.99/1
6				1.6×10 ⁴	1.19	1/0.73, 1/0.76	1/0.72
tri- block	PS		PS- <i>b</i> -PI		PS- <i>b</i> -PI- <i>b</i> -PtBuA		
	<i>M</i> _n	PDI	<i>M</i> _n	PDI	<i>M</i> _n	PDI	St/I/AA(HNMR)
	8.7×10 ³	1.06	4.3×10 ⁴	1.05	9.5×10 ⁴	1.08	1/4/4

^a M_n in unit of g/mol; ^b compositions by ¹H-NMR in unit of mol/mol; ^c I/*t*-BuA determined by vinyl and *tert*-butyl protons in ¹H-NMR; ^d I/*t*-BuA determined by vinyl and methine protons of PtBuA/PAA backbone in ¹H-NMR;

6.4.3 Self-assembly of PS-*b*-PI-*b*-PAA

PI-*b*-PAA diblock copolymers and PS-*b*-PI-*b*-PAA triblock copolymer dissolved in THF self-assembled into micelles with addition of water. Spherical micelles formed for diblock copolymers, as expected. For triblock copolymers, one of the objectives was to explore microphase separation behavior of “-PS-*b*-PI” in confinement and the impact of curvature of global aggregates (e.g. spheres, cylinders *etc.*) on the local/internal phase separation of the micelle core and *vice versa*. Structures formed by aqueous self-assembly are shown in Figure 6.3, observed *via* transmission electronic microscopy (TEM). The samples were stained by exposure to OsO₄ vapor for two hours, thus the PI phase appears dark and the PS phase appears white, while the PAA corona is invisible. As seen in these images, two types of global morphologies are observed for PS-*b*-PI-*b*-PAA micelles, worm-like cylinders and spheres. And the coexistence of the two morphologies cannot be excluded, as it can be seen that at the ends of the cylinders, spheres are being separated from or fused into cylinders (inset in Figure 6.3(a)). In these cylinders and spheres, multicompartment structures in the cores exist. Similar observations were reported previously for linear and star triblock terpolymers.^{8, 27-30} However, the fascinating phenomena reported here are the distinct local morphological structures (formed “-PS-*b*-PI block”) in the cores of cylindrical or spherical micelles, clearly visible due to staining with OsO₄. This exclusively happens in one homogenous system of narrowly dispersed triblock copolymers. To the best of our knowledge, such intriguing phase separating behaviors/structures under the same conditions have never been reported before. The worm-like cylinders show core-shell structures with white PS phases residing in the core



(a) (b)
Figure 6.3. TEM images of self-assembled structures of triblock terpolymers PS-*b*-PI-*b*-PAA in water.

center surrounded by dark PI phases (Figure 6.3(a)), which is consistent with the sequence of PAA-PI-PS where PAA as corona chains stabilize the hydrophobic “-PI-PS” cores in water. Surprisingly, for spheres, this internal morphology evolved to a dark shell-“white ring”-dark center structure (outside to inside) corresponding to a PI/PS/PI content sequence (Figure 6.3(a)), which is literally different from the molecular sequence of PAA-PI-PS (PAA has to be outside). Furthermore, high magnification TEM shows that “the ring” is not smooth. Instead it is “flower-like”, with a more complicated ultra-fine structure (Figure 6.3(b)).

The complex interplay among PS, PI, and PAA chains in aqueous media might cause complex self-assembly behavior in terms of global micellar morphology (e.g. spheres *vs* cylinders) and local PS/PI phase separation in the hydrophobic core of micelles. Both of global micelle structures and local phase separation in micellar cores are the results of compromises between local and global self-assembly. The interaction between them dictates the final morphologies of PS-*b*-PI-*b*-PAA in water. The coexistence of spheres and cylinders with different core morphologies may be at the minimum free energy of the whole system. The development of one level of self-assembly (global or local) will impact the other level of self-assembly (local or global). In other words, the global micellar structure transition (between sphere and cylinders) will impart the internal morphology change (between core shell and “ring-structure”) and vice versa. The interface between water/PAA and hydrophobic core is shelled by PI in both cases. And the PI chains folding away from the center of the core of spheres for the formation of the PS “ring” is probably due to the low T_g of the PI. More detailed investigations to address these issues are underway.

6.5 Conclusion

In summary, optimal conditions for hydrolyzing tertiary ester groups in the presence of polyisoprene were established. An efficient and scalable purification method for purifying the resulting PI-*b*-PAA and PS-*b*-PI-*b*-PAA block copolymers is also discussed. In principle, this method could be applied for any nonpolar-*b*-polar block copolymers based on the separation mechanism. Discoveries were made regarding micro phase separation in the confined spaces of micelles in aqueous media. Within the same PS-*b*-PI-*b*-PAA chains, cylindrical and spherical micelles have different ultra-fine structures in the micellar core consisting of PS and PI.

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Chapter 7 Polypeptide Grafted Hyaluronan: Synthesis and Characterization

Abstract

Poly(L-leucine) grafted hyaluronan (HA-g-PLeu) has been synthesized via a Michael addition reaction between primary amine terminated poly(L-leucine) and acrylate functionalized HA (TBAHA-acrylate). The precursor hyaluronan was first functionalized with acrylate groups by reaction with acryloyl chloride in the presence of triethylamine in N,N-dimethylformamide. ^1H NMR analysis of the resulting product indicated that an increase in the concentration of acryloylchloride with respect to hydroxyl groups on HA has only a moderate effect on functionalization efficiency, f . A precise control of stoichiometry was not achieved, which could be attributed to partial solubility of intermolecular aggregates and the hygroscopic nature of HA. Michael addition at high $[\text{PLeu-NH}_2]/[\text{acrylate}]_{\text{TBAHA}}$ ratios gave a molar grafting ratio of only 0.20 with respect to every repeating unit of HA, indicating upper limitation of grafting due to insolubility of the grafted HA-g-PLeu. Soluble HA-g-PLeu graft copolymers were obtained at lower grafting ratios (< 0.039) with $< 8.6\%$ mass of PLeu and were characterized thoroughly using light scattering, ^1H NMR, FT-IR and AFM techniques. Light scattering experiments showed that there is a strong hydrophobic interaction between PLeu chains, resulting in aggregates with segregated non-grafted HA segments. This yields local networks of aggregates as demonstrated by atomic force microscopy. Circular dichroism spectroscopy showed a β -sheet conformation for aggregates of poly(L-leucine).

7.1 Introduction

Hyaluronan (HA)¹ is a non-branched glycosaminoglycan consisting of disaccharide repeating units of D-glucuronic acid and N-acetyl-D-glucosamine. It exists as a gelatinous mass in human and animal tissues (vitreous body) and as a non-Newtonian viscous fluid in joint cavities (i.e., synovial fluid). It is also present as a gel in the extracellular matrix (ECMs) of cells, where it acts as a mechanical stress absorber. It is known that HA functions in many important biological processes including tissue hydration, diffusion of ions, nutrients and oxygen, supramolecular assembly of proteoglycans in the ECM,² cell differentiation and proliferation.³ Because of its unique structure and properties of biocompatibility and degradability, HA and its derivative hydrogels have broad applications in various technical and medical fields,⁴ for example, ophthalmologic surgery, cosmetics, tissue engineering and drug delivery.⁵⁻¹⁰

Over the past years, various modification strategies like crosslinking¹¹ and esterification¹² have been applied to hyaluronan in order to modify its mechanical and chemical properties to tailor materials for applications such as drug delivery, tissue engineering, joint lubrication, and cell adhesion and signaling.^{13, 14} Probably, the most widely used method for HA derivatization is coupling of water soluble hydrazides to the carboxylic acid groups of HA at pH 4.75, mediated by carbodiimides, for drug molecule attachment and hydrogel preparation.^{7, 9, 13, 15-19} The functionalization with alkanes using hydrazides method to improve the rheological property of HA has also been reported^{19c} Recently, several new methodologies were developed to prepare hyaluronan hydrogels by amidation, and photo-crosslinking.²⁰⁻²² Also, to improve hydrophobicity of HA for hot

molding or dissolution in organic solvents, alkanes, silylation and/or acylation have been explored.^{23,24}

Although various HA derivatives have been synthesized by a number of methods, only a few graft copolymers having HA as backbone have been reported using either “grafting onto” or “grafting from” strategies. For example, Ohya *et al.*^{10, 25} prepared HA-*graft*-poly(N-isopropylacrylamide) (HA-g-PNIPAM) copolymer by “grafting from” polymerization of NIPAM with “iniferter” dithiocarbamate functionalized HA. This graft copolymer served as a tissue adhesion prevention material and hemostatic aid.²⁵ Palumbo *et al.*²⁶ and Pravata *et al.*²⁷ synthesized poly(lactic acid) grafted copolymers of HA (HA-g-PLA) by means of “grafting onto” through esterification of primary hydroxyl groups on the HA backbone, yielding grafting ratios of 1.5-8.0 mole %. Detailed characterization was provided for the graft copolymers, showing hydrophobic interactions in aqueous solution.

Recently, well-defined poly(L-leucine) (PLeu) has been incorporated into block copolymers^{28, 29} because of its hydrophobic nature and ability to adopt secondary structure resulting in a rigid chain conformation. Additionally, Deming *et al.* reported that the incorporation of a PLeu segment into synthetic block copolypeptides significantly affected self-assembly^{30, 31} and the rheological properties of block polypeptides where rigid hydrogel formation was observed even at low concentrations.^{28, 32} Thus, PLeu grafted HA (HA-g-PLeu) may have advantages for mimicking natural processes occurring in proteins and thus have potential use in bio-medical applications. The combination of this polysaccharide HA and polypeptide apparently brings in the natural properties in terms of biocompatibility, which may generate a new class of

biomaterials that can be applied in tissue engineering as hydrogel and drug delivery system due to the strong hydrophobicity of PLeu rendering the possibility that hydrophobic drugs could be incorporated.

