Aqueous RAFT Synthesis of Stimuli-Responsive, Amphiphilic Block Copolymers and Self-Assembly Behavior in Solution and Incorporation Into LBL Films

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AQUEOUS RAFT SYNTHESIS OF STIMULI-RESPONSIVE, AMPHIPHILIC
BLOCK COPOLYMERS AND SELF-ASSEMBLY BEHAVIOR
IN SOLUTION AND INCORPORATION INTO LBL FILMS

by

Matthew Grady Kellum

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ABSTRACT

AQUEOUS RAFT SYNTHESIS OF STIMULI-RESPONSIVE, AMPHIPHILIC
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Of all the living radical polymerization techniques, reversible addition–
fragmentation chain transfer (RAFT) polymerization is arguably the most versatile in
terms of the reaction conditions (e.g. temperature and solvent selection), monomer
selection (e.g. neutral, anionic, cationic, and zwitterionic), and purification. Since the
introduction of RAFT in 1998, the McCormick research group and others including the
Lowe, Sumerlin, and Davis research groups have synthesized a wide range of
(co)polymers with predetermined molecular weights, low polydispersities, and advanced
architectures utilizing aqueous RAFT (ARAFT) polymerization. These research groups
have also studied how various block copolymers exhibit stimuli-responsive behavior due
to a change in temperature, solution pH, or electrolyte concentration. However the
stimuli-responsive behavior of unprotected, chiral, amino acid-based polymers had yet to
be reported. The incorporation of these homopolymers into stimuli-responsive block
copolymers will create novel polymer systems that can be reversibly “locked” under
facile conditions and have potential applications in sequestration and targeted delivery.
The overall goal of this research is to utilize the RAFT process for the synthesis of such
block copolymers directly in water, investigate the relationship between block copolymer
composition and solution properties on the self-assembly behavior of the copolymers, and
incorporate these micelles within films via the layer-by-layer technique to produce stimuli-responsive films for applications such as drug release from surfaces.

The first section concerns utilizing ARAFT polymerization for the successful synthesis of a series of novel pH-responsive block copolymers containing an unprotected amino acid-based block. Block copolymers containing a permanently anionically charged hydrophilic block of AMPS and a pH-responsive AAL block were subsequently synthesized and the aqueous self-assembly behavior was investigated. The aggregation behavior for a series of P(AMPS-b-AAL) was determined at varying pH values and salt concentrations. The effect of the permanently hydrophilic and responsive block lengths on the stimuli driven assembly behavior was examined. The second section details the cross-linking of these micelles using interpolyelectrolyte complexation (IPEC) with cationic polymers. This is the first report of a pH reversible IPEC cross-linked micellar system. The third section details work done in collaboration with Christopher Harris and concerns the incorporation of micelles possessing anionically charged coronas within layer-by-layer films. The effect of salt concentration on film thickness and morphology was studied. Also because the films are made using pH-responsive block copolymers, the responsive behavior of the polyelectrolyte multilayer films was also investigated.
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<td>2-vinylpyridine</td>
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<tr>
<td>4VP</td>
<td>4-vinylpyridine</td>
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<tr>
<td>AA</td>
<td>acrylic acid</td>
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<td>AaH</td>
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<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>EMP</td>
<td>2-ethylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid</td>
</tr>
<tr>
<td>EO</td>
<td>ethylene oxide</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier-transform infrared</td>
</tr>
<tr>
<td>GTP</td>
<td>group transfer polymerization</td>
</tr>
<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography</td>
</tr>
<tr>
<td>HPMA</td>
<td>hydroxypropyl methacrylamide</td>
</tr>
<tr>
<td>IPEC</td>
<td>interpolyelectrolyte complex</td>
</tr>
<tr>
<td>LbL</td>
<td>layer-by-layer</td>
</tr>
<tr>
<td>LCST</td>
<td>lower critical solution temperature</td>
</tr>
<tr>
<td>MAA</td>
<td>methacrylic acid</td>
</tr>
<tr>
<td>MAEDAPS</td>
<td>3[2-(N-methyl acrylamido)ethyldimethylammonio] propanesulfonate</td>
</tr>
<tr>
<td>MEMA</td>
<td>2-(N-morpholino)ethyl methacrylate</td>
</tr>
<tr>
<td>MPC</td>
<td>2-methacryloyloxyethyl phosphorylchloride</td>
</tr>
<tr>
<td>NAS</td>
<td>N-acryloxsuccinimide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>NIPAM</td>
<td>N-isopropylacrylamide</td>
</tr>
<tr>
<td>NMP</td>
<td>nitroxide mediated polymerization</td>
</tr>
<tr>
<td>PDI</td>
<td>polydispersity index</td>
</tr>
<tr>
<td>PEG</td>
<td>poly(ethylene glycol)</td>
</tr>
<tr>
<td>pHs</td>
<td>$p$-hydroxystyrene</td>
</tr>
<tr>
<td>PO</td>
<td>propylene oxide</td>
</tr>
<tr>
<td>Q4VP</td>
<td>quaternised 4-vinylpyridine</td>
</tr>
<tr>
<td>RAFT</td>
<td>reversible addition-fragmentation chain transfer</td>
</tr>
<tr>
<td>RMS</td>
<td>root mean square</td>
</tr>
<tr>
<td>SANS</td>
<td>small-angle neutron scattering</td>
</tr>
<tr>
<td>SCL</td>
<td>shell cross-linked</td>
</tr>
<tr>
<td>SEC</td>
<td>size exclusion chromatography</td>
</tr>
<tr>
<td>SFRP</td>
<td>stable free radical polymerization</td>
</tr>
<tr>
<td>SLS</td>
<td>static light scattering</td>
</tr>
<tr>
<td>SS</td>
<td>4-styrenesulfonate</td>
</tr>
<tr>
<td>tBA</td>
<td>tert-butyl acrylate</td>
</tr>
<tr>
<td>TCEP</td>
<td>tris(2-carboxyethyl)phosphine hydrochloride</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TMP</td>
<td>4-vinylbenzyl(trimethylphosphonium) chloride</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>V-501</td>
<td>4,4′-azobis(4-cyanopentanoic acid)</td>
</tr>
<tr>
<td>V-70</td>
<td>2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile)</td>
</tr>
<tr>
<td>VA-044</td>
<td>2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride</td>
</tr>
<tr>
<td>VBA</td>
<td>4-vinylbenzoate</td>
</tr>
<tr>
<td>VBTAC</td>
<td>ar-vinylbenzyl(trimethylammonium chloride)</td>
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</tbody>
</table>
CHAPTER I
INTRODUCTION

With the advent of controlled radical polymerization (CRP) techniques, opportunities for creating functional polymers with a wide variety of applications both in solution and on surfaces have arisen. Perhaps the most abundant area of research for solution-based functional polymers includes the creation of nanoscale vehicles for applications including targeted delivery of active agents, such as drugs and genes, and subsequent controlled release. Numerous factors must be taken into account for the rational design of polymeric systems for such applications including a housing area for the active species, retention of the vehicle structure throughout delivery, and a trigger function leading to the release of the payload at a specific area. A similar process is required for the functionalization of surfaces utilizing polymers, depending on the specific application desired. One of the most promising areas for surface modification lies with the aspiration to develop biological materials for film-based release of drugs. A major challenge associated with this application results from the ability to incorporate small, uncharged, hydrophobic therapeutics within thin films due to a lack of functionality. With these challenges in mind for both solution and film-based drug delivery, CRP techniques appear quite promising in the rational design of viable polymeric systems. Specifically, reversible addition-fragmentation chain transfer (RAFT) polymerization offers the most versatility, with its tolerance towards functional groups, ample reaction conditions, and selection of polymerization media and often allowing for direct polymerization in aqueous media. Because of these factors, RAFT polymerization offers the options for synthesis of a wide range of polymers for the
creation of such nanoscale vehicles which will inevitably play a large role in the future of diagnostics and drug delivery. In the following sections, the details of the RAFT polymerization process will be highlighted along with the construction of novel polymeric vehicles. Also, the stimuli-responsive, layered surfaces of polymeric films formed from RAFT synthesized homo- and block copolymers will be reported.

Reversible Addition-Fragmentation Chain Transfer Polymerization

Prior to the development of CRP techniques, control of polymer architecture, molecular weight, and molecular weight distribution were only obtainable by the use of cationic, anionic, or group transfer polymerization which are limited by monomer selection, have stringent reaction conditions and often require protecting group chemistry. With the advent of CRP techniques, polymers of pre designed molecular weight and architectures with low polydispersity indices (PDIs) can be synthesized without the limitations of traditional living polymerization techniques. Of particular interest, especially in the preparation of nanoscale vehicles, is the ability to create polymers with specific architectures, examples of which are shown in Figure I-1.
In order to control polymer architecture, it is necessary to maintain active chain ends and, hence, reduce the number of termination events that occur throughout the polymerization. CRP techniques significantly reduce termination events by lowering the concentration of radicals via establishing an equilibrium between dormant and active chain ends which predominately favors the dormant state. The major types of controlled radical polymerization are shown in Scheme I-1. Stable free radical polymerization (SFRP)\(^1\)\(^-\)\(^3\) and atom transfer radical polymerization (ATRP)\(^4\)\(^,\)\(^5\) rely on reversible termination mechanisms to impart control during the polymerization while reversible addition-fragmentation chain transfer (RAFT) operates under a degenerative chain transfer process.

**Figure I-1.** (Co)polymer architectures available with CRP techniques.
Scheme I-1. Equilibrium between active and dormant chains for the SFRP, ATRP, and RAFT methods.

The RAFT polymerization method was first introduced by the CSIRO group in a 1998 manuscript. Since this initial report, RAFT polymerization has been widely researched by a number of groups including ours. This technique is now considered the most versatile of the CRP methods allowing polymerization of effectively any vinyl monomer ranging from neutral to charged to zwitterionic in nature. Also a wide range of reaction conditions including room temperature reactions and direct polymerization of unprotected monomers in aqueous media can be accomplished. The popularity of this technique is exemplified in the numerous publications on the RAFT process/RAFT-made polymers within the past decade including a variety of reviews on the RAFT mechanism, computational studies, the RAFT process and RAFT in aqueous and heterogeneous media.
The RAFT Polymerization Mechanism

Radicals are generated using conventional free radical initiators, e.g. thermal or photochemical, with chemical decomposition of azo initiators dominating most reports in the literature (Figure I-2). The initiator (1) decomposes forming radicals which react with monomer to form a propagating radical, $P_a^\bullet$ (2). Eventually this polymer chain adds to the CTA (3), as shown in Scheme I-2. Upon addition, the intermediate radical (4) is formed. This intermediate species then fragments, expelling the leaving group $R^\bullet$ (6), which is free to initiate polymerization forming a new polymer chain, $P_m^\bullet$ (7). Once all of the R groups have initiated polymerization, the reaction has proceeded from the pre-equilibrium to the main equilibrium. In the main equilibrium two populations of polymer chains are present, dormant (either 5 or 9) and active (either 2 or 7), where the population of dormant species far exceeds that of the propagating species. By transferring the $S=C(Z)S^-$ group between active and dormant chains via addition-fragmentation equilibrium, all chains grow at a similar rate and uniformity, producing well-controlled polymers with narrow PDIs. However, chain termination events (e.g. radical-radical coupling), as in conventional free radical polymerization, can occur. The prevalence of these termination reactions and the efficiency of the addition-fragmentation steps determine the ‘livingness’ of the polymerization.
The RAFT Chain Transfer Agent

The RAFT process, depicted in Scheme I-2, is mediated by a chain transfer agent (CTA) which contains a thiocarbonylthio moiety. Examples of suitable classes of compounds include dithioesters, dithiocarbamates, trithiocarbonates and xanthates. All of these CTAs bear two kinds of functionality, Z and R groups. Both groups must be chosen with great care in order for the RAFT process to proceed efficiently with the characteristics of a controlled/“living” system, i.e. high transfer constants coupled with good reinitiating efficiency. The Z group plays an important role by both activating the C=S double bond for radical addition and potentially stabilizing the intermediate radical. Longer lived intermediate radicals can be achieved by choosing a Z group with a greater stabilizing effect, e.g. phenyl. The rate of polymerization is inversely related to the lifetime of the intermediate radical. The second functionality of the CTA, the R group,
must readily fragment homolytically, and the resulting radical, $R\cdot$, must be able to add to monomer freely in order for efficient degenerative chain transfer to take place.\textsuperscript{26}

\begin{align*}
\text{I.} & \quad \text{Initiator} \xrightarrow{k_d} 2I\cdot \\
& \quad 1^\cdot + \text{Monomer} \xrightarrow{} P_n^\cdot \\
\text{II.} & \quad P_n^\cdot + S\xrightarrow{k_{\text{add}}/k_{-\text{add}}} \overset{Z}{S} \xrightarrow{k_{j_i}} P_n^\cdot + \overset{Z}{S} \xrightarrow{R\cdot} Z-C-S-P_n + R\cdot \\
& \quad 2 \quad 3 \quad 4 \quad 5 \quad 6 \\
\text{III.} & \quad R\cdot + \text{Monomer} \xrightarrow{} P_m^\cdot \\
& \quad P_n^\cdot + \text{Monomer} \xrightarrow{} P_{n+x}^\cdot \\
\text{IV.} & \quad P_m^\cdot + Z-C-S-P_n \xrightarrow{k_{\text{add}}/k_{-\text{add}}} P_m^\cdot + Z-C-S-P_n \xrightarrow{k_{\text{add}}/k_{-\text{add}}} Z-C-S-P_m \xrightarrow{P_n^\cdot} \\
& \quad 7 \quad 8 \quad 9 \\
\text{V.} & \quad I^\cdot R\cdot P_n^\cdot P_m^\cdot \xrightarrow{k_t} \text{Dead Polymer}
\end{align*}

**Scheme I-2.** The RAFT mechanism.

*Molecular Weight Control*

According to the RAFT mechanism, polymer chains are derived from initiator fragments and the leaving group from the CTA. As such, the theoretical number-average molecular weight ($M_{n,\text{th}}$) of the polymer chains is defined as

\[
M_{n,\text{th}} = \frac{[M]_0 M_{MW} \rho}{[CTA]_0 + 2f[I]_0 (1-e^{-k_d t})} + CTA_{MW} \tag{1}
\]
where $[M]_0$ is the initial monomer concentration, $M_{MW}$ is the molecular weight of the monomer, $\rho$ is monomer conversion, $[CTA]_0$ is the initial concentration of CTA, $f$ is initiator efficiency, $[I]_0$ is the initial initiator concentration, $k_d$ is the decomposition rate constant of the initiator, $t$ is reaction time, and $CTA_{MW}$ is the molecular weight of the CTA. In typical RAFT polymerizations, the CTA:initiator ratio is kept high in order to ensure a low concentration of active radicals; therefore, less than 5% of polymer chains are derived from initiator radicals allowing simplification of Equation 1 to Equation 2.

$$M_{n,th} = \frac{[M]_0 M_{MW} \rho}{[CTA]_0} + CTA_{MW} \tag{2}$$

This equation shows that molecular weight increases linearly with conversion. By controlling conversion and the initial ratio of monomer to CTA, well defined polymers with predetermined molecular weights can be synthesized via RAFT polymerization.