In this chapter, we describe the synthesis of a new PLeu grafted hyaluronan (HA-g-PLeu) *via* a Michael addition reaction between acrylate-functionalized HA (TBAHA-acrylate) and primary amine terminated PLeu (PLeu-NH₂). Detailed characterization of the products by ¹H-NMR spectroscopy, dynamic light scattering (DLS), static light scattering (SLS), thermogravimetric analysis (TGA), atomic force microscopy (AFM), and circular dichroism (CD) has been performed. The functionalization of HA with acryloyl chloride and the solubility of TBAHA-acrylate and the final graft copolymers are discussed. The conformation of PLeu in the graft copolymer and the morphology of the graft copolymer in aqueous solution are also discussed.

7.2 Experimental Section

7.2.1 Materials Sodium hyaluronates (NaHA) with molecular weights of 74×10^3 and 132×10^3 g/mol were purchased from Lifecore Biomedical (Low and medium molecular weight HA were chosen for justification of chemistry applied in our study. It is believed that MW wouldn't be an obstacle if high MW HA is desired). Two separate tetrabutyl ammonium derivatives of HA (TBAHA) were obtained by passing an aqueous solution of NaHA through a column of cation exchange resin (Dowex 50wx8-100, Aldrich), followed by titration with tetrabutylammonium hydroxide (TBAOH, 40 wt% aqueous solution, Fisher) to pH 8 ~ 9, and then lyophilized to obtain solid TBAHA. To clarify, TBAHA-A is derived from 132k NaHA and TBAHA-B is from 74K NaHA. N,N-Dimethylformamide (DMF, Aldrich, HPLC grade), acryloyl chloride (Aldrich, 98%), and

triethylamine (TEA, Aldrich, 99.5%) were stirred over calcium hydride and freshly distilled prior to use. PLeu was obtained from Prof. Nikos Hadjichristidis' laboratory (University of Athens, Greece), and was prepared by ring opening polymerization of the monomer L-leucine *N*-carboxyanhydride ($M_n = 1500$ g/mol, ^1H NMR in *d*-TFA) *via* high vacuum techniques.³³

7.2.2 Synthesis of graft copolymer

7.2.2.1 Functionalization of TBAHA (TBAHA-acrylate)

In a typical experiment, a three-neck round bottom flask dried at 150 °C was equipped with inlet and outlet for high purity nitrogen and a magnetic stirring bar. Before sealing the flask with rubber septa, 0.87 g (4.3×10^{-6} moles) of tetrabutylammonium hyaluronate (TBAHA-A) (dried overnight under vacuum at 40 – 50 °C), dry DMF (100 ml) and TEA (3.2 ml) were added to the flask under constant nitrogen purge to form a clear solution. Next, 1.2 ml of acryloyl chloride solution (0.092 M in dry DMF or dry THF) was added drop-wise *via* syringe into the flask, which was subsequently immersed in a water bath. The mixture was left to stir overnight at room temperature. The acrylate-functionalized TBAHA (TBAHA-acrylate) was recovered by precipitating the reaction mixture into a large excess of diethyl ether, and further purified by rinsing with a large excess of ethyl ether several times, and finally dried in a vacuum oven at room temperature (batch process). In addition, a one-pot synthesis to obtain directly the graft copolymer was also developed. In this case, the reaction solution was directly used in the Michael addition reaction for synthesis of the graft copolymer as described below. Small amounts of solution were sampled for characterization, purified by direct dialysis against water, and lyophilized to yield 0.064 g of TBAHA-acrylate.

7.2.2.2 Michael addition to Prepare Graft Bioconjugates of HA and Poly(L-leucine)

Grafting of PLeu onto the backbone of TBAHA was performed using a Michael addition reaction between TBAHA-acrylate and PLeu-NH₂ (0.15 g) in the presence of TEA at room temperature. After the contents were combined, the reaction flask was covered with aluminum foil and stirred under N₂ atmosphere for 1 week. Excess/unreacted PLeu was removed by ultracentrifugation ($1.1\sim 1.4 \times 10^4$ rpm) at 25 °C to obtain a clear, transparent solution, followed by distillation of DMF under reduced pressure at mild temperatures. The viscous residue was again dissolved in water, neutralized with TBAOH and dialyzed against deionized water for three days (molecular weight cutoff of membrane: 3500 g/ml). The dialyzed solution was adjusted to pH ~ 7.0 before the graft copolymer was lyophilized to recover the white polymer solid (0.713 g, yield 74.6 %). An alternate way to purify the graft copolymer was to precipitate the reaction solution into a large excess of ethyl ether. The resulting solid was rinsed several times with ethyl ether, dissolved in water, neutralized, and dialyzed. Unreacted PLeu was removed by centrifugation ($1.1 \sim 1.4 \times 10^4$ rpm). Finally, the copolymer was recovered as a white solid after lyophilization.

Converting the tetrabutylammonium salt to the sodium salt was performed by dialyzing the TBA form of the graft copolymer (0.0323g polymer in 20ml water) against 0.1M NaCl solution for 3 days, and then against deionized water for another 3 days during which the NaCl solution and the deionized water was changed twice daily. The polymer solid was obtained by freeze-drying (0.02 g).

7.3 Characterization Methods

^1H -NMR spectroscopy was performed on Varian Mercury 300 MHz spectrometer with deuterated trifluoroacetic acid (TFA-*d*), D_2O , and dimethylsulfoxide (DMSO-*d*₆) as solvents. Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) was recorded on Varian Resolutions Pro instrument and data were averaged over 64 scans. Samples were prepared by placing several drops of aqueous solution onto aluminum foil and allowing it to dry in a hood at room temperature overnight. The thermal stabilities of TBAHA, PLeu, and the graft copolymer, as well as an estimation of PLeu composition in the graft polymer, were evaluated by TGA using a TA Q-50 instrument (temperature range: room temperature to 900 °C), with a 10 °C/min heating rate under nitrogen atmosphere.

Dynamic light scattering (DLS) and static light scattering (SLS) were used to characterize the conformations of NaHA, TBAHA, TBAHA-acrylate and the graft copolymer in 0.1 M NaCl aqueous solution. The hydrodynamic diameters of the polymers at 25 °C were measured on a PD Expert instrument (Precision Detectors) at a scattering angle of 95°. The diameters and polydispersity indices (PDIs) were averaged over 10 repetitive measurements.

Static light scattering (SLS) experiments were conducted using a DAWN[®] EOS[™] instrument having 18 scattering angles ranging from 13° to 147° (Wyatt Technology Corp., Santa Barbara, CA) with an operating wavelength $\lambda = 695$ nm and a He-Ne laser source. The micro-batch mode was used with a normalized scintillation vial (25 mL) where dilutions (for graft copolymer: 2.17×10^{-5} g/ml – 7.61×10^{-5} g/ml) were made from a concentrated stock solution (0.1M NaCl aqueous solution) at 25 °C. The polymer solutions and solvent for SLS were filtered with 0.45 and 0.2 μm Millipore nylon

membranes three times to remove dust. The specific refractive index increments (dn/dc) were determined using an Optilab DSP Interferometric Refractometer (Wyatt Technology Corp.) at $\lambda = 695$ nm, and the dn/dc values in 0.1M NaCl aqueous solution are 0.142, 0.152, 0.148, 0.139 mL/g for NaHA(132K), TBAHA-A, TBAHA-acrylate (A-acrylate) and graft copolymer (A-5), respectively. ASTRA for Windows software was used to collect and process data.

Atomic force microscopy (AFM) experiments were performed using a Nanoscope IIIa Microscope with Multimode Controller (Veeco Instrument) at ambient temperature and humidity. The tapping mode was employed with an antimony-doped Si tip (radius < 10 nm) at a line scanning frequency of 0.5 or 1 Hz. AFM samples were prepared as follows: Mica was pre-hydrated after cleavage at room temperature and humidity overnight. A droplet (~30 μ l) of solution (~ 1-10 μ g/ml) was deposited on the mica surface, allowed to sit for 2 minutes to allow polymer to be adsorbed, and then dried by gently blowing dry N₂ over the sample for 3-5min. Scanning was carried out immediately after the mica surface appeared dry.

Circular dichroism (CD) was used to investigate the secondary structure of poly(L-leucine) in the graft copolymers (A-5, B1) in aqueous solution. CD spectra were recorded on a Model 202, AVIV Instruments Inc. spectrometer under a nitrogen atmosphere. Experiments were performed in a quartz cell with a path length of 0.1 cm, over a range of 190 – 250 nm at 25 °C, and the data were collected and averaged over two scans. The polymer solutions used for CD were prepared in deionized water with a concentration of 0.2 – 0.5 mg/ml.