*Aqueous RAFT Polymerization Conditions*

With the appropriate selection of CTA, monomer, solvent, initiator and temperature, nearly all vinyl monomers can be polymerized utilizing the RAFT technique. Perhaps the most advantageous property of RAFT is the ability to conduct polymerizations directly in aqueous media. To date a variety of functional monomers have been polymerized via aqueous RAFT including those with neutral,\textsuperscript{27-37} anionic,\textsuperscript{27,38-41} cationic\textsuperscript{42-46} and zwitterionic\textsuperscript{47-51} functionality. Examples of such monomers are shown in Figure I-3.
**Figure I-3.** Examples of monomers polymerized by aqueous RAFT.
Although aqueous RAFT has proven to be a versatile technique for the synthesis of water-soluble polymers, certain considerations must be addressed in order to maintain control throughout the polymerization. Namely, the integrity of the thiocarbonylthio functionality must be retained throughout the polymerization, and any reaction which destroys this species reduces control. Many processes are detrimental to the CTA functionality including oxidation,\textsuperscript{52} hydrolysis,\textsuperscript{53} ultraviolet light\textsuperscript{54} and aminolysis.\textsuperscript{55,56} Oxygen free conditions are utilized in RAFT, which eliminate the effects of CTA oxidation. Similarly, the reaction solution is typically only exposed to a UV light source when a photoinitiator is used to generate radicals thus avoiding the effects of CTA degradation by ultraviolet light. The most significant factors contributing to CTA degradation in an aqueous RAFT polymerization are aminolysis and hydrolysis. Shown in Figure I-4 are the most common CTAs used in aqueous RAFT polymerization which might be susceptible to these degradation processes.

\textbf{Figure I-4.} Examples of water-soluble CTAs used in aqueous RAFT polymerizations.
Although thiocarbonylthio compounds are known to be thermodynamically unstable towards hydrolysis, there is a significant kinetic barrier to hydrolysis. Levesque and coworkers\textsuperscript{56} found that hydrolysis reactions could be reduced by more than fifty percent by lowering the reaction temperature from 35 to 20 °C and lowering the pH from 8.5 to 7.5. Similar results were found in our lab by Thomas and coworkers\textsuperscript{57} who studied the effect of solution pH on the hydrolysis of small molecule CTAs and macroCTAs of varying size. The hydrolysis of the CTA was assumed to be zero-order with respect to water due to a large excess of water; therefore, the rate of hydrolysis was expressed in terms of the apparent rate constant, $k_{\text{hyd}}$, shown in Equation 3.

$$-\frac{d[\text{CTA}]}{dt} = k_{\text{hyd}}[\text{CTA}]$$ (3)

The rates of hydrolysis of 4-cyanopentanoic acid dithiobenzoate, CTP (CTA\textsubscript{1}), and two macroCTAs of poly(sodium 2-acrylamido-2-methyl-1-propanesulfonate) (PAMPS) made with CTP were found to follow Equation 3 and were shown to increase at higher pH values. Also, the small molecule CTA was shown to be more susceptible to hydrolysis which was attributed to less steric hindrance as compared to the polymeric CTAs. Convertine and coworkers\textsuperscript{28} studied the effect of temperature on trithiocarbonates, specifically 2-(1-carboxy-1-methyl-ethylsulfanylthiocarbonylsulfanyl)-2-methylpropionic acid (CMP) (CTA\textsubscript{3}), and found that these species were more resistant to hydrolysis. They determined that near or below 50 °C, hydrolysis of the trithiocarbonate was negligible for over 24 hours.

Besides hydrolysis, another detrimental side reaction leading to degradation of CTAs is aminolysis. Aminolysis occurs when a primary or secondary amine reacts with the thiocarbonylthio moiety of CTAs. This reaction is known to be first order with
respect to the concentration of CTA and second order with respect to the amine concentration. Thomas and coworkers also conducted aminolysis experiments on CTP in a buffered media with ammonium hydroxide. By taking into account hydrolysis and aminolysis, the time dependent concentration of CTA can be determined according to Equation 4

\[
[CTA] = [CTA]_0 e^{-\left(k_{\text{hyd}} + ka[NH_3]^2\right)t}
\] (4)

where \(k_a\) is the rate constant of aminolysis and \([NH_3]\) is the concentration of ammonia. Utilizing this equation and the appropriate rate constants for CTP, Thomas et al. determined that after four hours, over 95% of CTP is destroyed which further emphasizes the importance of these factors on aqueous RAFT polymerizations. In order to maintain CTA functionality by minimizing hydrolysis and aminolysis (if necessary), moderately acidic conditions are optimal.

Prior to these reported results, monomers containing primary or secondary amines were excluded from RAFT polymerization in aqueous media. By lowering the solution pH to keep the amines protonated, aminolysis of the CTA functionality can be avoided. For example, Li et al. from our group reported the successful polymerization of the primary amine containing monomer N-(3-aminopropyl)methacrylamide, APMA (M7), mediated by CTP by maintaining a pH between 4 and 5. A later report by Alidedeoglu and coworkers also detailed the controlled polymerization of a primary amine containing monomer, 2-aminoethyl methacrylate (AEMA), in an acetate buffer at a pH of approximately 5 obtaining polymers with PDIs of > 1.2.
Stimuli-Responsive Block Copolymers

*Block Copolymer Micelles*

The capability of block copolymers to associate into supramolecular assemblies has become a vastly studied topic within the polymer science discipline. Such systems have been theorized to have a major impact on such fields as optics, electronics, cosmetics, and in particular biomedical applications including drug/gene delivery and diagnostic devices. The self-associative properties of block copolymers arise from their molecular composition where one block is dissolved in a liquid that is a thermodynamically good solvent while the same solvent is a precipitant for the other block. In general, micellization occurs in dilute solutions when the polymer concentration is greater than the critical micelle concentration (CMC). The CMC is dependent on several factors including the block composition and molecular weight. Below the CMC, the block copolymers exist as molecularly dissolved unimers, and at concentrations above the CMC, the block copolymers exist as micelles that are in dynamic equilibrium with unimers. These micellar systems can be characterized by several parameters including:

1) the equilibrium constant between micelles and unimers;
2) the critical micelle concentration;
3) the morphology of the micelles;
4) the molecular weight of the micelle ($M_m$);
5) the aggregation number of the micelle ($Z$), which can be defined as the molecular weight of the micelle divided by the molecular weight of the unimer ($M_u$) ($Z = M_m/M_u$);
6) the radius of gyration of the micelle ($R_g$);

7) the hydrodynamic radius of the micelle ($R_h$);

8) the shape factor of the micelle ($R_g/R_h$);

The characteristics of block copolymer micelles can be determined by a number of analytical techniques. The CMC can be determined by scattering methods, fluorescence or dye solubilization, and surface tension measurements. It should be noted that block copolymers have very low CMCs, and equilibrium conditions are only achieved after a very long time period. Because of this, fluorescence techniques are the preferred method for CMC determination for block copolymer micelles. Winnik and Reiss covalently attached fluoroprobes to determine the CMC of their micellar systems.\textsuperscript{60}

The relatively spherical morphology and narrow size distribution of block copolymer micelles of styrene and isoprene were determined by transmission electron microscopy by Price and Coworkers in the 1980s.\textsuperscript{61} More recently, cryo-TEM has been used to confirm these findings.\textsuperscript{62,63} Elliptical, rod-like, vesicles, crew-cut, and flower-like morphologies have also been reported.\textsuperscript{64}

The hydrodynamic radius ($R_h$), radius of gyration ($R_g$), and molecular weight of block copolymer micelles are determined by light scattering techniques. The $R_h$ is most often determined by dynamic light scattering (DLS). The micelle is assumed to be equivalent to a sphere using the Stokes-Einstein relationship which relates the $R_h$ to the translational diffusion coefficient ($D_{app}$) and is shown in Equation 5:

$$R_h = \frac{k_B T}{6 \pi \eta D_{app}}$$  \hspace{1cm} (5)

where $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, and $\eta$ is the viscosity of the solvent. The $R_g$ of block copolymer micelles is determined by static light
scattering (SLS) typically from the slope of a Zimm plot. A Zimm plot shows the
scattering intensity \(I_{ex}\) versus the square of the scattering vector \(q^2\) and is most valid
for particles < 100 nm. Above this size, a more suitable plot is a Berry plot \(I_{ex}^{-1/2} \text{ vs. } q^2\).
The molecular weight of the micelle can be determined from the intercept of these plots.
By knowing the molecular weight of the micelle and the molecular weight of the
polymers comprising the micelles, an aggregation number can be determined.

The advent of living polymerization techniques has allowed for the synthesis of a
large number of block copolymers; however, many limitations continue to make the
formation of micelles in aqueous solution a difficult process. In some instances, there is
a need for post polymerization modification. For example, in order to synthesize block
copolymers of polystyrene-\(b\)-poly(acrylic acid), Eisenberg and coworkers\(^65\) first
synthesized polymers of polystyrene-\(b\)-poly(\(tert\)-butyl acrylate) and then hydrolyzed the
\(tert\)-butyl ester groups using the catalyst \(p\)-toluene-sulfonic acid. Another difficulty often
comes in the preparation of the micelles. Because one block is permanently hydrophobic,
the block copolymer cannot be directly dissolved in water. Eisenberg and coworkers\(^66-68\)
reported several methods for micelle formation of such block copolymers. One such
method consists of directly dissolving the block copolymer in \(N,N\)-dimethyl formamide
(DMF) (a good solvent for both blocks) followed by the slow addition of water. The
solution is then dialyzed against water for one week to remove the DMF.

The introduction of CRP techniques provided a major breakthrough in the
preparation of block copolymers capable of forming micelles directly in aqueous
solution. These techniques, especially RAFT polymerization, allow for the
polymerization of a wide variety of functional monomers while maintaining control over
molecular weight and polydispersity. With these techniques, “smart” block copolymers could be synthesized where initially both blocks are hydrophilic and, therefore, directly dissolve in water. Upon application of an external stimulus, most often a change in temperature, pH, or electrolyte concentration, one block becomes hydrophobic leading to the self-assembly of the block copolymers into micelles (Scheme I-3). These “smart” block copolymers provide many advantages over their permanently hydrophilic-hydrophobic analogues, including the ease of micelle formation and dissociation by the addition and removal of the stimulus.


Applications of block copolymers have been reviewed and include uses such as dispersants, emulsifiers, wetting agents, foam stabilizers, flocculants, viscosity modifiers, and pharmaceutical formulations.\textsuperscript{69-71} Of particular current interest are applications related to the self-assembly of the block copolymers into micelles, especially those based on the solubilization of active compounds for targeted delivery. There are three major routes for delivering drugs from block copolymer micelles. These delivery schemes have recently been the subject of review by Kabanov and Alakov\textsuperscript{72} and include: block
copolymer micelles in which the drug is covalently linked to the block copolymer; block copolymer micelles in which the drug is incorporated into the core or “cargo area” of the block copolymer; and block copolymer micelles that form block ionomer complexes (BICs) which are polyelectrolyte complexes between a nucleotide and cationic sequence on the block copolymer. In the case of micellar drug delivery systems, important factors such as biocompatibility, biodegradability and particle size must also be taken into account. Block copolymers that can reversibly self-assemble into micelles in response to external stimuli such as pH and temperature appear to hold the most promise for targeted delivery applications.

*Thermally-Responsive Block Copolymers*

Temperature-responsive (co)polymers exhibit a sudden change in solvation at a critical temperature. Depending on the (co)polymer composition, either a lower critical solution temperature (LCST), where the polymer becomes insoluble upon heating, or an upper critical solution temperature (UCST), where the polymer becomes insoluble upon cooling, can be observed. This phenomenon is explained as a balance between enthalpic effects from hydrophobic interactions and hydrogen bonding and entropic effects from dissolution via the water molecules surrounding the polymer.

N-alkyl acrylamide monomers are the most commonly used in the preparation of temperature-responsive block copolymers. Among these, N-isopropylacrylamide, NIPAM (M3), has received the most attention due to its readily accessible LCST of ~32 °C, which is just below that of physiological temperature (37 °C). Due to the extensive amount of literature published concerning this monomer, only some of the most important contributions are highlighted.
In 2000, Gaunchaud and coworkers\textsuperscript{78} reported the polymerization of NIPAM via RAFT in organic solvents. Utilizing 2,2′-azobisisobutyronitrile, AIBN (11), as the radical source and either benzyl dithiobenzoate (in benzene) or cumyl dithiobenzoate (in 1,4-dioxane) as the CTA with a polymerization temperature of 60 °C, narrowly dispersed polymers with PDIs of > 1.5 were synthesized. Subsequently, Schilli \textit{et al.}\textsuperscript{79} polymerized NIPAM using cumyl and benzyl dithiocarbamates in 1,4-dioxane at 60 °C, obtaining polymers with PDIs around 1.3. This group later reported one of the first block copolymers containing PNIPAM, where a poly(acrylic acid) macroCTA was utilized in the polymerization of NIPAM in methanol at 60 °C.\textsuperscript{80} Aggregation of these block copolymers above the LCST of PNIPAM was demonstrated by dynamic light scattering and visualized with cryogenic transmission electron microscopy.

Yusa and coworkers\textsuperscript{81} later described the polymerization of sodium 2-acrylamido-2-methyl-1-propanesulfonate, AMPS (M16), in water using 4-cyanopentanoic acid dithiobenzoate, CTP (CTA1), and 4,4′-azobis(4-cyanopentanoic acid), V-501 (14), at 70 °C. This PAMPS macroCTA was then used to polymerize NIPAM in a methanol/water mixture (4/1 v/v). The self-assembly of this block copolymer was indirectly measured by \textsuperscript{1}H NMR and directly observed by light scattering. For a 0.1 wt % solution, aggregation was not observed until < 40 °C, which is well above the LCST value for PNIPAM, most likely attributed to an increase in hydrophilicity from the PAMPS block.

In our lab, Convertine and coworkers\textsuperscript{82} were the first to polymerize NIPAM at room temperature in DMF. Prior to this work, reports on RAFT polymerizations conducted at room temperature were limited to only a few accounts.\textsuperscript{83-85} Even more significant, Convertine \textit{et al.}\textsuperscript{29} later reported the room temperature RAFT polymerization
of NIPAM in aqueous solution. In that report, both ABA and AB type block copolymers were synthesized in order to produce temperature responsive block copolymers. The difunctional 2-(1-carboxy-1-methyl-ethylsulfanylthiocarbonylsulfanyl)-2-methylpropionic acid, CMP (CTA3), and novel monofunctional 2-ethylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid, EMP (CTA2), were employed as the CTAs in the polymerization of N,N-dimethylacrylamide, DMA (M2), using the water-soluble azo initiator 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride, VA-044 (I3), at 25 °C. These macroCTAs were subsequently used to polymerize NIPAM, creating block copolymers with a constant PDMA block length with varying PNIPAM block lengths. These ABA and AB block copolymers were shown to self-assemble into micelles when the solution temperature was raised above a critical value. Both the size of the micelles and the temperature required for micelle formation were shown to be highly dependent on the block length of PNIPAM. For example, dynamic light scattering results showed that a diblock copolymer of P(DMA100-b-NIPAM460) formed micelles at 34 °C with an apparent hydrodynamic diameter of ~ 80 nm. A polymer with the same PDMA block length and a shorter PNIPAM block length, P(DMA100-b-NIPAM71) was shown to aggregate into micelles at 45 °C with an apparent hydrodynamic diameter of ~ 25 nm.

Skrabania and coworkers recently reported the preparation of a series of BAB triblock copolymers, where the B blocks are made from thermo-responsive NIPAM (M3) and the A block is made from DMA (M2). They determined that the size of the micelles was dependent on how the solution was heated. For instance, a 0.1 wt % P(NIPAM_{242}-b-DMA_{242}-b-NIPAM_{242}) triblock solution was heated either slowly by one degree per ten
minutes or abruptly to 45 °C. When the slow heating technique was used, the triblock copolymer formed aggregates of varying sizes of 60, 100, and 3390 nm. Utilizing the fast heating technique, uniform micelles were formed with sizes of 173 nm. Kirkland et al.\textsuperscript{87} from our lab also reported studies utilizing similar BAB triblock copolymers. At polymer concentrations as low as 7.5 wt %, thermo-reversible gels were formed above the phase transition temperature of PNIPAM. According to this work, increasing the wt % of polymer in solution led to stronger gels and lower temperatures necessary for gelation to occur.

\textit{pH-Responsive Block Copolymers}

In 1997 one of the first examples of pH-responsive block copolymers was reported by Bütün and coworkers.\textsuperscript{88} These block copolymers were synthesized from 2-(dimethylamino)ethyl methacrylate, DMAEMA (M\textsubscript{8}), and 2-(diethylamino)ethyl methacrylate, DEAEMA (M\textsubscript{9}), by group transfer polymerization (GTP). At low pH values the block copolymers molecularly dissolve in aqueous solution due to protonation of their respective tertiary amine groups. Upon increasing the pH to 9.5, the PDEAEMA block becomes deprotonated undergoing a phase transition and leading to micelle formation. DLS results showed a drastic increase in size from unimers at pH 2.0 to micelles with apparent hydrodynamic diameters of approximately 20 nm at pH 9.5. Lee and coworkers\textsuperscript{89} later reported a more detailed study of the self-assembly behavior of these block copolymers. They reported, utilizing DLS, that the exact pH leading to micelle formation was 7.4. At this pH, a significant number of PDEAEMA units were deprotonated leading to aggregation. They also demonstrated that adding electrolytes leads to a lower critical pH value for micelle formation.
Bütün et al.\textsuperscript{90} later polymerized an AB block copolymer exhibiting what is now characterized as “schizophrenic” micellization behavior. The “schizophrenic” behavior results from the capability of both blocks to undergo a phase transition at different solution conditions. This polymer was synthesized by GTP from the monomers DEAEMA (M9) and 2-(N-morpholino)ethyl methacrylate (MEMA) leading to a narrowly dispersed polymer with a PDI of 1.05. As stated previously, the PDEAEMA block exhibits a hydrophilic to hydrophobic transition at higher pH values due to deprotonation of the tertiary amines. PMEMA undergoes a similar transition at neutral pH upon the addition of electrolytes such as Na\textsubscript{2}SO\textsubscript{4}.\textsuperscript{91} At neutral pH with no added salt, this block copolymer was found to be molecularly dissolved as unimers in solution. Increasing the pH led to micelle formation with PDEAEMA cores and a hydrodynamic diameter of 32 nm. At pH 6.7 and 1.0 M Na\textsubscript{2}SO\textsubscript{4}, micelles consisting of PMEMA cores with 26 nm diameters were observed. Bütün and coworkers\textsuperscript{92} later reported a more detailed study on this block copolymer system where block compositions were varied and studied by SLS and small-angle neutron scattering (SANS). Correlating the SLS and SANS data showed an increase in the mean micelle diameter and decrease in aggregation number for DEAEMA versus MEMA-core micelles. This led to the conclusion that the MEMA-core micelles are more dense and compact than those of the loosely packed DEAEMA-core micelles.

Liu and Armes\textsuperscript{93} later prepared “schizophrenic” diblock copolymers from 4-vinylbenzoate, (M20), and DEAEMA (M9) utilizing atom transfer radical polymerization (ATRP). Because these block copolymers are amphoteric, with the VBA block containing a carboxylic acid and the DEAEMA block containing a tertiary amine,
micellar aggregation occurs both at high and low pH values. The hydrodynamic diameter of the aggregates as a function of pH was monitored utilizing DLS. At pH 2, well-defined VBA-core micelles were formed with a size of 36 nm. Increasing the pH to 6 led to larger aggregates due to interactions between the deprotonated carboxylic acids and the protonated tertiary amines with sizes of 120 nm. Above pH 6 and below pH 8.5 the polymer precipitated from solution. At pH 10 well-defined micelles with compact DEAEMA cores of 35 nm in size were formed.

In 2001 Mitsukami et al.\textsuperscript{40} from our lab synthesized the first stimuli-responsive diblock copolymers in aqueous solution utilizing RAFT. In this report CTP (\textbf{CTA1}) and V-501 (\textbf{14}) were used to polymerize either sodium 4-styrenesulfonate, SS (\textbf{M19}), or ar-vinylbenzyl)trimethylammonium chloride, VBTAC (\textbf{M10}). These PSS and PVBTAC macroCTAs were subsequently used for blocking with 4-vinylbenzoate, (\textbf{M20}), and N,N-dimethylvinylbenzylamine, DMVBA (\textbf{M11}), respectively. The P(SS-\textit{b}-VBA) and P(VBATC-\textit{b}-DMVBA) polymers were then analyzed by DLS at varying pH values. At low pH, the P(SS-\textit{b}-VBA) formed multimolecular aggregates with a hydrodynamic diameter of 19 nm. At high pH, both blocks are ionized resulting in unimers of approximately 8 nm diameters. In the case of the P(VBATC-\textit{b}-DMVBA) polymer, high pH values result in unimeric species with a diameter of 8 nm due to protonation of the tertiary amine while high pH values render the PDMVBA block hydrophobic leading to micellar aggregates with hydrodynamic diameters of 38 nm. Mitsukami et al.\textsuperscript{94} later reported a more detailed study of the P(VBATC-\textit{b}-DMVBA) block copolymer system. In this study a series of block copolymers were synthesized where the PVBATC block was kept constant while the PDMVBA block lengths were varied. \textsuperscript{1}H NMR was utilized
to show the broadening of peaks associated with the PDMVBA block at high pH values indicating successful micelle formation. These results were confirmed by DLS data showing that below pH 8 the block copolymer exists as unimers while above this pH value they aggregate into micelles. Their DLS results also showed that as the block length of PDMVBA increases so too does the resulting hydrodynamic diameter of the micelles. Utilizing static light scattering, this increase in diameter was shown to correspond to an increase in aggregation number.

Following this work, Sumerlin et al.\textsuperscript{38} from our lab reported the synthesis of anionic acrylamido-based polymers directly in water utilizing the RAFT technique. This was accomplished by utilizing CTP (CTA\textsuperscript{1}) and V-501 (I\textsuperscript{4}) for the polymerization of either AMPS (M\textsuperscript{16}) or sodium 3-acrylamido-3-methylbutanoate, AMBA (M\textsuperscript{17}), producing controlled polymers with PDIs > 1.3. These macroCTAs were subsequently used for blocking with the opposite monomer to show that well-defined block copolymers were obtained regardless of the blocking order. Later work by Sumerlin et al.\textsuperscript{95} detailed the responsive behavior of these block copolymers in solution. A series of block copolymers were synthesized with a constant DP of 70 for the PAMPS block while the AMBA block length was varied from a DP of 62 to 16. Fluorescence spectroscopy studies demonstrated that below a pH of 5.5 the block copolymers assembled into micelles. DLS revealed that the sizes of the micelles at pH 1 were directly related to the block length of PAMBA, with diameters increasing as the PAMBA DP increases.

Interestingly, a very similar block copolymer system to the one discussed above was reported by Yusa and coworkers\textsuperscript{39} at approximately the same time. These block copolymers were also polymerized via RAFT using CTP (CTA\textsuperscript{1}) and V-501 (I\textsuperscript{4}) from
the monomers AMPS (M16) and sodium 6-acrylamidohexanoate (AaH). The P(AMPS-
b-AaH) polymers exhibited a phase transition at pH values ≤ 4.5 as determined by DLS. Also, as the pH decreased the size of the micelles decreased which was attributed to further protonation of the carboxylic acid groups and, hence, a more compact core.

Ma and coworkers\textsuperscript{96} reported ABA triblock copolymers comprised of pH-responsive outer blocks and a zwitterionic, biocompatible inner block. These polymers were made utilizing a bifunctional ATRP species to first polymerize 2-
(diisopropylamino)ethyl methacrylate (DPAEMA) followed by subsequent blocking with 2-methacryloyloxyethyl phosphorylchloride, MPC (M24). At pH values < 4, the polymers molecularly dissolved in aqueous solution; however, increasing the pH led to micelle formation with an intensity-average diameter around 68 nm as indicated by DLS studies in dilute solution (0.1 w/v %). Increasing the polymer concentration (> 5 to 10 w/v %, depending on the copolymer composition) led to pH reversible gelation.

In 2005 Determan and coworkers\textsuperscript{97} synthesized ABCBA pentablock copolymers based on commercially available Pluronic® block copolymers that exhibited both pH- and thermo-responsive behavior. The Pluronic® precursor, poly(ethyleneoxide-\textit{b}-
propyleneoxide-\textit{b}-ethyleneoxide) (PEO\textsubscript{100} \textit{b}-PPO\textsubscript{65} \textit{b}-PEO\textsubscript{100}), contained terminal hydroxyl groups which were subsequently reacted to form difunctional ATRP-based macroinitiators. These macroinitiators were utilized to polymerize a variety of tertiary amine-based monomers, namely DMAEMA (M8) and DEAEMA (M9). The pH-dependent micellization behavior of these pentablock copolymers was investigated by quasielastic light scattering and multi angle light scattering. The Pluronic® precursor showed no pH-responsive aggregation while polymers of P(DMAEMA\textsubscript{50} \textit{b}-EO\textsubscript{100} \textit{b}-

\textsuperscript{96} Ma and coworkers, \textsuperscript{97} Determan and coworkers.
PO<sub>65</sub>-b-EO<sub>100</sub>-b-DMAEMA<sub>50</sub>) and P(DEAEMA<sub>50</sub>-b-EO<sub>100</sub>-b-PO<sub>65</sub>-b-EO<sub>100</sub>-b-

DEAEMA<sub>50</sub>) aggregated into micelles when 50 and 20 % of the amines were protonated, respectively. The aggregates and sizes were also investigated via cryo-TEM analysis, showing relatively good agreement with light scattering results. These pentablock copolymers were also investigated at higher concentrations (20 wt %) to show variable gelation at different temperatures and pH values.

Zhang and coworkers<sup>98</sup> later describe the micellization behavior of a thermo- and pH-responsive triblock copolymer synthesized via ATRP. This triblock copolymer was synthesized using a poly(ethylene glycol) (PEG) macroinitiator by the sequential polymerization of 4-vinylpyridine, 4VP (M<sub>13</sub>), and NIPAM (M<sub>3</sub>). DLS measurements indicated that the polymer molecularly dissolves in water at room temperature at a pH of 2 with an average diameter of around 7 nm. Upon increasing the pH to 6.5 and deprotonating the P4VP block, the diameter increases to approximately 42 nm indicating micelle formation. Micelle formation was confirmed by performing SLS to obtain the R<sub>g</sub> of the aggregates (14 nm). The R<sub>g</sub>/R<sub>h</sub> value of 0.68 is indicative of spherical micelles.<sup>99-101</sup> TEM was also utilized to visualize the spherical nature of the micelles. A critical aggregation temperature at pH 2 was found to be approximately 35 °C which is slightly higher than the LCST for PNIPAM and is likely due to enhanced solubility from the PEG and P4VP. At 50 °C and pH 2, the micelle diameter was determined to be 130 nm. By increasing the pH to 6.5 while maintaining a temperature of 50 °C, both the P4VP and PNIPAM blocks are rendered insoluble, resulting in a smaller diameter of 118 nm.

In 2007 Zhang <i>et al.</i><sup>102</sup> utilized stopped-flow light scattering and fluorescence techniques to probe the pH-responsive micellization kinetics of a pyrene end-capped
diblock copolymer synthesized from DMAEMA ($M_8$) and DEAEMA ($M_9$). Taking advantage of the pyrene probe, fluorescence measurements of the excimer-to-monomer ratio ($I_E/I_M$) provide information on aggregation and compactness of the pyrene groups.\textsuperscript{103-106} Upon a pH jump from 4 to 9, both scattered light intensity and $I_E/I_M$ increased abruptly and then gradually to reach a plateau value. The fastest process, only observed by stopped-flow fluorescence, occurred within 4 ms and was attributed to the burst formation of small transient micelles comprising of only a few chains (too small to be detected by light scattering). These micelles then undergo rapid fusion to quasi-equilibrium micelles (ranging from 0.2 to 0.35 sec) with their final equilibrium state reached after approximately 10 to 20 sec.

Similar stopped-flow studies were reported by Zhang and coworkers\textsuperscript{107} on a pH- and thermo-responsive diblock copolymer polymerized from NIPAM ($M_3$) and DEAEMA ($M_9$) via RAFT. At pH 8 and room temperature, micelles with a hydrodynamic radius of 67 nm were observed by light scattering. At pH 4 and 42 °C, light scattering revealed micelles with a radius of 75 nm. According to the stopped-flow experiments, upon a pH jump from 4 to 12 at room temperature, two relaxation time constants in the ranges of 20-40 ms and 140-200 ms were observed depending on the polymer concentration. Because the slower time constant depended on polymer concentration, a micelle fusion mechanism was proposed. Upon a temperature jump from 20 to 45 °C at pH 4, relaxation time constants with a range of 1-3 sec and 20-25 sec were determined. The slower time constant was found to be independent of polymer concentration, suggesting that unimer insertion/expulsion was the more favorable
mechanism for micellar growth. This was attributed to electrostatic repulsion from the PDEAEMA corona making micelle fusion less favorable.

Wang and Lowe\textsuperscript{45} recently reported the aqueous RAFT polymerization of novel styrenic-based phosphonium monomers which were subsequently blocked to synthesize a pH-responsive block polyampholyte. Utilizing 2-(2-carboxy-ethylsulfanylthiocarbonylsufanyl) propionic acid, CEP (CTA\textsubscript{4}), as the CTA and V-501 (I\textsubscript{4}) as the initiator, 4-vinylbenzyl(trimethylphosphonium) chloride, TMP (M\textsubscript{12}), was polymerized via RAFT in deuterium oxide at 80 °C. According to aqueous size exclusion chromatography (SEC) analysis, the experimental polymer molecular weights were in good agreement with the theoretical values with vary narrow PDIs (> 1.1) resulting in the synthesis of well-defined homopolymers. This PTMP macroCTA was used in the polymerization of VBA (M\textsubscript{20}) for the synthesis of a block polyampholyte. Due to inherent difficulties with characterizing polyampholytes by either organic or aqueous SEC, the polymer molecular weight and PDI could not directly be determined. Fourier-transform infrared (FTIR) spectroscopy was, therefore, utilized to qualitatively demonstrate successful formation of block copolymers. The pH-responsive assembly of this diblock copolymer was investigated by examining \textsuperscript{13}C NMR spectra at pH values of 10 and 2. At pH 10, all resonances associated with PTMP and PVBA were present. Lowering the pH to 2 resulted in the disappearance of the carbonyl resonance and broadening of the aromatic resonances, consistent with a hydrophilic to hydrophobic phase transition. Subsequently, Lowe et al.\textsuperscript{44} reported the synthesis and self-assembly behavior of diblock copolymers of TMP (M\textsubscript{12}) and DMVBA (M\textsubscript{11}) synthesized via RAFT. The pH-responsive behavior was assessed by DLS and 1H NMR at pH values of 2 and 12. At pH 2, both blocks are
soluble due to the protonated tertiary amines on the PDMVBA block. An increase in the pH to 12 resulted in deprotonation and self-assembly with the PDMVBA block forming the hydrophobic micelle core, stabilized by the hydrophilic PTMP corona. As evidenced by DLS experiments, at low pH values, the polymers were reported to have a diameter between 3 to 6 nm, depending on the molecular weight of the polymer. At high pH values, the diameter increased to approximately 30 nm, indicative of aggregation.

Another pH-induced nanoassembly was later reported by Lee and coworkers\textsuperscript{108} from block copolymers synthesized via the nitroxide mediated polymerization (NMP) of acrylic acid, AA (M\textsubscript{21}) and $p$-hydroxystyrene ($p$HS) using protecting group chemistry. With pK\textsubscript{a} values of approximately 4 and 10 for the PAA and $p$HS blocks, respectively, the block copolymer was expected to form micelles below pH values of 10 and precipitate below pH values of 4. $^1$H NMR and DLS experiments were used to demonstrate the pH-induced self-assembly of these block copolymers while AFM and TEM were used to visualize the spherical nature of the micellar assemblies.