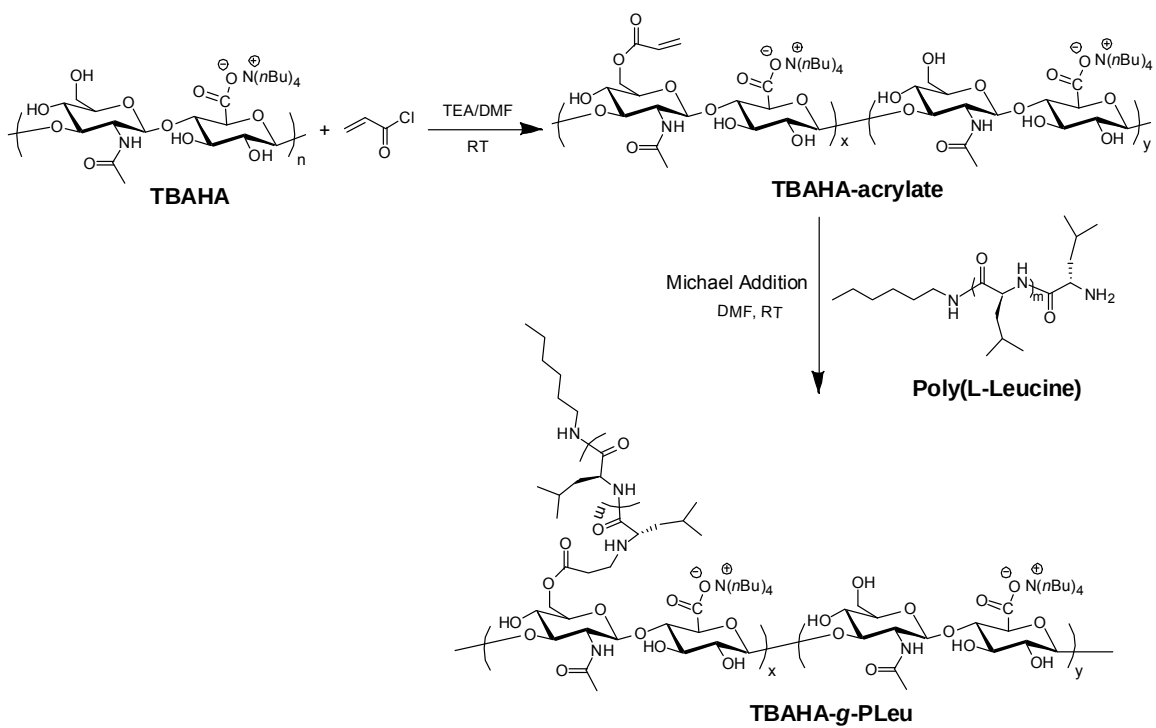
7.4 Results and Discussion

7.4.1 Functionalization of HA with acryloyl chloride (TBAHA-acrylate):

Sodium hyaluronate (NaHA) is a highly hydrophilic polysaccharide, which is soluble in water, but very difficult to dissolve in common organic solvents such as THF, DMF and DMSO. Improving solubility of NaHA in organic solvents is very important for many functionalization reactions. To this end, the dissolution of NaHA in polar organic solvents was enhanced through transformation of the metal counterion (Na^+) into the non-metal tetrabutyl ammonium (Bu_4N^+) counterion. This was accomplished by conversion of NaHA first to hyaluronic acid using ion-exchange resin and subsequent neutralization with tetrabutylammonium hydroxide (TBAOH). This method has been extensively applied in the past for modification of HA.^{10, 22, 24, 25, 34} The obtained tetrabutylammonium hyaluronate (TBAHA) exhibited improved solubility in DMF (~0.01 g/mL) compared to NaHA. In order to graft PLeu onto the back-bone of TBAHA, the primary hydroxyl groups of TBAHA were partially functionalized with acrylate. Predetermined amounts of acryloyl chloride were reacted with TBAHA in the presence of triethylamine in DMF, as shown in Scheme 7.1.

The primary hydroxyl group is known to be more reactive than the secondary hydroxyl groups on HA, thus esterification with acryloyl chloride most likely occurs at these sites. After the esterification, the acrylate functionalized TBAHA was recovered by precipitation in excess ether and the product was thoroughly washed in ether to remove TEA and hydrolyzed analog of acryloyl chloride, acrylic acid. Typical ^1H NMR spectra of TBAHA and acrylate functionalized TBAHA (TBAHA-acrylate) are shown in Figure 7.1 (Table 7.1, run 2).

Scheme 7.1: Synthesis of functionalized TBAHA (TBAHA-acrylate) and HA-g-poly(L-leucine)



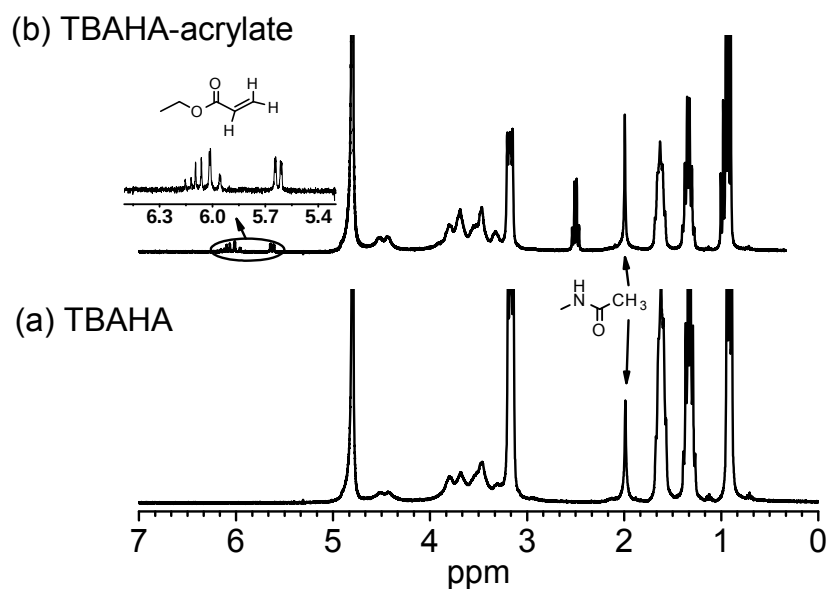


Figure 7.1: ^1H NMR spectra of (a) TBAHA: tetrabutylammonium hydroxide neutralized product of hyaluronic acid, in D_2O (b) TBAHA-acrylate (run 2): functionalized TBAHA by reacting TBAHA with acryloyl chloride, purified *via* dialysis against deionized water for three days, in D_2O with NaOH 0.2 mg/ml.

As shown in Figure 7.1(b), the vinyl protons observed between 5.4 and 6.3 ppm are attributed to the acrylate moiety on the HA backbone, indicating successful functionalization. The percentage of acrylate attached to the backbone was calculated by integration of vinyl protons and methyl protons of the acetamide group at 2.0 ppm in the TBAHA. The material shown in Figure 7.1(b) has a functionality approximately 0.5, which means that for every two disaccharide repeating units, there is one α , β -unsaturated carbonyl group attached. As the functionality was calculated on the basis of acetamide groups, attempt to identify the actual site of the esterification among the four hydroxyl groups was not made.

In order to control the degree of functionalization of TBAHA, varying amounts of acryloyl chloride were reacted with TBAHA as summarized in Table 7.1. The functionality increases as the ratio of acryloyl chloride and hydroxyl groups on HA backbone increases. However, it is difficult to achieve perfect stoichiometric control due to the highly hygroscopic nature of the reagents, even though TBAHA was pre-dried in vacuum.¹ The functionalization efficiency, f was determined using ^1H NMR spectroscopy as described previously. It was observed that at similar f values, the samples from two independent reactions showed distinctly different solubility characteristics (Table 7.1, run 3 and 4). Sample 4 was only swollen/gel-like in *d*-DMSO. We believe that this difference could result from specific conformation of HA and the position of esterification. Although the solubility of TBAHA-acrylate is expected to be much better than its precursor (TBAHA) in organic

Table 7.1 Reaction of TBAHA with acryloyl chloride in DMF at room temperature.^a

run	[TBAHA] 10 ⁻⁴ mol/L ^b	[Acryloyl-Cl] /[TBAHA]	[Acryloyl-Cl] /[⁻ OH ^c] _{TBAHA}	<i>f</i> ^d
1	0.42	1323	1	0.84 ^e
2	1.06	559	0.42	0.50 ^f
3	0.72	331	0.25	0.38 ^e
4	0.47	304	0.23	0.38 ^f

(a) ion-exchange and esterification of all reactions were performed step-by-step, while reaction 4 was performed in a one-pot process. The yields were close to 50 %.

(b) concentrations of TBAHA derived from NaHA ($M_w = 1.32 \times 10^5$ g/mol)

(c) every disaccharide repeating unit of TBAHA has 4 hydroxyl groups

(d) functionality of TBAHA : acrylate group per disaccharide repeating unit

(e) ¹H NMR in *d*₆-DMSO

(f) ¹H NMR in D₂O/NaOH

solvents like DMSO due to the incorporation of hydrophobic functionality ($\text{CH}_2=\text{CHCO}-$), solubility apparently depends on the nature of solvent and the aggregation behavior of hydrophobically modified polysaccharides. The results suggest that the dissolution of TBAHA-acrylate is in equilibrium with different types of aggregates in a particular solvent. The nature of the solvent determines the extent of dissolution. For instance, the TBAHA-acrylate forms a viscous gel-like solution in *d*-DMSO or D_2O at high functionalization (Table 7.1, run 2), but the solution began to readily flow with the addition of tetrabutylammonium fluoride or sodium chloride. The f for sample 2 is 1.00 in the presence of salts in DMSO. The same sample in D_2O in the presence of NaOH or *d*-trifluoroacetic acid completely dissolved forming a clear, transparent solution with functionalities of 0.5 and 0.82, respectively. The gel formation in DMSO in the absence of salt is possibly caused by inter-chain hydrogen bonding due to complexation of the acid hydrogen with TEA (detected as signals at 2.5 and 0.9 ppm in Figure 7.1b), which are broken down by an ionic strength change upon addition of salt or base, although the dialyzed TBAHA-acrylate had been neutralized with TBAOH.

These solubility differences and the presence of different intermolecular aggregates limit accurate quantification of the functionalization.³⁵ All of these observations suggest that the conformation change of HA leading to complex solubility is due to incorporation of acrylate functional groups into the HA chains, which is further confirmed by light scattering experiments in the following discussion.

7.4.2 Michael addition to form graft conjugates:

The graft copolymer PLeu-*g*-HA was synthesized by Michael addition reaction between the telechelic poly(L-leucine) bearing primary amine terminal functional group and the

TBAHA-acrylate. Since HA degrades readily under strongly basic or acidic conditions and because the Michael addition reaction between a primary amine and vinyl (acrylate type) group can be conducted under mild conditions, the Michael addition reaction for grafting poly(L-leucine)-NH₂ (PLEu-NH₂) onto TBAHA-acrylate was conducted in DMF in the presence of TEA at room temperature. Similar mild conditions for Michael addition using aprotic or protic solvents at relatively low temperatures have been extensively reported in the literature.^{16, 36-39}

Accordingly, different concentrations of PLEu-NH₂ with respect to acrylate functionalized TBAHA-acrylate were used for the reaction. Table 7.2 summarizes the reaction conditions and results of the two types of grafting reactions: batch and one-pot synthesis, where the difference is whether TBAHA-acrylate is separated/purified (batch process, A1-2, A4) or used directly (one-pot process, A3, A5-6, B1-2). It is clear that the one pot process gives a much higher yield due to losses during the purification step in the batch process. Precipitating the final reaction solution into ether to recover polymer may cause loss of HA-oligomers and losses may occur during centrifugation of aqueous dialyzed solution.