Recently, Sumerlin’s group\textsuperscript{109} prepared novel water-soluble boronic acid block copolymers of poly(4-vinylphenylboronic acid-$b$-DMA), P(APBA-$b$-DMA), via RAFT polymerization. Later the same group\textsuperscript{110} reported the synthesis of a poly(3-acrylamidophenylboronic acid-$b$-DMA) block copolymer which showed novel pH- and sugar-responsive behavior. Boronic acids are uniquely stimuli-responsive to both pH and solution diol concentration. Acidic conditions render the acids neutral (typically insoluble) while basic conditions result in boronates which are anionically charged (soluble). In the presence of vicinal diols such as glucose, water-soluble boronate esters are formed. DLS measurements were used to study the unique solution behavior
exhibited by this block copolymer. P(APBA-b-DMA) molecularly dissolves at pH 10.7 to give unimers with a diameter of 7 nm. Decreasing the pH below the pKₐ of PAPBA (∼9) leads to aggregate formation with hydrodynamic diameters of 35 nm. As previously mentioned, boronate esters are formed in the presence of diols; therefore, upon addition of 45 mM glucose at pH 8.7, the PAPBA resolublized, resulting in a decrease in the diameter to 9 nm. Sumerlin’s group¹¹¹ later synthesized block copolymers of P(APBA-b-NIPAM) exhibiting triply-responsive behavior to temperature, pH, and sugar concentration. The aggregation behavior was studied via DLS experiments. At 25 °C and pH 11, both blocks are soluble resulting in a hydrodynamic diameter of 8 nm. Lowering the solution pH to 8.7 leads to a phase transition of the PAPBA block and formation of aggregates of approximately 55 nm. By adding glucose under these same conditions, aggregate dissociation occurs. The temperature responsive behavior was demonstrated by increasing the temperature to 50 °C at pH 11 where aggregates with diameters of 78 nm were observed.

Cross-Linked Nanoassemblies

The use of block copolymer assemblies as drug delivery vehicles has been well documented; however, certain limitations of these nanostructures prohibit the realization of their use in practical applications. Namely, dilution-induced dissociation of the aggregates into unimers after administration in vivo is the major disadvantage of such systems. When the polymer concentration falls below the critical aggregation concentration (CAC), the nanostructures dissociate, resulting in premature release of the payload. In order to circumvent such stability issues, cross-linking techniques have been developed.
In 1996, Wooley and coworkers\textsuperscript{112} first reported shell cross-linked (SCL) micelles utilizing a diblock copolymer of polystyrene and 4-(chloromethyl)styrene-quaternized P4VP. Shell cross-linking of the precursor micelles was achieved by radical oligomerization of the pendent styrenyl groups on the coronal P4VP blocks. Unlike conventional micelles, these SCL micelles were stable at infinite dilution. Since this seminal work, a number of chemistries have been developed for the preparation of SCL nanoassemblies. For example, Ding and Liu\textsuperscript{113} reported the synthesis of an AB block copolymer of poly(styrene-b-2-cinamoylethyl methacrylate), P(S-b-CEMA), where the PCEMA shells were cross-linked by photolysis with UV light. Later, Wooley and coworkers developed a novel cross-linking method utilizing carbodiimide coupling.\textsuperscript{114-119} First, block copolymers of poly(styrene-b-acrylic acid) were synthesized either by anionic polymerization or ATRP. Next, the polymers were dissolved in THF followed by the addition of water to induce micelle formation. Shell cross-linking was achieved by activation of the carboxylic acid groups utilizing 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide. The addition of 2,2′-(ethylenedioxy)bis(ethylamine) subsequently resulted in SCL micelles.

Alternative cross-linking techniques have also been developed to accomplish cross-linking directly in water. Armes’ group\textsuperscript{120} reported one of the first examples of a SCL micelle with a tunable hydrophilic/hydrophobic core. In this report, a P(DMAEMA-\textit{b}-MEMA) diblock copolymer was synthesized by GTP followed by selective quaternization of 30 % of the PDMAEMA block. At pH 10 and 60 °C, the PMEMA block becomes hydrophobic, forming the core of the micelle. Shell cross-linking of the unquaternized DMAEMA units was achieved by adding 1,2-bis-(2-iodoethoxy)ethane
Prior to cross-linking, the micelles had a diameter of 36 nm, while afterwards the size decreased to 28 nm. Upon cooling and rendering the PMEMA block hydrophilic, the SCL micelles remained intact with a size of 30 nm. Since this preliminary work, Armes’ group has reported numerous examples of stimuli-responsive micelles cross-linked with BIEE. Another cross-linking technique introduced by this group utilizes a Michael addition between divinyl sulfone and pendent hydroxyl functionalities.

Although both of these techniques could be accomplished under mild reaction conditions, the reagents are mutagenic and less toxic alternatives were desirable. As such, Weaver and coworkers utilized polyelectrolyte complexation for ionic cross-linking micelles possessing a charged shell. In that report, a PEO-based ATRP macroinitiator was utilized to polymerize DMAEMA and subsequently blocked with DEAEMA. At pH values greater than 7.5, micelles with diameters ranging from 35 to 50 nm were formed. The cationic PDMAEMA block was utilized for interpolyelectrolyte complexation (IPEC) with either a P(EO-b-SS) or PSS polymer. Subsequent lowering of the pH to resolublize the PDEAEMA block resulted in the maintenance of micelles in solution due to the IPEC cross-linking. These polyelectrolyte cross-linked complexations offer several advantages over the previously discussed cross-linking methods including low toxicity, relatively fast physical cross-linking, lack of small molecule byproducts, and reversibility by the addition of salt.

Since this preliminary work, our group has investigated several stimuli-responsive block copolymer systems capable of forming SCL micelles and vesicles utilizing IPEC formation. In 2006, Li and coworkers reported the formation of temperature-responsive
vesicles from P(APMA-\textit{b}-NIPAM) block copolymers. At increased temperatures, vesicle formation occurred with hydrodynamic diameters of approximately 280 nm. Since PAPMA contains a primary amine, the degree of protonation can be varied with pH; therefore, the size of the vesicles could be tailored depending on the pH of the solution (from 310 nm at pH 3 to 220 nm at pH 10.8). The cationic PAPMA shells were subsequently utilized for the formation of IPEC with the anionic homopolymer, PAMPS (Scheme I-4). After cross-linking, the diameter decreased from 270 to 140 nm which was attributed to neutralization of the shell. Successful cross-linking was demonstrated by lowering the temperature to resolubilize the PNIPAM blocks where the size of the vesicles remained constant. The cross-linking was then shown to be reversible by increasing the electrolyte concentration to 0.8 M NaCl.

\textbf{Scheme I-4.} Formation of vesicles from P(APMA-\textit{b}-NIPAM) and subsequent IPEC with PAMPS.\textsuperscript{43}
Lokitz et al.\textsuperscript{129} in our lab later demonstrated the successful shell cross-linking of micelles derived from an amino acid-based monomer. Tri- and pentablock copolymers of DMA (M2), N-acryloylalanine (AAL) (M18), and NIPAM (M3) were polymerized using either EMP (CTA2) or CMP (CTA3) via aqueous RAFT polymerization. Upon increasing the temperature, the block copolymer self-assembled into micelles with PNIPAM cores. DLS experiments showed that increasing the PNIPAM block length led to an increase in the size of the formed micelles and a decrease in the critical aggregation temperature at which micellization occurs. At 50 °C, the micelles were cross-linked utilizing IPEC between the PAAL block and a cationic homopolymer of PVTAC with an equimolar amount of cationic to anionic repeats as outlined in Scheme I-5. The cross-linked micelles remained intact upon decreasing the temperature where the PNIPAM block becomes water-soluble. The cross-linked micelle sizes decreased as the temperature was lowered which was attributed to the loss of electrostatic charges from the PAAL block. The cross-linked micelles remained intact up to the addition of 0.4 M NaCl. Interestingly, micellar aggregates were reformed above 0.8 M NaCl due to the “salting out” of the PNIPAM block. Later work by Lokitz and coworkers\textsuperscript{130} demonstrated the temperature/pH-responsive behavior of a similar block copolymer. In that case, a PDMA macroCTA was utilized to polymerize a statistical block containing both NIPAM (M3) and N-acryloylvaline (AVAL). Due to the incorporation of AVAL within the PNIPAM block, the self-assembly behavior was dependent on both pH and temperature. The carboxylic acids from the incorporated AVAL also rendered this system amenable to shell cross-linking through IPEC formation. DLS experiments were utilized to show the formation of micelles at pH 4.5 and 50 °C with sizes of 97 nm. Upon
the addition of an equimolar ratio of VBTAC:AVAL units, the cross-linked micelles exhibited a size decrease to approximately 52 nm due to charge neutralization.

“Locking” of the micelles was demonstrated by decreasing the temperature to 25 °C where the micelles remained intact with diameters of 57 nm. The reversibility of this system was also demonstrated with the addition of NaCl.

**Scheme 1-5.** Temperature induced micellization of P(DMA-b-AAL-b-NIPAM) and subsequent IPEC formation with PVBTAC.\(^{129}\)

Another novel cross-linking technique developed in our lab consists of the reaction of a difunctional amine with an activated ester moiety incorporated in the shell of nanoassemblies. Recently, Li and coworkers\(^ {131}\) reported the synthesis of a PEO macroCTA used to polymerize a statistical block of DMA (M2) and N-acryloylsuccinimide (NAS) followed by blocking with NIPAM (M3). At 45 °C after micellization is achieved, the NAS moieties were subsequently reacted with ethylene diamine to form SCL micelles. The reaction proceeded rapidly with over 95 %
completion in two hours. The micellar structure was maintained as monitored by DLS as the temperature was lowered. By incorporating less NAS in the statistical copolymer, a more swollen micelle was observed at low temperatures due to an overall lower degree of cross-linking. Although this is a facile method for the preparation of SCL micelles, the cross-linking cannot be reversed. In order to circumvent this, Li and coworkers utilized a cystamine, disulfide-containing diamine cross-linker with the same block copolymer system. The resulting disulfide cross-links were cleaved through the reduction by a thiol exchange reaction with either dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine hydrochloride (TCEP) as outlined in Scheme I-6. By removal of these reducing agents at increased temperatures, addition of cystamine results in the reformation of the SCL micelles through a thiol/disulfide exchange reaction. These micelles were also loaded with dipyridamole, a model hydrophobic compound, with the release monitored by UV-vis spectroscopy. A comparison between cross-linked and uncross-linked micelles at 25 °C showed that the SCL micelles significantly retarded the release rate; however, only 75 % was released after seven days with the remaining stabilized within the core. Release studies at 37 °C with and without DTT showed that release in the presence of DTT was much faster due to cleavage of the cross-links, making these SCL micelles good candidates as drug delivery vehicles.
Scheme I-6. Formation of reversible SCL micelles from P(EO-\text{-}b-(DMA-s-NAS)-b-NIPAM) cross-linked with cystamine.$^{132}$

Xu \textit{et al.}$^{133}$ recently reported the use of a cleavable, temperature-responsive polymeric cross-linker for the formation of SCL micelles. In that study, a PEO-based macroCTA was synthesized from 4-cyano-4-(propylsulfanylthiocarbonyl)sulfanylpentanoic acid, CPP (CTA5), and utilized to polymerize APMA (M7) and DPAEMA. This triblock copolymer exhibited pH-responsive behavior, forming micelles above a pH of 6 due to deprotonation of the PDPAEMA block. The polymeric cross-linker was synthesized by the RAFT polymerization of NIPAM (M3) utilizing CMP (CTA3) followed by end group functionalization to an activated ester via carbodiimide coupling. The primary amine functionality in the shell of the micelle was reacted with the polymeric cross-linker. After cross-linking, these SCL micelles slightly decreased in diameter from 55 to 46 nm. Upon lowering the pH to protonate the PDPAEMA block, swelling of the micelles to 82 nm occurs, indicating successful cross-linking. A later report by Xu \textit{et al.}$^{134}$ utilized the same pH-responsive triblock copolymer to achieve a “one-pot” synthesis of reversible SCL micelles. Here, a water-soluble, reversibly
cleavable cross-linker, dimethyl 3,3-dithiobispropionimide (DTBP), was employed to “lock” the micelles. This disulfide-containing DTBP cross-linker provided a reversibly cleavable site similar to the work done by Li et al.\textsuperscript{132}

In 2007, Li and coworkers\textsuperscript{135} from our lab introduced another novel cross-linking technique based on the in situ formation of gold nanoparticles. In this report, block copolymers were synthesized from DMAEMA (M\textsubscript{8}) and NIPAM (M\textsubscript{3}) and the thermo-responsive behavior was studied utilizing DLS measurements. At room temperature, the polymers are molecularly dissolved with diameters of 8 nm while increasing the temperature above 38 °C led to the formation of vesicles with diameters of 140 nm. Previous reports showed that small molecule amines could be used as reducing agents in the formation of gold nanoparticles.\textsuperscript{136} Also, Armes et al.\textsuperscript{137} showed that PDMAEMA could be used to reduce \( \text{AuCl}_4^- \) to zero-valent gold while, at the same time, stabilizing the resulting gold nanoparticles. Therefore, a solution with a 10:1 ratio of DMEAMA units:NaAuCl\textsubscript{4} was kept at 50 °C for two days, after which the solution temperature was lowered to 25 °C. DLS analysis detected no dissociation, indicating successful “locking” of the vesicle structure. TEM images of the SCL vesicles showed that the morphology was maintained after cross-linking.

Subsequently, Smith et al.\textsuperscript{138} in our lab utilized this same cross-linking technique to “lock” the morphology of several block copolymer nanostructures. In that report, block copolymers of P(DMAEMA\textsubscript{-}b-NIPAM) were synthesized with a constant PDMAEMA DP of 165 while the PNIPAM DP was varied (either 100, 200, or 435). These block lengths were chosen in order to vary the hydrophilic/hydrophobic mass fractions in hopes of obtaining various morphologies.\textsuperscript{139} The effect of temperature,
solution pH, and NaCl concentration on the aggregation of these block copolymers was investigated utilizing DLS. The polymer aggregate morphologies were then “locked” for TEM analysis using the same gold cross-linking procedure reported by Li et al.\(^\text{135}\)

**Layer-by-Layer Films**

Layer-by-layer (LbL) assembly, introduced by Decher and co-workers,\(^\text{140}\) is a promising method for the creation of structural and functional thin films on solid substrates. By using this technique, thin films can be fabricated on almost any substrate composition or topology, even colloidal particles,\(^\text{141}\) with many different materials incorporated into the individual multilayers. A major advantage of LbL assembly is that multiple driving forces including hydrogen bonding,\(^\text{142-145}\) charge transfer,\(^\text{146,147}\) acid-base pairs,\(^\text{148}\) metal-ion coordination\(^\text{149,150}\) and covalent bonds\(^\text{151}\) can complement electrostatic assembly. Due to the ease and versatility of electrostatic assembly, it is the dominant LbL process reported in the literature.

Since the early 1990s, LbL films have been explored for a variety of applications. Additionally, the applicability of the LbL technique extends beyond polyelectrolyte systems. Almost any type of charged species, including inorganic molecular clusters,\(^\text{152}\) nanoparticles,\(^\text{153}\) nanotubes and nanowires,\(^\text{154,155}\) nanoplates,\(^\text{156}\) organic dyes,\(^\text{157}\) biological polysaccharides,\(^\text{158}\) polypeptides,\(^\text{159}\) nucleic acids and DNA,\(^\text{160}\) proteins,\(^\text{161}\) and viruses,\(^\text{162}\) can be incorporated components used in LbL films. The broad scope of the LBL process has catalyzed the rapid development of polyelectrolyte multilayer films for potential biomedical applications. This translates into a wide variety of structural characteristics
and functional properties, including multilayer films that are responsive to various stimuli, e.g. light, pH, salt, and temperature.