The highest acrylate containing TBAHA-acrylate (0.013 mol/L) at high [PLEu-NH₂]/[acrylate]_{TBAHA} gave molar grafting ratio of 0.2 indicating a limiting grafting density for the grafting reaction (Table 7.2, A1). This could be attributed to the insolubility of the modified TBAHA-acrylate as the 0.2 molar ratio PLEu grafted product is not soluble in water or DMF after the purification process. Thus, the reaction ratio of acrylate to hydroxyl groups was reduced and the amount of PLue-NH₂ used in the reaction was also controlled in order to have soluble product. At low ratio of

[acryloylchloride] / [-OH]_{TBAHA} the acrylate signals could not be detected in the ¹H NMR spectra due to low concentration. However, the presence of α, β-unsaturated ester group in the product (TBAHA-acrylate) could be identified in the FT-IR spectrum at 1738 cm⁻¹.

Although quantifying the functionality of HA modification by ¹H NMR spectroscopy is complicated, a moderate control of the functionality can be achieved through varying the ratio of acryloyl chloride/TBAHA as shown in Table 7.1. The graft copolymer synthesized using TBAHA-acrylate containing low concentration of acrylate groups produced soluble graft copolymer. Typical ¹H NMR spectra of purified graft copolymer (A5, Table 7.2), TBAHA (A), and PLeu in *d*-TFA are shown in Figure 7.2, illustrating the successful attachment of PLeu chains onto HA. Comparison of the spectra in Figure 7.2 shows that the characteristic chemical shifts of PLeu and TBAHA can be differentiated from each other, and both are observed in the final graft copolymer: 4.6 ppm (-CO-CH-NH-, PLeu), 0.95-0.82 ppm ((CH₃)₂-CH-, PLeu) and at 2.25 ppm (-NH-CO-CH₃, TBAHA). The grafting ratio was determined from these signals according to their peak areas ((CH₃)₂-CH-in PLeu, -NH-CO-CH₃ in TBAHA), which is defined as number of PLeu chains in every disaccharide repeating unit in TBAHA backbone. For instance, the grafting efficiency, *f*' determined by the grafting mole ratio shown in Figure 7.2(c) (Table 7.2, A5) is 0.037. This value corresponds to 3 or 4 poly(L-leucine) chains per every 100 disaccharide repeating units. There are no differences found in grafting efficiency when two different molecular weights of precursor HA were used in the study.

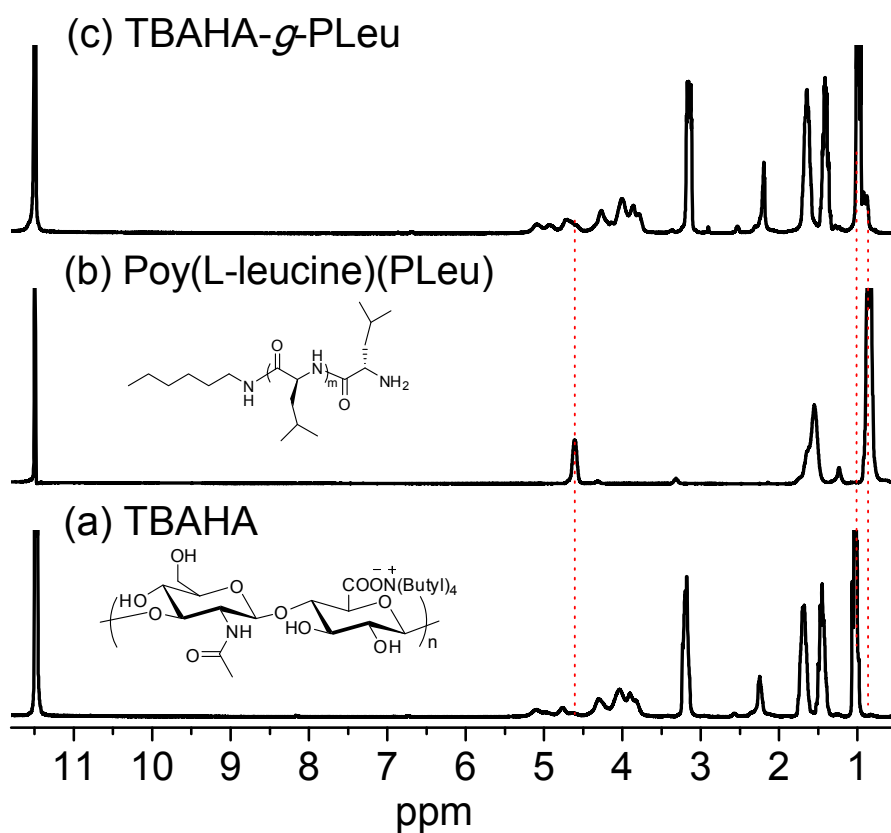


Figure 7.2: ^1H NMR spectra in *d*-TFA of (a) TBAHA: tetrabutylammonium hydroxide neutralized product of hyaluronic acid (b) PLeu (c) HA-*g*-PLeu: Michael addition reaction product, purified by centrifugation in DMF and dialysis against deionized water.

Although the reaction solution was transparent after centrifugation to remove insoluble PLeu, a very pronounced Tyndall effect was observed indicating the presence of aggregates even in the DMF reaction medium. Thus, the graft copolymer is less soluble in water than in DMF due to the hydrophobic nature of PLeu. At high grafting ratio, the grafted polymer (large aggregates) in water could be removed by ultra-centrifugation, thus leading to low yield. This would also explain the differences observed for entries B1 and B2 (Table 7.2), where the final yield decreased with increasing grafting ratio when using DMF and the same purification conditions. This suggests that there exists an upper limit of solubility in DMF for the final graft copolymer due to precipitation during the reaction. Nevertheless, it is shown that the grafting ratio can be moderately controlled by $[\text{acryloyl chloride}]/[-\text{OH}]_{\text{TBAHA}}$ and/or the $[\text{PLeu-NH}_2]/[\text{acrylate}]_{\text{TBAHA}}$. The highest grafting ratio for a water-soluble product appears to be nominally less than 4.3 molar %, which is supported by the fact that product B2 (Table 7.2) is not soluble in water. It was reported that a grafting ratio of 7.8 molar % for poly(lactic acid) grafted HA resulted in a dramatic decrease in water solubility,²⁶ which supports this current work since PLeu is significantly more hydrophobic than poly(lactic acid). In fact, the ^1H NMR spectrum of the graft copolymer in D_2O showed no signals attributed to PLeu, suggesting formation of solid aggregates of PLeu. In theory, the formation of such aggregates would be driven by strong hydrophobic interactions between PLeu chains, which is substantiated by light scattering experiments and AFM as discussed in the following.

Attempts were made to convert the TBA cation back to sodium by dialysis of aqueous solutions of TBAHA-g-PLeu against NaCl solution, followed

Table 7.2 Reaction Conditions and Results for Synthesis of Graft Copolymer HA-g-PLeu

No .	[acryloylchloride] /[-OH] _{TBAHA}	[acrylate] ^a mol/L	[PLeu-NH ₂] /[acrylate] _{TBAHA}	Yield (%)	grafting ratio of TBAHA-g-PLeu ^e	
					molar ratio, f	mass %
A1	1	0.013 ^b	1.79 ^c	10	0.200	32.7
A2	0.25	0.011 ^b	1.24 ^c	NA	0.110	21.1
A3	0.23	0.014	0.12 ^d	46	0.013	3.1
A4	0.42	0.006 ^b	1.13 ^c	33	0.037	8.2
A5	0.02	0.001	1.00 ^d	74.6	0.037	8.2
A6	0.04	0.002	0.69 ^d	75.6	0.039	8.6
B1	0.08	0.004	0.21 ^d	79.3	0.015	3.5
B2	0.26	0.014	0.15 ^d	67.5	0.043	9.5

*, A1, A2 and A4 are batch process; A3, A5, A6 and B1, B2 are one-pot process. The precursors HA with M_w 132K for A and with M_w 74K for B were used.

(a) concentration of acrylate group of TBAHA in system, while it is concentration of acryloyl chloride for one-pot process

(b) Calculated using the relationship: $\frac{m_{\text{TBAHA-acrylate}}}{M_n} \times f \times \frac{1}{V}$, in which M_n is molecular weight of repeating unit of TBAHA-acrylate, is assumed

to be: $617(\text{TBAHA}) + 55(\text{CH}_2=\text{CHCO}) \times f$ (functionality of TBAHA-acrylate)

(c) ratio of molar of PLeu-NH₂ and acrylate group of TBAHA in batch process

(d) ratio of molar of PLeu-NH₂ and acryloyl chloride added into the solution in one-pot process.

(e) f , number of PLeu chains for every disaccharide repeating unit as determined by ¹H NMR in *d*-TFA and mass % = $f \times 1500 / (f \times 1500 + 617)$.

by dialysis against water to remove excess NaCl. The ^1H NMR spectrum of the graft copolymer product in *d*-TFA showed characteristic signals from PLeu ($((\underline{\text{CH}}_3)_2\text{-CH-}$, 0.96 ppm; $(\text{CH}_3)_2\text{-CH-CH}_2\text{-}$, 1.67 ppm) chains without residual signals corresponding to TBA ($-\underline{\text{CH}}_2\text{CH}_2\text{CH}_2\text{CH}_3$, 3.18 ppm). Thus, it is possible to exchange the counter ion, if desired.