**Electrostatic Layer-by-Layer Process**

Prior to film formation, an appropriate solid substrate must be selected. Many surfaces, such as metals, silicones, and glasses, have net negative charges in solution due to surface oxidation and hydrolysis. Often, a silicon wafer is utilized as the substrate due to reflective properties which enable ellipsometricconfirmation of film thickness and also due to the smooth surface which facilitates characterization by atomic force microscopy (AFM). While a silicon substrate is ideal for the characterization methods described, the LbL technique is not limited to 2-dimensional substrates, which is another advantage over other coating techniques.

Once an appropriate substrate has been selected, electrostatic layer-by-layer assembly is achieved by alternating exposure of the substrate between solutions containing species of complementary affinities. Strong electrostatic attractions form between charged molecules in solution and an oppositely charged surface leading to the adsorption of charged molecules and surface charge reversal to that of the adsorbed molecules. This charge reversal has two major consequences including the ability of an oppositely charged molecule to be absorbed on top of the first one, and the restriction of a single deposited layer due to the repulsion of equally charged molecules. Scheme I-7 illustrates the assembly process schematically where steps 1 and 3 are the deposition steps of the cationic and anionic layers, respectively, and steps 2 and 4 are washings.
Scheme I-7. (A) Schematic of the multilayer film assembly process. Steps 1 and 3 represent the deposition of the cationic and anionic layers, respectively. Steps 2 and 4 are washing steps. (B) Simplified picture of the deposition of the first two layers of a multilayered film starting with an anionic surface; counter ions omitted for clarity.

The concentration of polymer in solution is normally in the few milligrams per milliliter range for typical multilayer films. Such a high concentration ensures that the solutions do not become depleted during the preparation of films with multiple layers. Between each adsorption step, a rinsing step of varied time is employed to help avoid contamination of the next adsorption solution along with the removal of weakly adsorbed polyelectrolytes.¹⁷⁰
Factors Influencing Film Growth

The electrostatic LBL process has been explained by the over-compensation of a charge at each adsorption step. Each exposure deposits a reproducible quantity of charged polyelectrolytes and reverses the top surface charge, making it ready for the next adsorption step. The adsorption behavior of polyelectrolytes is influenced by many factors including ionic strength\textsuperscript{171,172} and/or the pH\textsuperscript{173,174} of the polymer assembly solution, solvent quality,\textsuperscript{172,173,175} and charge density.\textsuperscript{176-179} Recent experimental\textsuperscript{180,181} and theoretical\textsuperscript{182-184} studies reported that nonelectrostatic short-range interactions have an important role in multilayer film formation. Short-range interactions that have been reported include van der Waals and hydrogen bonding.\textsuperscript{182,185}

Past experimental and theoretical studies focused on the use of strong polyelectrolytes where the charge density does not change dramatically over a wide pH range. These systems solely depend on electrostatic interactions for film formation and film properties are mainly affected by the molecular weight and charge density of the polyelectrolytes. Recently, incorporation of weak polyelectrolytes has been used as a way to create stimuli-responsive multilayer systems in which the pH of the polyelectrolyte solution governs the ionization of the polymer chains.\textsuperscript{141,173,174,186-194}

Initial studies demonstrated the applicability of LbL for strong or weak synthetic and natural polyelectrolytes. One such study by Choi and Rubner\textsuperscript{195} investigated a series of weak polyelectrolytes, poly(allylamine hydrochloride) and poly(acrylic acid), and the relationship of charge density to film growth. This study demonstrated that thin, flat layers formed during assembly of high charge density polyelectrolytes, while much thicker adsorbed layers were observed for low charge density polyelectrolyte LbL films.
Analysis of systems containing strong polyelectrolytes was performed by Sui and coworkers\textsuperscript{196} who prepared a series of films incorporating various combinations of strong polyelectrolytes and weak polyelectrolytes. The films assembled exclusively from strong polyelectrolytes exhibited thicknesses under ten nanometers for ten bilayer films, while systems incorporating weak polyelectrolytes were consistently thicker, with a thickness of 45 nm for a 10 bilayer film. The effect of polymer molecular weight was investigated; however, the strength of the charge present was determined to be the main factor in determining film thickness. Strong polyelectrolytes exhibit intramolecular charge-charge repulsions creating linear, extended conformations in solution which result in deposition of thinner films than those created from weak polyelectrolytes. Weak polyelectrolytes exist in a conformation closer to a random coil, which explains the increased thickness at each deposited layer within the studied films.

The effect of polymer microstructure was evaluated by Morgan et al.\textsuperscript{197} This was the first report of the use of well defined polymers synthesized by RAFT to produce homopolymers and block copolymers with precise molecular weights, polymer architectures, and narrow polydispersity indices (PDIs) for use in LbL films. In this study several distinct molecular architectures were incorporated into LbL films and the resulting morphologies investigated. Small changes in polymer architecture produced significant effects in LBL film morphology and thickness. Block copolymers containing P(AMBA-\textit{b}-AMPS), weak and strong polyanions, respectively, were synthesized at three different ratios of block lengths (40 \%, 50 \%, and 60 \% relative to the PAMBA block) and compared to a statistical copolymer of the same composition. As the length of the strong anionic block (PAMPS) increased, the overall relative thickness of the films
decreased, as did the measured roughness. This work elucidates the concept that linear, extended strong polyelectrolytes form thin layers compared to weakly charged polyelectrolytes. When systems of mixed layers of strong and weak polyelectrolytes were formed, intermediate thicknesses were observed, in between the low thicknesses reported for strong-strong polyelectrolyte systems (PAMPS layered with quaternized PDMAEA) and the high thicknesses reported for weak anionic polymers layered with weak cationic layers (PAMBA layered with protonated PDMAEA). When the copolymer architecture was random as opposed to blocky, the film thickness was double that of the block copolymer (246 nm and 124 nm respectively), even though the chemical composition was exactly the same. The pH responsiveness of LBL films formed from the different copolymer combinations was also evaluated with respect to the strength of the polyelectrolyte interactions.

Recently, the impact of substrate on structural characteristics was demonstrated in a study by Buron et al.\textsuperscript{198} where it was reported that the functionality of the substrate directly impacted the coverage of the films in the initially deposited bilayers, leaving incomplete coverage until a significant number of bilayers were deposited. Micropatterned silica was used to demonstrate this effect, with varying chemical modifications shown to affect water contact angle measurements and coverage properties. This study demonstrated an important method for controlling morphology via chemical modification. A major limitation of this study was due to thermal cleaning at 1000°C, which yielded an oxidative layer with a measured thickness of 100 nm, about four to five times thicker than can be achieved though chemical oxidative cleaning. While micropatterned regions display differing morphology in the AFM height images
produced, little can be concluded about which morphological features are attributed to the underlying substrate versus the chemical functionality on the surface of the substrate.

In 2007, Hammond et al. reported using amphiphilic dendrimers to incorporate a drug, triclosan, into LbL films. This study demonstrated that the growth of *S. aurus* bacteria was inhibited by a ten bilayer film containing triclosan. Release profiles, fluorescence spectroscopy, and UV-vis measurements were utilized to characterize the release profile of these films. Although significant, this report does not include analysis of the morphology of the film surface, which has been demonstrated to affect cellular adhesion and growth.

*Micelles in Layer-by-Layer Films*

Attempts have been made to functionalize surfaces with micelles. In 2000, Emoto et al. reported the creation of a multilayer film from the homopolymer polyallylamine layered with poly(ethylene glycol)-co-poly(D,L-lactide), which was demonstrated to act as an antifouling coating. This system relied on hydrophobic effects to organize the multilayers, which were covalently bound to each other. The core of the micelles was cross-linked to ensure that the micelle structure was retained within the multilayer film. The thicknesses and morphologies of the multilayer films were not reported, but the release profiles for the protein BSA and pyrene (which had been loaded into the cores of these micelles) were generated from fluorescence spectroscopy monitored as a function of time. The change in fluorescence of aminated glass coated with a hexapoid layer of micelles was demonstrated after exposure to the pyrene-loaded micelle solution.

More recently, Ma et al. proved the existence of diblock copolymer micelles of poly(acrylic acid-*b*-styrene) (PAA-*b*-PS: anionic PAA corona) as a second layer on top of
an initial layer of linear poly(diallyldimethylammonium chloride) using atomic force microscopy. The obtained AFM images showed that the glassy nature of the PS cores allows for excellent image resolution of the incorporated micelle structure. Different degrees of polymerization resulted in different sized micelles.

Building on the idea of micelle incorporation, LbL films composed solely of diblock copolymer micelles have been reported recently. For example, Qi et al.\textsuperscript{203} constructed alternating multilayers composed of micelles of poly(quaternised 4-vinylpyridine)-\textit{b}-poly(\textit{tert}-butyl acrylate), (PQ4VP-\textit{b}-PrBA: cationic PQ4VP corona) and poly-(acrylic acid)-\textit{b}-poly(4-vinylpyridine), (PAA-\textit{b}-P4VP: anionic PAA corona). Cho et \textit{al.}\textsuperscript{204} reported the formation of micellar multilayers based on P4VP-\textit{b}-PS and PAA-\textit{b}-PS (with cationic P4VP and anionic PAA coronas, respectively). In these cases, the retention of the relatively robust, high glass-transition cores of the micelles deposited during multilayer construction was inferred from UV-vis spectroscopy, fluorescence spectroscopy, SEM or tapping mode AFM.

In addition to micelle-micelle multilayer construction on planar substrates, Biggs \textit{et al.}\textsuperscript{205} recently reported the first example of a LbL film of stimulus-responsive diblock copolymer micelles on a particulate substrate. Furthermore, the authors demonstrated increased color intensity with each layer deposition, utilizing the preloading of a hydrophobic dye within the micelle cores to infer the retention of micelle character for each new layer. However, direct visualization of the actual retained core-shell structure was not possible. They concluded that the use of a multilayer of block copolymer micelles may enhance the stability of the adsorbed layer due to local chain interactions within each layer, as well as offer a potential route to optimizing the rate of release of
entrained active species, either via diffusion through the film or by limiting the loading to specific layers.

Most recently a report by Tan and coworkers\textsuperscript{206} demonstrated temperature-induced reversible swelling transitions in a multilayer film. This was achieved by incorporating a P(DMAEMA-\textit{b}-PO-\textit{b}-DMAEMA) triblock copolymer as the cationic layer. Due to the temperature responsive behavior of PPO, swelling was demonstrated when the film was submersed into solutions at low temperatures. The swelling ratio reached a maximum of 4 in DI water; however, by changing the salt concentration or pH of the submersion solution, this value was increased to 6.
CHAPTER II

OBJECTIVES OF RESEARCH

Of all the living radical polymerization techniques, reversible addition–fragmentation chain transfer (RAFT) polymerization is arguably the most versatile in terms of the reaction conditions (e.g. temperature and solvent selection), monomer selection (e.g. neutral, anionic, cationic, and zwitterionic), and purification. Since the introduction of RAFT in 1998, the McCormick research group and others including the Lowe, Sumerlin, and Davis research groups have synthesized a wide range of (co)polymers with predetermined molecular weights, low polydispersities, and advanced architectures utilizing aqueous RAFT (ARAF) polymerization. These research groups have also studied how various block copolymers exhibit stimuli-responsive behavior due to a change in temperature, solution pH, or electrolyte concentration. However the stimuli-responsive behavior of unprotected, chiral, amino acid-based polymers had yet to be reported. The incorporation of these homopolymers into stimuli-responsive block copolymers will create novel polymer systems that can be reversibly “locked” under facile conditions and have potential applications in sequestration and targeted delivery. The overall goal of this research is to utilize the RAFT process for the synthesis of such block copolymers directly in water, investigate the relationship between block copolymer composition and solution properties on the self-assembly behavior of the copolymers, and incorporate these micelles within films via the layer-by-layer technique to produce stimuli-responsive films for applications such as drug release from surfaces.
Specific objectives of the research are to:

1) Synthesize a well-defined series of block copolymers of sodium 2-acrylamido-2-methyl-1-propanesulfonate (AMPS) (M14) and N-acyloyl-L-alanine (AAL) (M16);

2) Investigate the effect of block length, solution pH, and electrolyte concentration on aqueous assembly behavior;

3) Characterize the assembled polymeric micelles or vesicles using dynamic and static light scattering, transmission electron microscopy, atomic force microscopy, and $^1$H NMR;

4) Utilize interpolyelectrolyte complexation to cross-link the assemblies using polymers synthesized from either N-[3-(dimethylamino) propyl] acrylamide (DMAPA) (M5) or N,N-dimethylaminoethyl methacrylate (DMAEMA) (M8);

5) Study the effect of the cationic polymer used for cross-linking on the pH reversibility of the cross-linked assemblies;

6) Incorporate the assembled micelles into layer-by-layer (LbL) films and examine film morphology via atomic force microscopy;

7) Investigate the stimuli-responsive behavior of the micelle-incorporated LbL films;

8) Examine the release of pyrene from micelles within the LbL films at varying solution pH values.

The above objectives have been attained as described in the three sections of Chapter IV. The first section concerns utilizing ARAFT polymerization for the successful synthesis of a series of novel pH-responsive block copolymers containing an unprotected amino acid-based block. Block copolymers containing a permanently anionically
charged hydrophilic block of AMPS and a pH-responsive AAL block were subsequently synthesized and the aqueous self-assembly behavior was investigated. The aggregation behavior for a series of P(AMPS-\textit{b}-AAL) was determined at varying pH values and salt concentrations. The effect of the permanently hydrophilic and responsive block lengths on the stimuli driven assembly behavior was examined. The second section details the cross-linking of these micelles using interpolyelectrolyte complexation (IPEC) with cationic polymers. This is the first report of a pH reversible IPEC cross-linked micellar system. The third section details work done in collaboration with Christopher Harris and concerns the incorporation of micelles possessing anionically charged coronas within layer-by-layer films. The effect of salt concentration on film thickness and morphology was studied. Also because the films are made using pH-responsive block copolymers, the responsive behavior of the polyelectrolyte multilayer films was also investigated.
CHAPTER III

EXPERIMENTAL

Materials

All reagents were purchased from Aldrich at the highest purity available and used as received unless otherwise stated. 4-Cyanopentanoic acid dithiobenzoate, CTP (CTA1), was synthesized according to literature procedures.40 4,4′-Azobis(4-cyanopentanoic acid), V-501 (I4), was donated by Wako Chemicals and was recrystallized twice from methanol before use. 2-Acrylamido-2-methyl-1-propanesulfonic acid, AMPS (M16), was recrystallized twice from methanol prior to use. N,N-Dimethylaminopropyl acrylamide, DMAPA (M5), was purchased from TCI and vacuum distilled prior to use. N,N-Dimethylaminoethyl methacrylate, DMAEMA (M8), was dried with CaH2 and vacuum distilled prior to use. N-acryloyl-L-alanine, AAL (M18), was synthesized according to literature procedures.129
General Procedure for the RAFT Polymerization of AMPS

A solution of AMPS (M₁₆) (13.1 g, 63.1 mmol), CTP (CTA₁) (56.3 mg, 0.2 mmol), and V-501 (I₄) (11.3 mg, 0.04 mmol) were added along with deionized (DI) water (55 mL) to a round-bottom flask, and the solution pH was adjusted to 6.5. After purging with nitrogen for 30 min, the polymerization was allowed to proceed at 70 °C for 2 hours. The reaction was terminated by cooling the reaction flask in liquid nitrogen followed by exposure to air. The resultant PAMPS₁₁₀ (P₁) (Mₐ = 25,100 g/mol, PDI =
1.10) and PAMPS\textsubscript{225} (\textbf{P2}) (\(M_n = 51,400\) g/mol, PDI = 1.14) macroCTAs were purified by dialysis against DI water for three days and isolated by lyophilization (Figure III-2).