7.4.3 Light Scattering Characterization

The grafting of hydrophobic polypeptide onto HA is expected to impart conformational changes in terms of hydrodynamic volume and radius of gyration, which were measured by light scattering experiments. A typical Zimm plot for graft copolymer (A5) in 0.1 M NaCl aqueous solution is shown in Figure 7.3.

Light scattering data (weight-average molecular weight (M_w), hydrodynamic radius (R_H), radius of gyration (R_g), and second virial coefficient (A_2) for PLeu-g-HA graft copolymer (Entry A5, Table 7.2), as well as precursors TBAHA, TBAHA-acrylate, and parent NaHA in 0.1 M NaCl aqueous solution, are summarized in Table 7.3. As shown in Table 7.3, the molecular weight of TBAHA decreases from that of the parent NaHA possibly due to degradation of HA under acidic conditions⁹ during the ion exchange process, while R_H and R_g do not change significantly because of incorporation of the bulky tetrabutylammonium cation. The values reported in Table 7.3 for R_H and R_g of NaHA are very close to those previously reported for $M_w = 160$ K NaHA⁴⁰ and indicate a random coil conformation in 0.1M NaCl.⁴¹ It is observed that the

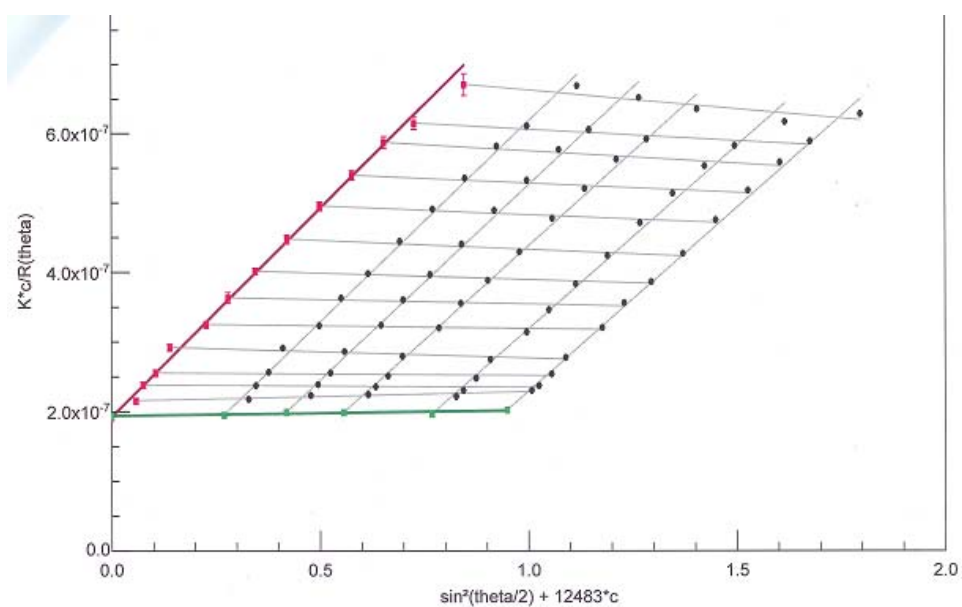


Figure 7.3: Static light scattering Zimm plot of PLeu-g-HA graft copolymer (Entry A5, Table 7.2) in 0.1M NaCl aqueous solution at 25 °C.

Table 7.3 Dilute Solution Properties of NaHA, TBAHA, TBAHA-acrylate, TBAHA-g-PLeu in 0.1 M NaCl Aqueous Solution

Sample	$M_w/$ ($\times 10^5$ g/mol)	R_H (nm)	R_g (nm)	2nd virial coefficient, A_2 ($\times 10^{-3}$ mol mL/g ²)
NaHA (132K)	1.332	15.9	35.7 $\pm$ 1.6	4.02 $\pm$ 0.12
TBAHA (A)	0.874	18.9	32.6 $\pm$ 1.7	2.81 $\pm$ 0.06
TBAHA-acrylate	8.946	49.8	80.4 $\pm$ 1.0	-0.24 $\pm$ 0.04
TBAHA-g-PLeu (A5)	51.740	155.0	125.7 $\pm$ 1.5	0.05 $\pm$ 0.04
Poly(L-Leucine)	0.015	NA	NA	NA

functionalization of HA affects the polymer conformation, the M_w of TBAHA-acrylate being 10-fold larger than that of its precursor TBAHA. Similarly, R_H increased to 49.8 from 18.9 nm, and R_g increased to 80.4 from 32.6 nm further confirming a substantial change in conformation. The second virial coefficient is a parameter characterizing the solubility of a polymer in a particular solvent, or, more formally, it represents the thermodynamic interactions between polymer and solvent. In 0.1 M NaCl aqueous solution, A_2 decreases upon transitioning from NaHA to TBAHA to graft copolymer, and even becomes negative for TBAHA-acrylate. This indicates that the solubility become poorer in water as the grafting process proceeds due to the incorporation of hydrophobic groups.

These results confirm the association behavior of TBAHA-acrylate chains, which could be prevented by dilution of the polymer solution, as demonstrated by DLS experiments in Figure 7.4. There is a concentration dependence of R_H of TBAHA-acrylate, experiencing an abrupt R_H decrease when solution was diluted below ~ 0.07 mg/ml. This was not the case for the graft copolymer, where R_H remained relatively constant with variation of concentration (Figure 7.4, TBAHA-g-PLeu). From ^1H NMR spectroscopy and TGA (PLeu mass percent is about 7.3 %) measurements, it is estimated that on average there are 12 PLeu chains attached onto every HA chain, assuming there was no degradation during the reaction. The molecular weight of HA-g-PLeu in sodium salt form is calculated to be: $12 \times 1500 + 132000 = 1.5 \times 10^5$ g/mol, which is substantially lower than the value measured by light scattering. Apparently, there exists strong association between the grafted chains, which could

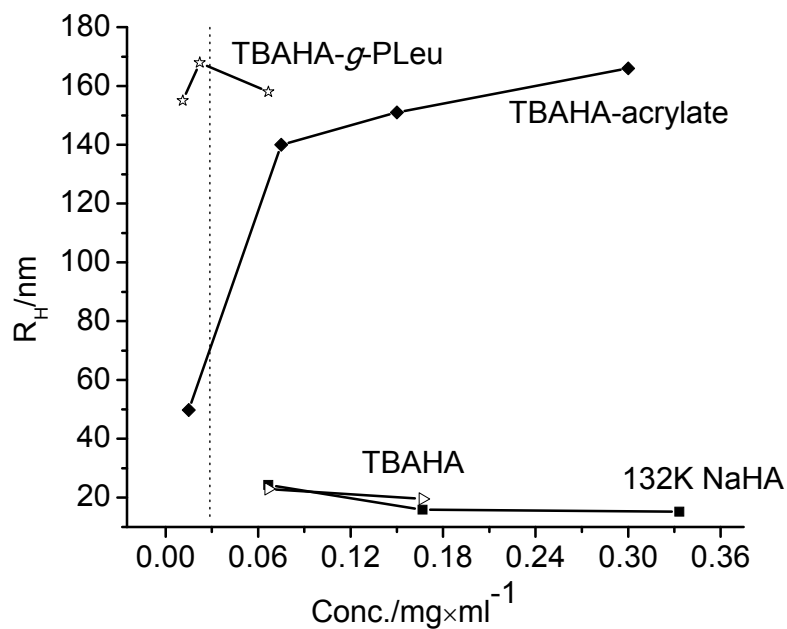


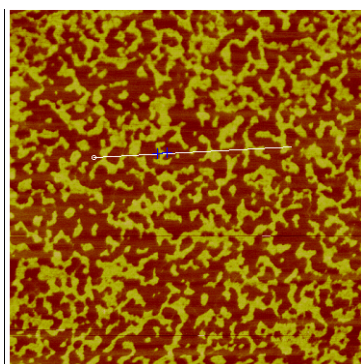
Figure 7.4: Concentration dependence of R_H determined by dynamic light scattering experiments for NaHA, TBAHA, TBAHA-acrylate, and HA-g-PLeu in reaction A5 (Table 7.2) in 0.1M NaCl aqueous solution at 25 °C

account for such a dramatic difference in apparent molecular weight due to the amphiphilic nature of the graft copolymer. This association or aggregation is observed even though the light scattering experiments were carried out at very low concentrations (the lowest concentration was $\sim 20 \mu\text{g/ml}$). We would expect the critical micelle concentration (CMC) for our graft copolymer in water to be much lower than this concentration. It is possible that no unimers of the PLeu-g-HA graft copolymer exist in aqueous solution due to the strong hydrophobic interactions between the PLeu chains.

7.4.4 Atomic force microscopy (AFM) and Circular dichroism (CD):

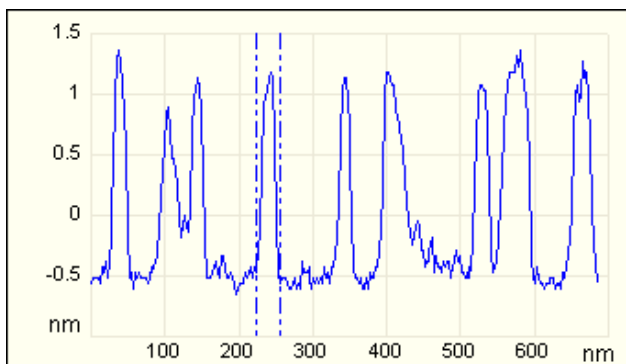
Atomic force microscopy (AFM) in tapping mode was previously used to visualize a single HA chain, taking advantage of its interaction with a mica surface, in order to investigate the conformation of HA.^{42, 43} We used AFM to study the conformation and morphology of TBAHA-g-PLeu. Figures 7.5 and 7.6 show the AFM height images obtained in tapping mode for TBAHA (A) and the graft copolymer (A5), respectively.