**General Procedure for the RAFT Synthesis of P(AMPS-b-AAL)**

As outlined in Scheme III-1, a series of block copolymers were synthesized via the chain extension of PAMPS\textsubscript{110} with AAL. As an example, a solution of PAMPS\textsubscript{110} macroCTA (\textbf{P1}) (0.60, 0.024 mmol), AAL (\textbf{M18}) (1.06 g, 7.4 mmol), and V-501 (\textbf{I4}) (1.3 mg, 0.005 mmol) along with deionized (DI) water (8 mL) to a round-bottom flask, and the solution pH was adjusted to 6.5. After sparging with nitrogen for 30 min, the polymerization was allowed to proceed at 70 °C for 5.5 hours. The reaction was terminated by cooling the reaction flask in liquid nitrogen followed by exposure to air. The products P(AMPS\textsubscript{110}-b-AAL\textsubscript{185}) (\textbf{P3}) (\(M_n = 51,800\) g/mol, PDI = 1.23), P(AMPS\textsubscript{110}-b-AAL\textsubscript{305}) (\textbf{P4}) (\(M_n = 68,700\) g/mol, PDI = 1.27), P(AMPS\textsubscript{110}-b-AAL\textsubscript{490}) (\textbf{P5}) (\(M_n = 95,100\) g/mol, PDI = 1.23) were purified by dialysis against DI water for 3 days and isolated by lyophilization (Figure III-2).

Additionally, a series of block copolymers were synthesized via the chain extension of PAMPS\textsubscript{225} with AAL. As an example, a solution of PAMPS\textsubscript{110} macroCTA (\textbf{P2}) (1.23, 0.024 mmol), AAL (\textbf{M18}) (2.00 g, 14.0 mmol), and V-501 (\textbf{I4}) (1.3 mg, 0.005 mmol) along with deionized (DI) water (8 mL) were added to a round-bottom flask, and the solution pH was adjusted to 6.5. After sparging with nitrogen for 30 min, the polymerization was allowed to proceed at 70 °C for 5.5 hours. The reaction was terminated by cooling the reaction flask in liquid nitrogen followed by exposure to air. The products P(AMPS\textsubscript{225}-b-AAL\textsubscript{350}) (\textbf{P6}) (\(M_n = 101,300\) g/mol, PDI = 1.22), P(AMPS\textsubscript{225}-b-AAL\textsubscript{660}) (\textbf{P7}) (\(M_n = 145,900\) g/mol, PDI = 1.26), P(AMPS\textsubscript{225}-b-AAL\textsubscript{1000})
(P8) \((M_n = 198,400 \text{ g/mol}, \text{PDI} = 1.29)\) were purified by dialysis against DI water for 3 days and isolated by lyophilization (Figure III-2).

**Scheme III-1.** Preparation of pH-responsive block copolymers of AMPS and AAL via RAFT polymerization.
General Procedure for the RAFT Polymerization of DMAPA

As outlined in Scheme III-2, DMAPA (M5) (10.4 g, 66.6 mmol) was polymerized directly in an aqueous acetic buffer (pH = 5.2, 0.27 mol/L acetic acid and 0.73 mol/L sodium acetate) (50 mL) at 70 °C, employing V-501 (I4) (0.003 g, 0.011 mmol) as the primary radical source and CTP (CTA1) (0.015 g, 0.055 mmol) as the RAFT chain transfer agent (CTA). Polymerizations were performed with an initial monomer concentration ([M]₀) of 1.0 M and an initial CTA to initiator ratio ([CTA]₀:[I]₀) of 5:1 under a nitrogen atmosphere in a 100 mL round-bottom flask equipped with a magnetic stir bar and sealed with a rubber septum. The products PDMAPA₂₀₀ (P9) (Mₙ = 30,800

Figure III-2. PAMPS macroCTAs and responsive block copolymers of P(AMPSₓ-b-AALᵧ) synthesized by RAFT polymerization.
g/mol, PDI = 1.04) and PDMAPA_{995} (P^{10}) (M_n = 155,500 g/mol, PDI = 1.06) were purified by dialysis against deionized water for three days and isolated by lyophilization (Figure III-3).

Scheme III-2. Synthesis of the cationic homopolymer PDMAPA.

General Procedure for the RAFT Polymerization of DMAEMA

As outlined in Scheme III-3, a solution of CTP (CTA_{1}) (0.015 g, 0.055 mmol), DMAEMA (M_{8}) (2.00 g, 12.7 mmol), and V-501 (I_{4}) (0.003 g, 0.011 mmol) in 6.5 mL of dioxane were added to a 25 mL round bottom flask sealed with a rubber septum. The solution was sparged with nitrogen for 30 min and the flask was placed in a preheated oil bath at 70 °C. The reaction was terminated after 8 h (70 % conversion) by cooling in liquid nitrogen followed by exposure to air. The product PDMAEMA_{160} (P^{11}) (M_n = 25,200 g/mol, PDI = 1.11) was purified by dialysis against deionized water for three days and isolated by lyophilization (Figure III-3).
Scheme III-3. Synthesis of the cationic homopolymer PDMAEMA.

Figure III-3. Cationic homopolymers of PDMA and PDMAEMA synthesized by RAFT polymerization.

Self-Assembly and Cross-Linking of Block Copolymers

Self-Assembly of the $P(AMPS_x\text{-b-}AAL_y)$ Block Copolymers

Copolymers were dissolved directly in HPLC grade water at a concentration of 1 mg/mL (0.1 wt %). The pH of the solution was subsequently adjusted to 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 using 1 M HCl. The salt (NaCl) concentration of these solutions was varied from 0 to 2 M in increments of 0.1 M. Micellization occurred at a critical pH and salt concentration.
Shell Cross-Linking of P(AMPS-b-AAL) Nanostructures via IPEC

Solutions of 0.05 wt % P(AMPS-b-AAL) were prepared at a pH value of 1.0 to induce self-assembly. An aqueous solution of PDMAPA<sub>990</sub>, PDMAPA<sub>200</sub>, or PDMAEMA<sub>160</sub> was prepared at a concentration of 0.05 wt % and adjusted to pH 1.0. These cationic polymer solutions were then slowly added to the P(AMPS-b-AAL) micelle solution at a rate of 0.05 mL/min and allowed to stir for one hour upon completion. The final mole ratio of AMPS:cationic repeat was varied from 8:1, 4:1, 3:1, 2:1, and 1:1.

(Co)Polymer Characterization

**Size Exclusion Chromatography**

Size exclusion chromatography (SEC) was used to determine the number-average molecular weight (M<sub>n</sub>) and polydispersity indices (PDI) for all homo- and block copolymers. The PAMPS macroCTAs and block copolymers of P(AMPS<sub>x</sub>-b-AAL<sub>y</sub>) were analyzed by aqueous size exclusion chromatography (ASEC) using an aqueous eluent of 20%/80% acetonitrile/0.05 M Na<sub>2</sub>SO<sub>4</sub> (aq). A flow rate of 0.3 mL/min, TOSOH Biosciences TSK-GEL columns (G3000 PWXL, <50000 g mol<sup>−1</sup>, 200 Å) and G4000PWXL (2000–300000 g mol<sup>−1</sup>, 500 Å), Wyatt Optilab DSP interferometric refractometer, and a Wyatt DAWN EOS multiangle laser light scattering detector (690 nm) were employed in the analysis. The dn/dc of PAMPS (0.181 mL/g) and P(AMPS-b-AAL) (0.170 mL/g) in the ASEC eluent were determined at 35 °C. The absolute molecular weights and polydispersities of PDMAPA and PDMAEMA were determined by ASEC using SynChropak CATSEC columns (100, 300, and 1000 Å; Eichrom Technologies Inc.), Wyatt Optilab DSP interferometric refractometer, a Wyatt DAWN
DSP multiangle laser light scattering detector ($\lambda = 690$ nm), and 1 wt % acetic acid/0.1 M Na$_2$SO$_4$ (aq) as the eluent at a flow rate of 0.25 mL/min. The $dn/dc$ of PDMAPA (0.163 mL/g) and PDMAEMA (0.160 mL/g) in the cationic eluent was determined at 35 °C.

Copolymer Characterization Using $^1$H NMR

$^1$H NMR measurements were performed with a Mercury Innova spectrometer operating at a frequency of 499.8 MHz. P(AMPS-b-AAL) samples were prepared in D2O (HOD internal standard) at 0.05 wt % and spectra were obtained at pD values of 7.0 and 1.0. A PDMAPA sample was prepared in D2O (HOD internal standard) at 0.05 wt % and a spectrum was obtained at a pD value of 7.0. Upon formation of IPEC cross-linked micelles, spectra were recorded at pD values of 1.0, 7.0, and 9.0.

Characterization of Self-Assembled Nanostructures

Dynamic and Static Light Scattering

Dynamic light scattering measurements were conducted on the block copolymer series at a concentration of 1.0 g/L in aqueous solution using a Malvern Instruments Zetasizer Nano ZS series instrument equipped with a 4 mW He-Ne laser operating at $\lambda = 632.8$ nm at an angle of 173°, an avalanche photodiode detector with high quantum efficiency, and an ALV/LSE-5003 multiple $\tau$ digital correlator electronics system. Dispersion Technology Software 5.03 (Malvern Instruments) was used to record and analyze the data to determine particle size distributions.

Variable-angle DLS and SLS measurements were made using incident light at 633 nm from a Spectra Physics HeNe operating at 40 mW. The angular dependence of the autocorrelation functions was measured using a Brookhaven Instruments BI-200SM goniometer with an avalanche photodiode detector and TurboCorr correlator. Correlation
functions were analyzed according to the method of cumulants using the companion software. All data reported correspond to the average decay rate obtained from the second cumulant fit. Apparent diffusion coefficients ($D_{app}$) were obtained from the slope of the relaxation frequency ($\Gamma$) versus the square of the scattering vector ($q^2$) where

$$q = \frac{4\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right)$$  \hspace{1cm} (6)

$\lambda$ is the wavelength of the incident laser (633 nm), $\theta$ is the scattering angle, and $n$ is the refractive index of the media. The hydrodynamic radius ($R_h$) was then calculated from the Stokes-Einstein equation (Eq. 5)

$$R_h = \frac{k_B T}{6\pi \eta D_{app}}$$  \hspace{1cm} (5)

where $k_B$ is the Boltzmann constant, $T$ is the temperature, and $\eta$ is the viscosity of the medium.

Angular–dependent static light scattering (SLS) experiments were performed on aqueous polymer solutions with the same instrument as described above. The radius of gyration ($R_g$) of the assemblies was determined from the angular dependence of the scattering intensity. A Zimm plot of the scattering intensity ($I_{ex}$) versus the square of the scattering vector ($q$) was used to determine the radius of gyration ($R_g$). A Berry plot ($I_{ex}^{-1/2}$ vs. $q^2$) is used in instances where a Zimm treatment results in upward curvature of the data when $qR_g \geq 1$.

Solutions for SLS experiments were prepared by dissolving the polymer in purified water at a concentration of 0.01 wt%. Samples were agitated to ensure complete dissolution and then filtered through a 0.45 $\mu$m PVDF syringe-driven filter (Millipore)
directly into the scattering cell. Samples were then sonicated and allowed to reach thermal equilibrium prior to measurements.

**Transmission Electron Microscopy**

Transmission electron microscopy (TEM) measurements were conducted using a JEOL JEM-2100 electron microscope at an acceleration voltage of 200 kV. Samples were prepared by placing a 5 µL drop of the 0.1 wt % nanoparticle solution on a Formvar-coated copper grid followed by water evaporation in an incubator at 45 °C.

**Atomic Force Microscopy**

Atomic force microscopy (AFM) imaging was performed with a Veeco Dimension 3000 AFM (Veeco Instruments Inc.). Morphologies were investigated in tapping mode operation in air. An RTSP, silicon cantilever (Veeco Probes, Santa Barbara, CA) was used for dry imaging. Samples were prepared by placing a 5 µL drop of the 0.1 wt % nanoparticle solution on a neat silicon wafer followed by water evaporation at room temperature.

**Preparation and Characterization of Layer-by-Layer Films**

**Preparation of Multilayer Films**

Silicon wafers, approx 1.5 × 3 cm², were cleaned with piranha solution (70:30 concentrated H₂SO₄/35 wt % H₂O₂) overnight to ensure a clean and uniform oxide surface. Wafers were then rinsed with DI water and ethanol and dried with a gentle stream of nitrogen. The clean silicon wafers were used immediately. Polyelectrolyte deposition solutions were prepared by dissolving the polymer in HPLC-grade water with a 1 mg/mL concentration. The pH of the solution was adjusted to 1.0 ± 0.1 for both anionic and cationic polymers by adding appropriate amounts of concentrated HCl.
Multilayer films were produced by alternately exposing the substrate to the respective solutions of polycation and polyanion for 15 min. The substrate was rinsed for 2 min between each deposition using 2 different beakers containing pH 1.0 HPLC-grade water. Five thickness measurements were then conducted via ellipsometry. Error bars represent the standard deviation from five measurements taken on the film. Note throughout this work that a layer is defined as one polyanion or polycation layer and a bilayer is defined as the layer pair of a polyanion and a polycation.

*Ellipsometry*

Ellipsometry measurements were performed on a Gaertner Scientific LSE-Stokes ellipsometer with an angle of incidence of 70° using a 632.8 nm He-Ne laser and Gaertner GEMP software. The real part of the refractive index of the silicon wafers was fixed to 3.85 and its imaginary part to -0.02. The real refractive index value of the films has been chosen equal to 1.455.

*Atomic Force Microscopy*

Atomic force microscopy imaging and film thickness measurements were made with a Veeco Dimension 3000 AFM (Veeco Instruments Inc.). Film morphology of dried assembled multilayer films was investigated in tapping mode operation in air in a temperature and humidity controlled room. Macroscopically separated areas were imaged for each film and representative images shown. An RTSP, silicon cantilever (Veeco Probes, Santa Barbara, CA) was used for dry imaging and surface scratching was used to determine surface thickness on samples of thickness greater than ~100 nm. The film was scraped from the silicon surface using a razor blade and the film edge imaged. Step-height thickness measurements and general image processing were performed using
Veeco version 5.30R3.Sr² software and Gwyddion v2.9 software. The image root-mean-square roughness (RMS) is calculated as the root-mean-square average of the height deviations taken from the mean data plane. The errors reported for the film thickness are the standard deviations of all of the measurements taken.