It was reported that a single HA chain (sodium form) can be absorbed either as an extended chain or in a condensed conformation on a freshly cleaved mica surface, depending on sample preparation conditions.⁴² In this study, a strongly condensed conformation of single TBAHA chains was observed on pre-hydrated mica (Figure 7.5a), with apparent heights of $\sim 1.6 \text{ nm}$ and widths of $\sim 29 \text{ nm}$ (averaged in Figure 7.5b); the previously reported values for NaHA were 0.8 nm and 20 nm for height and width, respectively.⁴² This difference may be attributed to the bulky TBA cation, while the more contracted conformation could suggest weaker adsorption of TBAHA on the mica surface. From SLS and DLS results, it was found that aggregation occurred in aqueous solution with or without salt for the graft copolymer due to its strongly amphiphilic



1.2 μm $\times$ 1.2 μm

(a)



(b)

Figure 7.5: (a) AFM height image for TBAHA (A) on pre-hydrated mica under tapping mode; (b) Section analysis of arbitrary part of (a).

character. The AFM images of TBAHA-g-PLeu in solution without salt could be visualized as aggregates on mica surface (Figure 7.6). It is interesting to notice that the independent aggregates appear to be connected to each other by partially non-grafted and extended HA chains that form a local network. The connecting chain is about 0.57 nm in height and about 13.1 nm in width, which is in good agreement with that expected for an extended HA chain^{42,43}.

For the aggregates, their size is about 1.3 nm in height and roughly 40.7 nm to 75.6 nm in width (calculation based on averaging area in Figure 7.6b) suggesting each aggregate self-assembles into a long, thin sheet. However, the width is likely overestimated due to convolution of the scanning tip of AFM.⁴⁴ One explanation for a fully extended chain conformation is absorption of aggregates on the mica surface and the so-called “combing force” during sample preparation.⁴² It is plausible that the surface interaction of TBAHA-g-PLeu and hydration characteristics of the mica surface could lead to the formation of such a networked morphology. The aggregated regions are not correlated to the amount of PLeu grafted on the chain which indicates that the intermolecular aggregates are induced by hydrophobic modification of HA. The aggregates formed in aqueous media clearly reflect self-assembly driven by the hydrophobic polypeptide grafted onto the HA backbone.

As mentioned previously, the individual aggregates are more or less like thin sheets, a typical structure for self-assembled polypeptides that is governed by the ability to adopt secondary structure. To this end, circular dichroism spectroscopy (CD) was used to examine the secondary structure of PLeu in the aggregates. In Figure 7.7, we

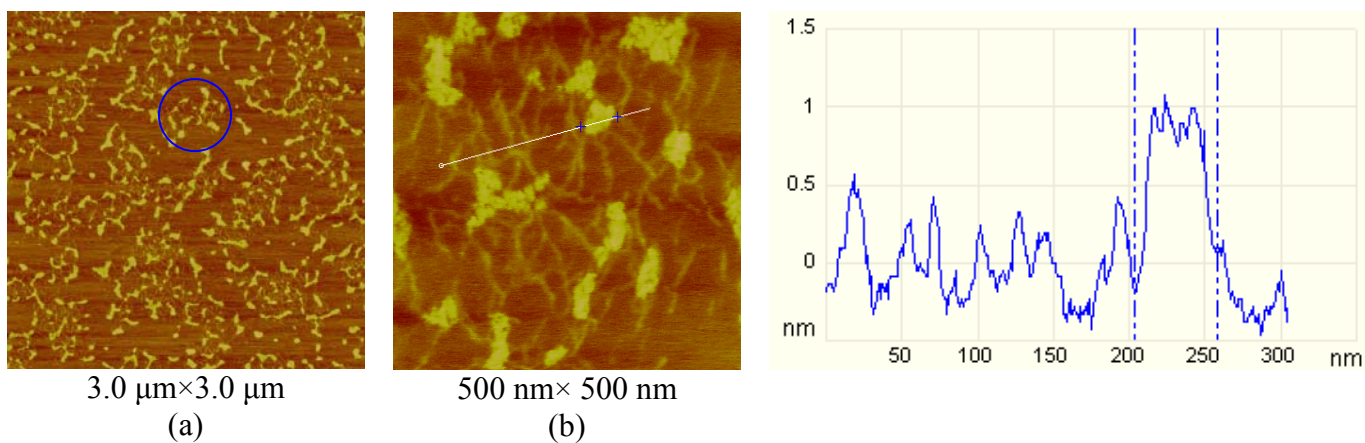


Figure 7.6: (a) AFM height images for graft copolymer (A5) on pre-hydrated mica under tapping mode, circle showing independent local network; (b) Zoom in scanning of (a); (c) Section analysis of arbitrary part of (b), white line.

compare the CD spectra of TBAHA and PLeu-g-HA from two different HA precursors (A and B). Since TBAHA itself would give a CD signal mainly due to the $n\text{-}\pi^*$ transition of the amide chromophore of acetamido group, which minimizes the CD band at about 210 nm,⁴⁵ (as shown in Figure 7.7(A and B) where molar ellipticity $[\theta]$ of TBAHA was calculated with disaccharide repeating unit as residuals), this effect has to be taken into account when analyzing the CD spectra of our graft copolymers. The molar ellipticity of PLeu was obtained by normalization of TBAHA concentration and subtraction of TBAHA spectra from graft copolymer spectra (Figure 7.7: PLeu in B1 and A5). We used this simple subtraction to obtain an approximate analysis of the secondary structure of the polypeptide, although this could be problematic⁴⁶ because of potential conformational changes impacting the CD spectra of TBAHA through interaction with polypeptides. From Figure 7.7, PLeu in both graft copolymers, A5 and B1, show a single negative maximum at about 215 nm and a positive maximum at 197 nm, characteristics of a typical β -sheet structure, but with different $[\theta]$ for A5 and B1. As well known, the percentage of each unique conformation of protein can be calculated by deconvoluting CD spectra into the three basic secondary structures (coil, sheet and helix)⁴⁷. Thus, the percentages of each conformation of PLeu in each graft copolymer were calculated and are summarized in Table 7.4.

It appears that in the sample A5, the β -sheet is the main secondary structure, with a significant presence of coils. The morphology observed in AFM possibly contains stacks of β -sheets of PLeu²⁹ as well as intermolecular aggregates with partially non-grafted HA segments. In the B1 graft copolymer, PLeu mainly adopts the β -sheet conformation.

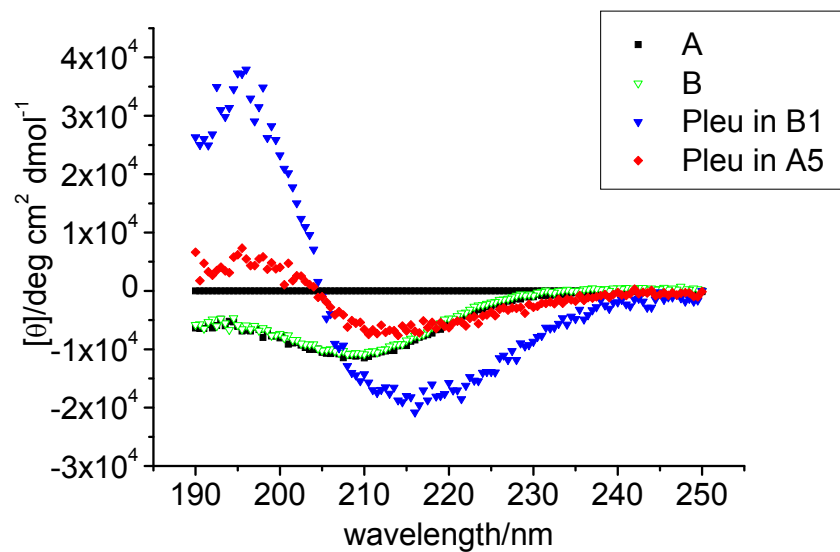


Figure 7.7: Circular dichroism spectra of TBAHA (A from M_w 132K and B from 74K NaHA), poly(L-leucine) in graft copolymer(signal obtained by subtracting TBAHA from graft copolymer, PLeu in graft copolymers in reaction B1 and A5, Table 7.2)

Table 7.4 Secondary Structure Characterization of Poly(L-leucine) in graft copolymer TBAHA-g-PLeu

Conformation (%) Sample	helix	sheet	coil
A5	0	67.23	32.77
B1	3.55	91.18	5.27

Compared to traditional graft copolymers such as HA-g-PNIPAM or HA-g-PLA as mentioned in Introduction, although all of them could potentially form hydrogels due to the hydrophobic interaction between grafted braches, none of them had been visioned with aggregates and secondary structure (β -sheet) which had been demonstrated in our lab with PLeu grafted HA. And also our graft copolymers provide a possibility of specific interaction between body protein and poly(L-Leucine), which might be of importance to the application of this material. A detailed rheological characterization of a peptide-modified hyaluronic acid (HA) derivative is under way in our laboratory. The solution morphology of this PLeu modified HA showed substantial increase in viscosity and can be used as associative thickeners in biomedical applications. The preliminary results indicate that it self-assembles under aqueous conditions to form long-lived physical networks and the results will be published very soon.