Fluorescence Spectroscopy

A 0.5 mg/mL stock solution of pyrene in acetone was prepared. To a 150 mL 1 mg/mL micelle solution, 6 mL of the pyrene solution was added. After filtration, this solution was used to prepare LbL films on quartz slides. Release of pyrene was monitored via fluorescence measurements of the film at varying submersion intervals. Fluorescence emission spectra were recorded with a PTI QuantaMaster™ 40 steady state spectrofluorometer. An excitation of 334 nm was used while the scan range was from 355 to 500 nm with a 1 nm stepsize, integration of 0.5 seconds, and averaging of 2 scans. FeliX32 software was used for data analysis.
CHAPTER IV

RESULTS AND DISCUSSION

This work may be divided into three sections. The first section concerns utilizing aqueous reversible addition-fragmentation (ARAF) polymerization for the successful synthesis of a series of novel pH-responsive block copolymers containing an unprotected amino acid-based block. Block copolymers containing a permanently anionically charged hydrophilic block of sodium 2-acrylamido-2-methyl-1-propanesulfonate, AMPS (M16), and a pH-responsive N-acryloyl-L-alanine, AAL (M18), block were subsequently synthesized and the aqueous self-assembly behavior was investigated. The aggregation behavior for a series of P(AMPS-\text{-}b\text{-}AAL) was determined at varying pH values and salt concentrations. The effect of the permanently hydrophilic and responsive block lengths on the stimuli-driven assembly behavior was examined. The second section details the cross-linking of these micelles using interpolyelectrolyte complexation (IPEC) with cationic polymers. This is the first report of a pH-reversible IPEC cross-linked micellar system. The third section details work done in collaboration with Christopher Harris and concerns the incorporation of micelles possessing anionically charged coronas into layer-by-layer films. The effect of salt concentration on film thickness and morphology was studied. Also because the films are made using pH-responsive block copolymers, the responsive behavior of the polyelectrolyte multilayer films was also investigated.
Section I. Development of Novel pH/Salt-Responsive ARAFT-Synthesized P(AMPS-b-AAL) Block Copolymers

Overview

Recently, a great deal of interest has been focused on the synthesis of well-defined, water-soluble block copolymers capable of self-assembling in response to external stimuli. These polymers typically contain a permanently hydrophilic block and a responsive block which upon application of an external stimulus (i.e. temperature, pH, or electrolyte concentration) is rendered hydrophobic. Upon conversion to a hydrophilic-hydrophobic copolymer, self-assembly into higher order structures such as micelles and vesicles are possible.\textsuperscript{129,207-210} The ability to control the assembly/disassembly process through environmental cues makes these materials attractive candidates for controlled release applications in which hydrophobic agents are loaded into the core of the structure, and subsequently carried until exposed to an external stimulus.\textsuperscript{116,117,122,123,211-221} In many cases, control of the solution pH is more convenient than manipulation of temperature or salt concentration.\textsuperscript{89,93,222-224} Moreover, pH-induced micelles have been reported to yield cores with more hydrophobic character than temperature-induced micellization.\textsuperscript{223}

Herein, we report the strategic design of pH-responsive, micelle-forming block copolymers which contain an anionically charged corona and an insoluble protonated core at low pH. The block copolymers which comprise the micelles were synthesized \textit{via} aqueous reversible addition-fragmentation chain transfer (ARAF) polymerization and consist of a hydrophilic, anionically charged poly(sodium 2-acrylamido-2-methyl-1-propanesulfonate) (PAMPS) block and a pH-responsive poly($N$-acyryloyl-$L$-alanine)
(PAAL) block. These block copolymers undergo a reversible unimer-to-micelle transition upon lowering the solution pH.

**ARAFT Synthesis of Responsive P(AMPS-b-AAL) Block Copolymers**

A series of block copolymers of AMPS (M16) and AAL (M18) were synthesized according to Scheme IV-1. Two PAMPS macro-CTAs with targeted degrees of polymerization (DPs) of 100 and 200 were first prepared by employing CTP (CTA1) to control the polymerization at 70 °C, using V-501 (I4) as the primary radical source and maintaining a solution pH of 6.5 in order to reduce CTA hydrolysis. Monomer conversion was approximately 35 % in order to maintain the dithioester chain-end functionality for efficient blocking with AAL. The resultant PAMPS\textsubscript{110} (P1) and PAMPS\textsubscript{225} (P2) macroCTAs had number average molecular weight (M\textsubscript{n}) and polydispersity index (PDI) values of 25,100 Da (1.10) and 51,400 Da (1.14), respectively. The PAMPS\textsubscript{110} macroCTA was chain extended with AAL to give three block copolymers with DPs of 185 (P3), 305 (P4), and 490 (P5). The PAMPS\textsubscript{225} macroCTA was chain extended with AAL to give three block copolymers with DPs of 350 (P6), 660 (P7), and 1000 (P8). These block copolymers were specifically designed to obtain PAAL block weight fractions of 0.75, 0.65, and 0.50 in hopes of tailoring their solution morphology upon self-assembly. SEC chromatograms of the two copolymer series are shown in Figures IV-1 and IV-2. All of the SEC traces are unimodal with narrow PDIs (< 1.3) indicating near-quantitative blocking efficiency and controlled polymerization. The molecular weight, PDI, and composition data for these series of block copolymers are summarized in Table IV-1.
Scheme IV-1. Synthetic Pathway for the Preparation of P(AMPS$_x$-b-AAL$_y$) Block Copolymers via Aqueous RAFT Polymerization.

Figure IV-1. GPC chromatograms for the PAMPS$_{110}$ macroCTA and subsequent chain extension yielding three P(AMPS$_{110}$-b-AAL$_{185}$) block copolymers using reversible addition-fragmentation chain transfer polymerization.
Figure IV-2. GPC chromatograms for the PAMPS<sub>225</sub> macroCTA and subsequent chain extension yielding three P(AMPS<sub>225</sub>-b-AAL<sub>x</sub>) block copolymers using reversible addition-fragmentation chain transfer polymerization.
Table IV-1. Molecular Weight ($M_n$), Polydispersity Index (PDI), and AMPS/AAL Weight Percents for P(AMPS$_x$-b-AAL$_y$) Block Copolymers.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$ (kDa)$^b$</th>
<th>PDI$^b$</th>
<th>AMPS/ALAla (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(AMPS$_{110}$)</td>
<td>25.1</td>
<td>1.10</td>
<td>100/0</td>
</tr>
<tr>
<td>(P1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(AMPS$<em>{110}$-b-AAL$</em>{185}$) (P3)</td>
<td>51.8</td>
<td>1.23</td>
<td>48/52</td>
</tr>
<tr>
<td>P(AMPS$<em>{110}$-b-AAL$</em>{305}$) (P4)</td>
<td>68.7</td>
<td>1.27</td>
<td>36/64</td>
</tr>
<tr>
<td>P(AMPS$<em>{110}$-b-AAL$</em>{490}$) (P5)</td>
<td>95.1</td>
<td>1.23</td>
<td>26/74</td>
</tr>
<tr>
<td>P(AMPS$_{225}$)</td>
<td>51.4</td>
<td>1.14</td>
<td>100/0</td>
</tr>
<tr>
<td>(P2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(AMPS$<em>{225}$-b-AAL$</em>{350}$) (P6)</td>
<td>101.3</td>
<td>1.22</td>
<td>51/49</td>
</tr>
<tr>
<td>P(AMPS$<em>{225}$-b-AAL$</em>{660}$) (P7)</td>
<td>145.9</td>
<td>1.26</td>
<td>35/65</td>
</tr>
<tr>
<td>P(AMPS$<em>{225}$-b-AAL$</em>{1000}$) (P8)</td>
<td>198.4</td>
<td>1.29</td>
<td>26/74</td>
</tr>
</tbody>
</table>

$^a$ Subscripts represent the degree of polymerization (DP) for the respective blocks. $^b$ As determined by aqueous size exclusion chromatography (ASEC).

**Self-Assembly Behavior of P(AMPS-b-AAL)**

The self-assembly behavior of the block copolymers was followed by dynamic light scattering (DLS). Below the $pK_a$ of PAAL (3.5), segments are protonated rendering them hydrophobic and leading to precipitation. PAMPS, a strong polyacid, which remains negatively charged even at extremely low pH values and high salt concentrations, is permanently hydrophilic across the pH and salt concentrations utilized in this research. As such, the block copolymers P(AMPS$_x$-b-AAL$_y$) are expected to form AAL-core and AMPS-shell nanostructures when the solution pH is lowered below the $pK_a$ of PAAL. However, the block copolymers did not form aggregates around the $pK_a$.
of the PAAL block in water at ambient conditions. After further investigation, it was determined that aggregation could be induced by further lowering the pH to protonate the entire PAAL block (pH = 1) or by adding small molecule electrolytes at pH values \( \leq 3.5 \). It is reasoned that the addition of salt promotes assembly by screening the charges of the PAMPS and any unprotonated carboxylic acids on the PAAL. Similar results have been previously reported by Armes and coworkers\(^8^9\) for PDMAEMA/PDEAEMA block copolymers.

The aggregation behavior of the block copolymer series synthesized from the PAMPS\(_{110}\) macro-CTA was studied utilizing DLS. For these studies the solution pH values were varied from 4.0 to 1.0 in 0.5 increments. Given the salt dependent assembly, the electrolyte concentration was also varied in order to determine the critical salt concentration (CSC) necessary for the formation of aggregates at specific solution pH values. Figure IV-3 shows the hydrodynamic diameter of P(AMPS\(_{110}-b\)-AAL\(_{185}\)) (P3) (50 wt % PAAL) at designated pH and salt concentrations. When no electrolyte is present, the block copolymers remain unimers in solution (approximately 15 nm) throughout the entire pH ranged studied. By adding electrolytes to the solution, aggregation is promoted by screening charges of the highly ionized PAMPS block and any residually deprotonated carboxylate groups on the PAAL block and breaking up hydrogen bonding between water molecules and the protonated carboxylic acids on the PAAL block. DLS results determined that at a salt concentration of 0.15 M NaCl and a pH value of 1.0, aggregation occurred resulting in nanostructures with a hydrodynamic diameter of approximately 56 nm. By further increasing the salt concentration, self-assembly could be induced at higher pH values. At a salt concentration of 1.8 M NaCl,
aggregation occurred at the pKa value of the PAAL block (3.5); however, above this pH even with increased electrolyte concentration, self-assembly could not be achieved, likely due to the presence of an abundant amount of deprotonated carboxylic acids. As shown in Figure IV-3, a general trend is observed demonstrating that as the solution pH increases so does the ionic strength required for aggregate formation.

Figure IV-3. Hydrodynamic diameter versus solution pH for a 0.1 wt % P(AMPS$_{110}$-b-AAL$_{185}$) (P3) solution shown at salt concentrations of 0.15 (■), 0.2 (○), 0.3 (▲), 0.7 (▼), 1.2 (△), and 1.8 M NaCl (▲).

The same study was conducted on P(AMPS$_{110}$-b-AAL$_{305}$) (P4) (65 wt % PAAL) to determine the effect of increasing the weight percent of the responsive block on the self-assembly behavior of these block copolymers. This block copolymer also remained as unimers in solution throughout the entire pH range evaluated without the presence of
electrolytes; however, at pH 1.0 a salt concentration of only 0.05 M NaCl (0.1 M NaCl less than for the previously studied polymer) was required to promote aggregation resulting in nanostructures with a hydrodynamic diameter of approximately 60 nm. The lower CSC values obtained at all pH values studied for this block copolymer compared with the previous one was typical. Shown in Figure IV-4 are the DLS data demonstrating the hydrodynamic diameter for P(AMPS\textsubscript{110-b-AAL\textsubscript{305}}) (P4) within the pH range of 1.0 to 4.0 at varying salt concentrations.

![Figure IV-4](image-url)

**Figure IV-4.** Hydrodynamic diameter versus solution pH for a 0.1 wt % P(AMPS\textsubscript{110-b-AAL\textsubscript{305}}) (P4) solution shown at salt concentrations of 0.05 (■), 0.1 (●), 0.2 (▲), 0.5 (▼), 1.0 (▲), and 1.6 M NaCl (◄).

DLS was also utilized to study the CSC at the varying pH values for the P(AMPS\textsubscript{110-b-AAL\textsubscript{490}}) (P5) (75 wt % PAAL) block copolymer (Figure IV-5). When no electrolyte is present, the block copolymers remained unimers in solution (approximately 15 nm) above pH 1.0; however, at pH 1.0 the block copolymers aggregated into micelles
with average hydrodynamic diameters of approximately 65 nm. From these results, two trends were observed for the $\text{P(AMPS}_{110}\text{-b-AAL}_{\gamma})$ series. As the DP of the PAAL block increases (185, 305, and 490) and, hence, the hydrophobicity of the block copolymer, their respective aggregates increase in size (56, 60, and 65 nm) along with lower CSC values required for aggregate formation at a specific pH value (0.15, 0.05, and 0 M NaCl at pH 1.0). Shown in Figure IV-5 are the DLS data demonstrating the hydrodynamic diameter for $\text{P(AMPS}_{110}\text{-b-AAL}_{490})$ (P5) within the pH range of 1.0 to 4.0 at varying salt concentrations.

**Figure IV-5.** Hydrodynamic diameter versus solution pH for a 0.1 wt % $\text{P(AMPS}_{110}\text{-b-AAL}_{490})$ (P5) solution shown at salt concentrations of 0 (■), 0.05 (●), 0.15 (▲), 0.3 (▼), 0.8 (▲▲), and 1.4 M NaCl (◄).
In order to better represent the effect of the responsive block length on the aggregation behavior of each of these block copolymers, a plot of the CSC required for micelle formation as a function of solution pH for P(AMPS\textsubscript{110-}b-AAL\textsubscript{185}) (P\textsubscript{3}), P(AMPS\textsubscript{110-}b-AAL\textsubscript{305}) (P\textsubscript{4}), and P(AMPS\textsubscript{110-}b-AAL\textsubscript{490}) (P\textsubscript{5}) was constructed as seen in Figure IV-6. By maintaining a constant PAMPS block length, the effect of the PAAL block length on self-assembly can be determined. As demonstrated in the figure, increasing the PAAL block length leads to a decrease in the CSC required for micelle formation at a specific pH value. Although the percentage of protonated PAAL units remains the same for each block copolymer at a specific pH value, the overall hydrophobicity of the polymers increases as the PAAL block length increases resulting in less screening necessary for micelle formation. This is in some ways analogous to block copolymers consisting of PNIPAM as the responsive block where the critical micelle temperature for smaller NIPAM blocks is higher than those with longer NIPAM blocks.\textsuperscript{225,226}
Figure IV-6. Critical salt concentration (CSC) required for the formation of micelles as a function of pH as determined from dynamic light scattering for P(AMPS$_{110}$-b-AAL$_{185}$) (P3) (■), P(AMPS$_{110}$-b-AAL$_{305}$) (P4) (●), and P(AMPS$_{110}$-b-AAL$_{490}$) (P5) (▲).

Evaluation of the P(AMPS$_{225}$-b-AAL$_y$) block copolymer series was performed in a similar manner in order to validate the results from the previous study. Figure IV-7 shows a plot of the hydrodynamic diameter of P(AMPS$_{225}$-b-AAL$_{350}$) (P6) (50 wt % PAAL) as a function of pH at varying salt concentrations. It is interesting to note that when no salt is present, the block copolymer formed aggregates at pH values up to 1.5 whereas P(AMPS$_{110}$-b-AAL$_{185}$) (P3), having the same responsive block weight percent, requires the addition of electrolytes even at a pH of 1.0 for aggregation to occur. The nanostructures were determined to have sizes ranging between 90 and 100 nm, depending on the pH and salt concentration.
Figure IV-7. Hydrodynamic diameter versus solution pH for a 0.1 wt % P(AMPS\textsubscript{225}-\textit{b}-AAL\textsubscript{350}) (\textbf{P6}) solution shown at salt concentrations of 0 (■), 0.1 (●), 0.4 (▲), 0.9 (▼), and 1.8 M NaCl (♦).

DLS results for the hydrodynamic diameter of P(AMPS\textsubscript{225}-\textit{b}-AAL\textsubscript{660}) (\textbf{P7}) (65 wt % PAAL) as a function of pH at varying salt concentrations are shown in Figure IV-8. The nanostructures formed by this block copolymer system had hydrodynamic diameters of approximately 110 nm. This polymer self-assembled below a pH value of 2.5 without the need for added electrolytes while its counterpart from the previous series, P(AMPS\textsubscript{110}-\textit{b}-AAL\textsubscript{305}) (\textbf{P4}), required the addition of salt for aggregate formation even at a pH value of 1.0. These results suggest that the weight percent of the responsive block is not the key factor contributing to aggregation.
Figure IV-8. Hydrodynamic diameter versus solution pH for a 0.1 wt % P(AMPS$_{225}$-$b$-AAL$_{660}$) (P7) solution shown at salt concentrations of 0 (■), 0.2 (●), 0.7 (▲), and 1.6 M NaCl (▼).

Finally, the aggregation behavior of the block copolymer P(AMPS$_{225}$-$b$-AAL$_{1000}$) (P8) (75 wt % PAAL) was examined utilizing DLS as shown in Figure IV-9. This polymer self-assembled below a pH value of 2.5 without the need for added electrolytes forming aggregates with hydrodynamic diameters of approximately 160 nm. From these results, the same trends observed for the P(AMPS$_{110}$-$b$-AAL$_{y}$) series were confirmed. As the DP of the PAAL block increases (350, 660, and 1000) and, hence, the hydrophobicity of the block copolymer, their respective aggregates increase in size (100, 110, and 160 nm) along with lower CSC values required for aggregate formation at a specific pH value (0.4, 0.2, and 0.1 M NaCl at pH 2.5).
Figure IV-9. Hydrodynamic diameter versus solution pH for a 0.1 wt % P(AMPS$_{225}$-b-AAL$_{1000}$) (P₈) solution shown at salt concentrations of 0 (■), 0.1 (●), 0.6 (▲), and 1.4 M NaCl(▼).