7.5 Conclusion

A new method to synthesize poly(L-leucine) grafted hyaluronan via a “grafting onto” strategy using a mild Michael addition reaction between amine terminated PLeu-NH₂ and acrylate functionalized HA (TBAHA-acrylate) has been described. Although other methods like “grafting from” could be used to potentially increase grafting efficiency by functionalizing HA backbone with amine groups which initiate ring opening polymeriazation of leucine monomer, it will suffer from such disadvantages as limited chain length of PLeu as well as its characterization. The grafting efficiency, f' of PLeu on TBAHA could be moderately controlled by functionality of TBAHA (f) and [PLeu-NH₂]/[acrylate]_{TBAHA} or by their multiply according our study, thus it is believed the reaction could be reproduced to get desired grafting ratios. Grafted TBAHA-g-PLeu

copolymer with a grafting ratio of 3.7 mole % formed intermolecular aggregates and networks in aqueous solution. At higher ratios, the graft copolymers were not soluble in water. The functionalized TBAHA and graft copolymers were thoroughly characterized by ^1H NMR and FT-IR spectroscopies, and TGA. Conformational information, the nature of the aggregates of the graft copolymer in solution, and the secondary structure of PLeu in the aggregates were studied using a combination of DLS, SLS, AFM and CD spectroscopy techniques. The results showed that there exists strong hydrophobic inter/intra molecular interactions between PLeu chains in the TBAHA-g-PLeu copolymer leading to intermolecular aggregates with partially non-grafted HA segments. Thus, it is very possible to form a physical hydrogel with unique secondary structure information given by polypeptide, by adjusting the HA backbone molecular weight and grafting ratio, which may be applied in medical treatment as injectable associative thicker, tissue engineering and drug delivering system.

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Chapter 8 Conclusions

The fundamental role of electrostatics in the micro-phase separation of charged block copolymers has been discussed and the experimental and theoretical results were thoroughly reviewed in Introduction. Although considerable insights into the morphological behavior of charged block copolymers have been accomplished, more efforts are clearly needed in order to provide a complete or universal picture in terms of their structure-property relationships. By synthesizing well-defined ionic block copolymers with different structures and systematic characterization of these materials, combined with theoretical modeling/calculation development, predictive capabilities may be developed for these materials.

In this dissertation, well-defined block copolymers PS-*b*-PI, PS-*b*-fPI and *s*PS-*b*-fPI were synthesized having different compositions. Complete saturation of the PI blocks can be accomplished by optimizing reaction conditions with difluorocarbene to yield well-defined structures. By using the acetyl sulfate sulfonation method and sulfonation using the complex of TEP/SO₃, block copolymers of PS-*b*-fPI can be successfully sulfonated, yielding a broad range of sulfonation degrees. And all these materials were used to investigate bulk morphologies as a function of sulfonation degree in bulk and in aqueous solution. In bulk, low sulfonation degrees disorder the well phase separated structures of precursors and high sulfonation degrees promote long range ordered structures. The domain spacing is shown to be a function of sulfonation degree and can be explained by considering ion condensation and chain stretching. In aqueous media, these model block copolymers show unique self-assembly behavior, forming novel tapered rods and ribbon-like micelles. It is believed that a distribution of sulfonation degrees and residing in the SSSR drive the self-assembly of these materials in unique

ways, accounting for axial dimensional changes and interfacial flatness. The concept of spatial distribution of sulfonation degrees across asymmetrical micelles, opens up a new approach to tuning self-assembly of block copolymers.

Strong electrolyte-containing diblock copolymers of styrene and sulfonated cyclohexadiene, with different microstructures in the PCHD block, were also presented in this dissertation. It was demonstrated that the sulfonation chemistry had no selectivity toward the different microstructures of PCHD and could be controlled to yield the same SD in diblock copolymers containing PCHD blocks having different microstructures. For the first time, we discovered that a sample having about 70 wt% of the sulfonated PCHD block and the highest 1,4-microstructure of PCHD formed vesicles, while all other micelles were spherical aggregates. The electrostatic repulsion in charged PCHD chains and the poor thermodynamic interactions between corona chains and water contribute to the vesicle formation. For these materials, it was shown that hydrophilicity of *s*PCHD increases with 1,2-microstructure in PCHD block as indicated by A_2 .

Optimal conditions were found to prepare PI-*b*-PAA and PS-*b*-PI-*b*-PAA block copolymers. Discoveries were made regarding micro phase separation in the confined spaces of micelles in aqueous media. Within the same PS-*b*-PI-*b*-PAA chains, cylindrical and spherical micelles have different ultra-fine structures in the micellar core consisting of PS and PI.

In this dissertation, we also described synthesis and characterization of graft copolymers of HA and polyleucine in details. The solution properties were studied to show local-network aggregates formed from these graft copolymers. These materials have the potential applications in medical treatments.

Appendix

Chapter 4 Asymmetrical Self-assembly From Fluorinated and Sulfonated Block Copolymers in Aqueous Media

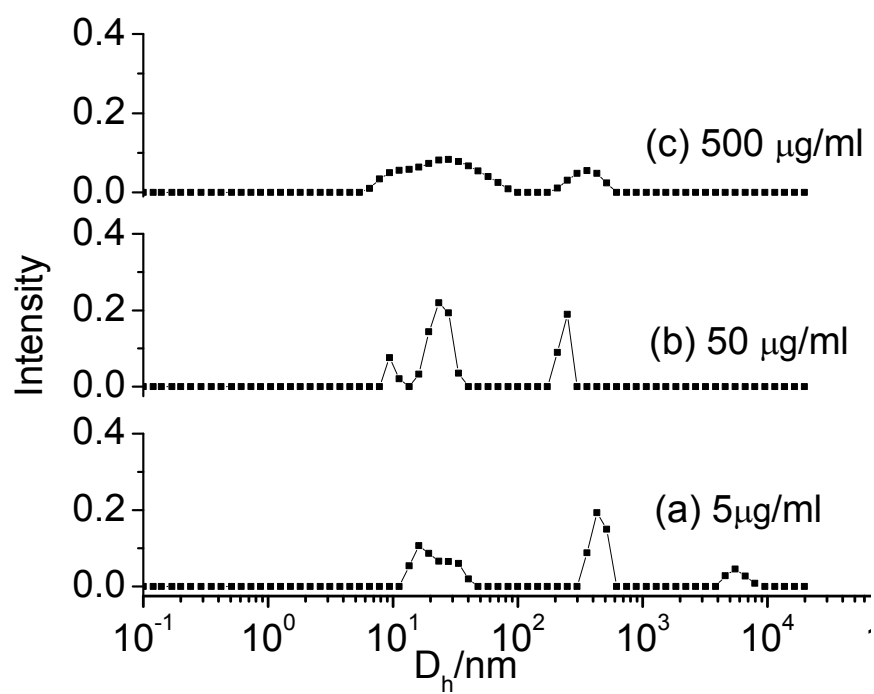


Figure A0. Concentration dependence of D_h distributions of freshly made sample Series 2-SD29.6 at a) 5 µg/ml; b) 50 µg/ml, c) 500 µg/ml after aging 4 days.

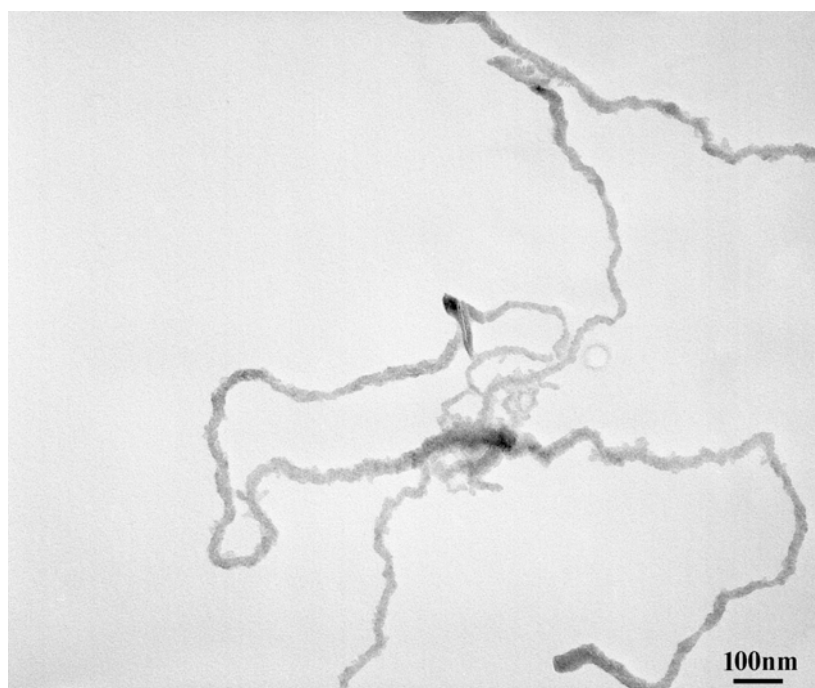
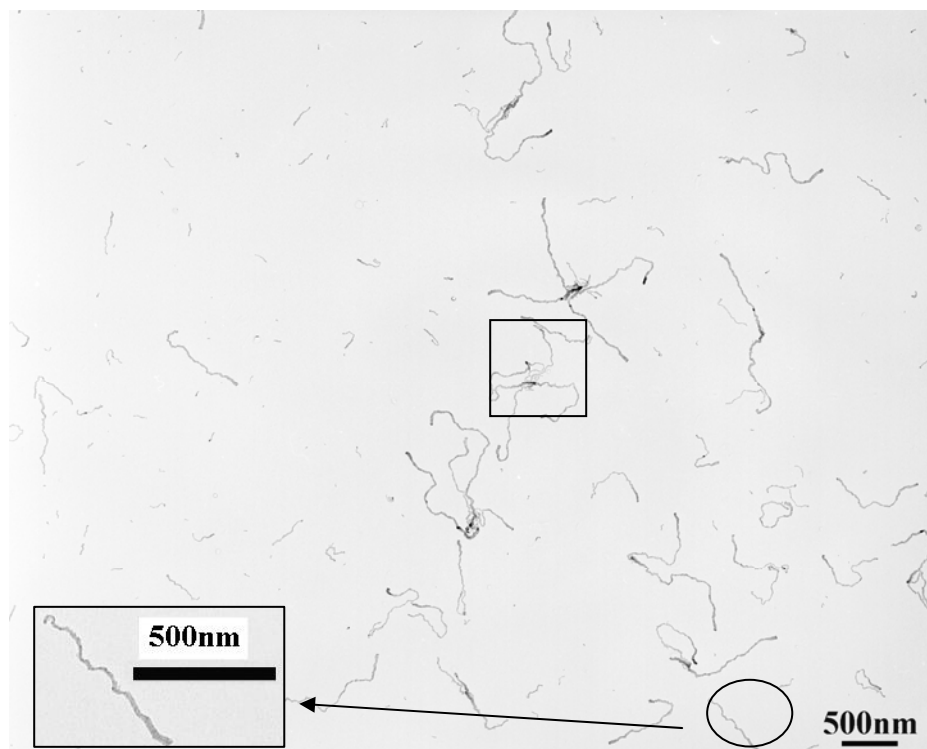


Figure A1. Large area TEM image same as the one shown in main text(Figure 1(b)) and TEM image of the position squared with high magnification.

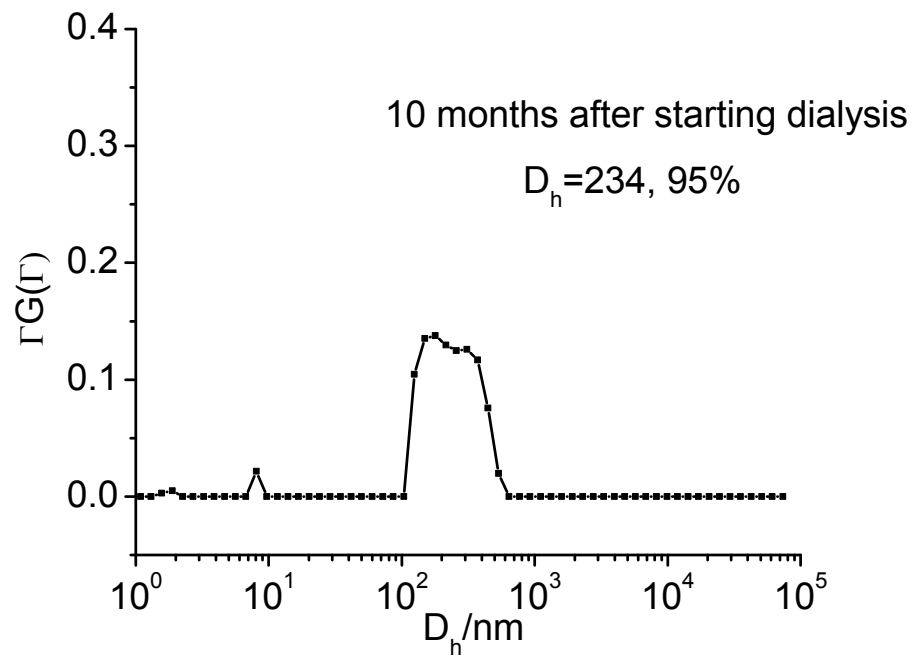


Figure A2. 10 months after starting dialysis for sample Series1-SD38.8 in aqueous solution.

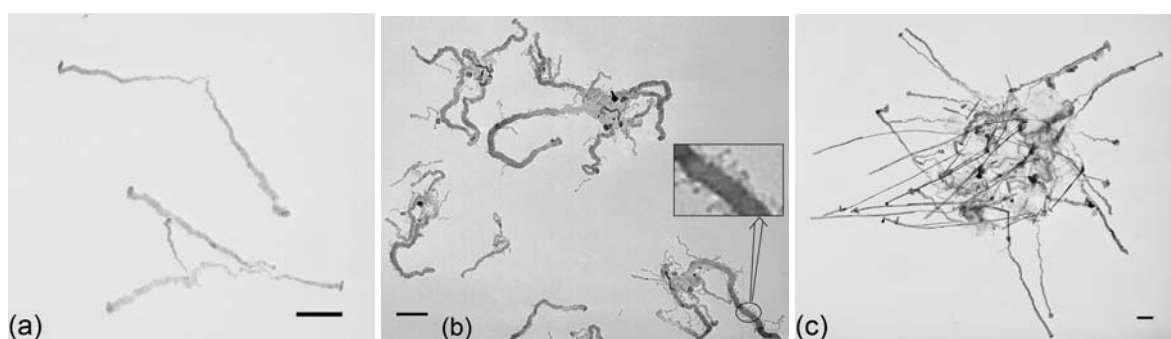


Figure A3. TEM images of Series 2-SD29.6, three different morphologies were observed after aging 60 days: a) extended tapered rods; b) hairy worms; c) smooth rigid “fibers”. Scale bar 250 nm

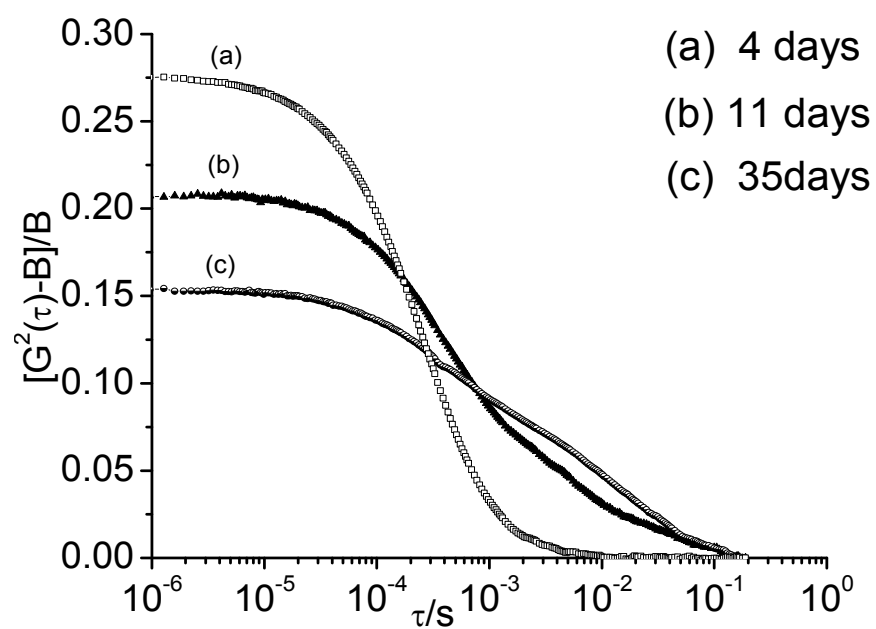


Figure A4. Intensity-intensity time correlation functions by DLS at 45° scattering angle as a function of time: (a) 4 days;(b) 11days; (c) 35 days of sample Series 2-SD29.6 at 0.5 mg/ml.

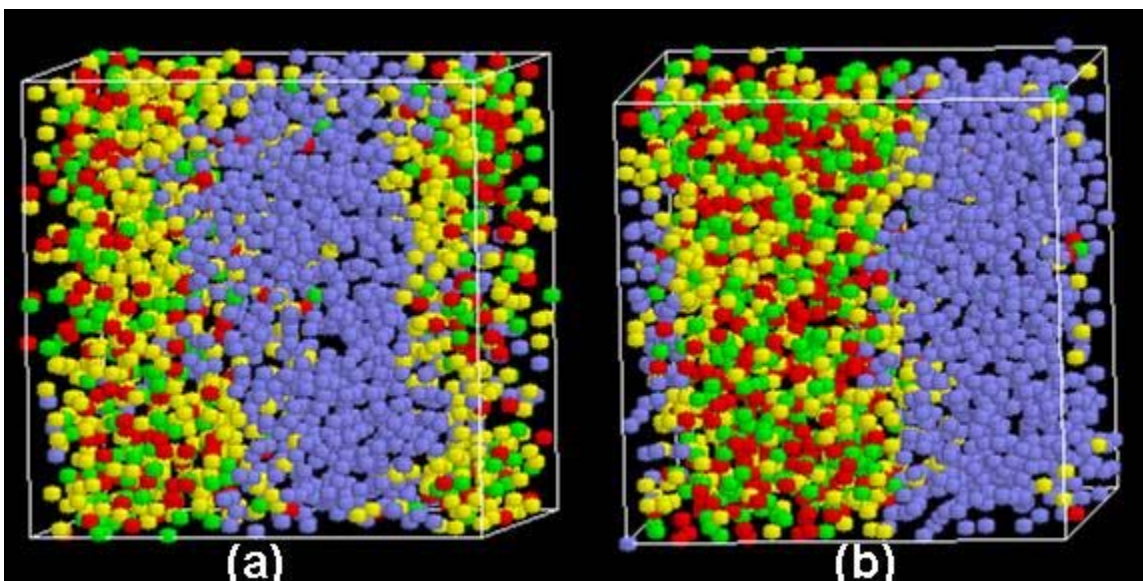


Figure A5. Snapshot of the system at the end of the simulation run for (a) 28.1% and (b) 46.9% of charges on the charged block. The diblock consists of 50-50 uncharged and charged blocks. The blue dots are uncharged block, red dots are the counterions and the yellow and green dots are the uncharged and charged monomeric sites of the charged block. A comparatively flat interface are seen to be formed preferentially with micro phase separated charged and uncharged block. The higher charged states on the charged block forms flatter interface.

Table A1 D_h of aggregates in THF and Water at 25 °C

Solvent	Sample	Hydrodynamic diameter (nm)		
		Series1-SD13.4	Series1-SD38.8	Series1-SD98.0
THF		6.0	7.5/150.6	20.6
Water		231	252	260

Vita

Xiaojun Wang was born in a beautiful village in Fuyang, China. He attended East China University of Science and Technology from 2000 to 2004 and Fudan University from 2004 to 2007, where he received his B.E. in Material Science and M.S. in Macromolecular Chemistry and Physics, respectively, in Shanghai, China. He was enrolled as a graduate student in Department of Chemistry at the University of Tennessee-Knoxville and joined Professor Jimmy W. Mays' group in August, 2007. His PhD work mainly concerned synthesis of well-defined biomaterials and neutral, charged block copolymers, characterization and self-assembly study in melts and aqueous system. Xiaojun Wang received a Doctor of Philosophy Degree in Polymer Chemistry from the University of Tennessee in March, 2012.