In order to better represent the effect of the responsive block length on the aggregation behavior of each of this series of block copolymers, a plot of the CSC required for micelle formation as a function of solution pH for P(AMPS$_{225}$-b-AAL$_{350}$) (P₆), P(AMPS$_{225}$-b-AAL$_{660}$) (P₇), and P(AMPS$_{225}$-b-AAL$_{1000}$) (P₈) was constructed as seen in Figure IV-10. Similarly to the previously studied block copolymer system, increasing the PAAL block length leads to a decrease in the CSC required for micelle formation at a specific pH value. However, comparison between block copolymers of the same PAAL weight percent does not result in the same CSC. For example, while P(AMPS$_{110}$-b-AAL$_{490}$) (P₅) and P(AMPS$_{225}$-b-AAL$_{1000}$) (P₈) have the same weight percent of PAAL, the former forms aggregates at pH 2.0 without the addition of...
electrolytes while at the same pH value the latter requires at least 0.15 M NaCl to induce aggregation. Although the percentage of protonated PAAL units remains the same for each block copolymer at a specific pH value, the overall hydrophobicity of the polymers increases as the PAAL block length increases resulting in less screening necessary for micelle formation.

**Figure IV-10.** Critical salt concentration (CSC) required for the formation of micelles as a function of pH measured as determined from dynamic light scattering for P(AMPS\textsubscript{225-}b-AAL\textsubscript{350}) (P\textsubscript{6}) (■), P(AMPS\textsubscript{225-}b-AAL\textsubscript{660}) (P\textsubscript{7}) (○), and P(AMPS\textsubscript{225-}b-AAL\textsubscript{1000}) (P\textsubscript{8}) (▲).
Table IV-2. Radius of Gyration (\(R_g\)), Hydrodynamic Radius (\(R_h\)), and \(\rho\) (\(R_g/R_h\)) for P(AMPS\(_x\)-b-AAL\(_y\)) Block Copolymers.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Radius of Gyration ((R_g)) (nm)(^a)</th>
<th>Hydrodynamic Radius ((R_h)) (nm)(^b)</th>
<th>(\rho)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(AMPS(<em>{110})-b-AAL(</em>{185})) (P3)</td>
<td>19.4(^c)</td>
<td>26.9(^c)</td>
<td>0.721</td>
</tr>
<tr>
<td>P(AMPS(<em>{110})-b-AAL(</em>{305})) (P4)</td>
<td>22.9(^c)</td>
<td>29.2(^c)</td>
<td>0.784</td>
</tr>
<tr>
<td>P(AMPS(<em>{110})-b-AAL(</em>{490})) (P5)</td>
<td>26.4(^c)</td>
<td>35.4(^c)</td>
<td>0.746</td>
</tr>
<tr>
<td>P(AMPS(<em>{225})-b-AAL(</em>{350})) (P6)</td>
<td>31.1</td>
<td>42.9</td>
<td>0.725</td>
</tr>
<tr>
<td>P(AMPS(<em>{225})-b-AAL(</em>{660})) (P7)</td>
<td>34.8</td>
<td>45.0</td>
<td>0.773</td>
</tr>
<tr>
<td>P(AMPS(<em>{225})-b-AAL(</em>{1000})) (P8)</td>
<td>40.9</td>
<td>69.6</td>
<td>0.588</td>
</tr>
</tbody>
</table>

\(^a\) As determined by static light scattering (SLS) in solution at pH 1. \(^b\) As determined by dynamic light scattering (DLS) in solution at pH 1. \(^c\) As determined in solution at pH 1 and 0.2 M NaCl.

In order to determine the structural properties of these block copolymers, dynamic light scattering (DLS) and static light scattering (SLS) were used to determine the hydrodynamic radii (\(R_h\)) and radii of gyration (\(R_g\)), respectively. The ratio of \(R_g\) to \(R_h\), or \(\rho\), is known as the shape factor, with spherical micelles and vesicles having values of approximately 0.775 and 1.00, respectively.\(^{227}\) Recently, Discher and Eisenberg developed an empirical relationship between block copolymer composition and the resultant morphologies.\(^{228}\) Spherical micelles are expected for polymers with hydrophobic mass fractions \(f\) less than 55 %, while copolymers with \(f\) greater than 55 % typically assemble into vesicles. Therefore, the block copolymers of 65 and 75 wt % PAAL were expected to form vesicles; however, according to the experimental values of
R_g, R_h, and \( \rho \) shown in Table IV-2, the shape factors indicate that all the block copolymers in this study form spherical micelles. Although these results seem atypical, the f values predicted by Discher and Eisenberg were based on permanently hydrophilic/hydrophobic block copolymers rather than stimuli-responsive block copolymers. As stated previously, the DLS show that the hydrodynamic radii for P(AMPS_{110}\text{-}b\text{-}AAL_{185}) (P_3), P(AMPS_{110}\text{-}b\text{-}AAL_{305}) (P_4), and P(AMPS_{110}\text{-}b\text{-}AAL_{490}) (P_5) increase as the AAL block length increases. The same trend is observed for the block copolymer series derived from PAMPS_{225}. These results are consistent with previous results from our lab^{29} and from Perrier’s research group^{225}.

Transmission electron microscopy (TEM) and atomic force microscopy (AFM) were used as complementary means to determine the morphological properties. Figure IV-11 shows the TEM and AFM images of the micelles formed at pH 1.0. Both techniques confirm the spherical nature of the micelles and reveal radii of approximately 25 and 27 nm, respectively. These values are lower than those observed by DLS (45 nm) which is consistent with the literature reports of dehydration during sample measurement in both TEM^{122,229} and AFM^{230,231}. Sizes determined from AFM and TEM images are in good agreement.
Figure IV-11. Transmission electron microscopy (left) and atomic force microscopy (right) images of micelles assembled from P(AMPS<sub>225-b-AAL<sub>660</sub>) (P7).
Section II. Reversible IPEC Shell Cross-linking of Micelles Derived from Stimuli-Responsive Diblock Copolymers

Overview

In the previous section, the ability to induce micelle formation for a series of block copolymers of AMPS (M16) and AAL (M18) was discussed. Although this stimuli-responsive behavior could prove promising for drug delivery, practical applications remain limited due to dilution effects, specifically the disassembly of micelles into unimers as the concentration of polymer falls below the critical micelle concentration. In order to circumvent dilution effects, researchers have developed a number of methods to either permanently or reversibly cross-link the micelles. Although advantageous, many of these cross-linking techniques are limited by low reaction efficiency, reagent insolubility, irreversibility, and extensive purification techniques to remove small molecule byproducts. An alternate approach involves the careful design of polymeric micelles containing charged segments for complexation with oppositely charged polymers to form interpolyelectrolyte complexed micelles.\(^{128}\) This technique provides many advantages over traditional cross-linking reactions including near instantaneous cross-linking, solvent selection (aqueous environment), lack of byproducts, and reversibility in the presence of added electrolytes. Previously, our group designed both temperature\(^{129,130}\) and pH\(^{50}\) responsive systems capable of forming these interpolyelectrolyte complexed micelles and demonstrated their reversibility with added electrolytes. Although reversible, a large salt concentration (> 1 M) is required to disrupt these cross-linked micelles rendering them impractical for use as drug delivery vehicles. In order to circumvent these inefficiencies, we have designed a novel micelle-forming,
pH-responsive block copolymer system cross-linked via interpolyelectrolyte complexation in which the cross-linking is reversibly induced by a change in solution pH. Even more importantly, we demonstrate that the extent of pH induced dissociation of shell cross-linked micelles can be altered by simply changing the cationic homopolymer used to form the interpolyelectrolyte complex.

Herein, we report the use of the previously designed pH-responsive, micelle-forming block copolymers which contain an anionically charged corona and an insoluble protonated core at low pH. These block copolymers undergo a reversible unimer-to-micelle transition upon lowering the solution pH. The micelles can be cross-linked via interpolyelectrolyte complexation utilizing the anionic PAMPS shell and a cationic homopolymer, in the current case either protonated poly(N-[3-(dimethylamino)propyl]acrylamide) (PDMAPA) or poly[(N,N-dimethylaminoethyl methacrylate) (PDMAEMA). As outlined in Scheme IV-2, the pH reversibility of these systems is demonstrated by increasing the solution pH to deprotonate the polycation cross-linker resulting in dissociation of the cross-linked micelles to their respective water-soluble unimer components.
Scheme IV-2. Micelle Formation, Shell Cross-linking, Solvation of the PAAL Block, and Dissociation of Shell Cross-linked Assembly.

*Synthesis of Cationic Polymers*

In this work, we sought to combine the stimuli-responsive behavior of a doubly hydrophilic polyanionic diblock copolymer and interpolyelectrolyte complex (IPEC) formation to prepare “locked” nanoscale micellar assemblies which could readily dissociate with changes in pH. In order to prepare these IPECs, two cationic homopolymers made from DMAPA (M5) and DMAEMA (M8) were synthesized utilizing RAFT polymerization. Two homopolymers of PDMAPA with targeted degrees of polymerization (DPs) of 200 and 1000 were first prepared by employing CTP (CTA1) to control the polymerization at 70 °C, using V-501 (I4) as the primary radical source and maintaining a solution pH of 6.5 in order to reduce CTA hydrolysis. Monomer conversion was kept under approximately 80 % in order to maintain control during the
polymerization. The resultant PDMAPA$_{200}$ (P9) and PDMAPA$_{995}$ (P10) had number average molecular weight ($M_n$) and polydispersity index (PDI) values of 30,800 Da (1.04) and 155,500 Da (1.06), respectively. The SEC traces of these polymers are narrow and unimodal indicating successful control (Figure IV-12). These polymers were specifically designed to determine whether the homopolymer molecular weight had any effect on the cross-linking/pH-reversibility of the IPECs. A homopolymer of PDMAEMA was prepared by utilizing CTP (CTA1) as the CTA to control the polymerization at 70 °C, using V-501 (I4) as the primary radical source in dioxane. The resultant PDMAEMA$_{160}$ (P11) had a number average molecular weight ($M_n$) and polydispersity index (PDI) of 25,200 Da (1.11), respectively. The SEC plot for this polymer is shown to be narrow and unimodal, also (Figure IV-13). The two cationic homopolymers of PDMAPA and PDMAEMA were chosen due to their different respective pK$_a$ values of 8.5 and 7.3.
**Figure IV-12.** GPC chromatograms for PDMAPA_{995} (P19) and PDMAPA_{200} (P10) using reversible addition-fragmentation chain transfer polymerization.

**Figure IV-13.** GPC chromatograms for PDMEAMA_{160} (P11) using reversible addition-fragmentation chain transfer polymerization.
Interpolyelectrolyte Complexation

In the previous study, block copolymers of P(AMPS<sub>x</sub>-b-AAL<sub>y</sub>) were shown to aggregate into micelles. Due to the very low pKa value of the PAMPS block, even at pH values of 1.0 the micelles maintain their anionic corona. The anionic corona of the block copolymer micelles can be utilized for formation of interpolyelectrolyte complexes (IPECs) using the previously described cationic homopolymers with different pK<sub>a</sub> values. In turn, the cross-linked micelles should exhibit pH-dependent reversibility. Because interpolyelectrolyte complexes have been shown to be reversible with the addition of electrolytes, only the previously studied block copolymers that could assemble without the need for added electrolytes were utilized in this study, which consists of P(AMPS<sub>110</sub>-b-AAL<sub>490</sub>) (P<sub>5</sub>), P(AMPS<sub>225</sub>-b-AAL<sub>350</sub>) (P<sub>6</sub>), P(AMPS<sub>225</sub>-b-AAL<sub>660</sub>) (P<sub>7</sub>), and P(AMPS<sub>225</sub>-b-AAL<sub>1000</sub>) (P<sub>8</sub>). Micelles assembled at low pH from these block copolymers were ionically cross-linked with cationic polymers as outlined in Scheme IV-2.

Effect of Charge Ratio on IPEC Formation

Utilizing DLS to monitor size distributions, the effect of the AMPS:DMAPA ratio was studied for the cross-linking of P(AMPS<sub>110</sub>-b-AAL<sub>490</sub>) (P<sub>5</sub>) and P(AMPS<sub>225</sub>-b-AAL<sub>660</sub>) (P<sub>7</sub>) micelles at pH 1.0 with PDMAPA<sub>995</sub> (P<sub>9</sub>). Prior to cross-linking the respective sizes of these micelles were 65 and 90 nm. As expected, a 1:1 ratio led to precipitation of both polymer systems due to the lack of excess charge to stabilize the cross-linked micelles. Cross-linking of P(AMPS<sub>225</sub>-b-AAL<sub>660</sub>) (P<sub>7</sub>) was conducted at varying ratios of 2:1, 3:1, 4:1, and 8:1. When a ratio of 2:1 was applied, the cross-linked micelles did not precipitate from solution; however, two size distributions were observed
with values of approximately 90 and 295 nm. These results suggest that aggregation occurs during cross-linking due to an insufficient number of charges remaining on the micelles. By lowering the cross-linking ratio to 3:1, a unimodal peak of 90 nm is observed indicating successful cross-linking without aggregation. Figure IV-14 compares the size distribution (volume %) versus hydrodynamic diameter of the cross-linked micelles at ratios of 2:1 and 3:1. These same results were observed when ratios of 4:1 and 8:1 were utilized. When the pH of the solution is increased causing the PAAL block to become soluble, the cross-linked micelles should maintain their structure. Increasing the solution pH of the cross-linked micelles applying an 8:1 ratio led to dissociation of the IPECs to unimers with sizes of 23 nm, likely due to an inadequate cross-linking density to preserve the micelle structure.

Figure IV-14. Size distribution (measured by dynamic light scattering) of P(AMPS<sub>225</sub>-b-AAL<sub>660</sub>) (P7) cross-linked with PDMAPla<sub>995</sub> (P9) at AMPS:DMAPA ratios of 3:1 (solid line) and 2:1 (dashed line).
Cross-linking of P(AMPS_{110-b-AAL490}) (P5) was conducted at varying ratios of 3:1, 4:1, 6:1, and 8:1. When a ratio of either 3:1 or 4:1 was applied, the cross-linked micelles did not precipitate from solution; however, two size distributions were observed with values of approximately 70 and 255 nm, suggesting aggregation due to an insufficient number of charges remaining on the micelles. By lowering the cross-linking ratio to 6:1, a unimodal peak of 70 nm is observed indicating successful cross-linking without aggregation. Figure IV-15 compares the size distribution (volume %) versus hydrodynamic diameter of the cross-linked micelles at ratios of 4:1 and 6:1. These same results were observed when a ratio of 8:1 were utilized; however, increasing the solution pH of these cross-linked micelles led to dissociation of the IPECs to unimers with sizes of 17 nm due to an inadequate cross-linking density to preserve the micelle structure.

**Figure IV-15.** Size distribution (measured by dynamic light scattering) of P(AMPS_{110-b-AAL490}) (P5) cross-linked with PDMA_{995} (P9) at AMPS:DMAPA ratios of 6:1 (solid line) and 4:1 (dashed line).
By comparing these results, the ratio of AMPS:DMAPA was determined to be a critical factor of IPEC formation. Too high a ratio led to aggregation while too low a ratio led to unwanted dissociation. Also, the length of the PAMPS corona was crucial when optimizing the cross-linking ratios. The micelles with a shorter PAMPS block required a lower ratio to prevent aggregation during cross-linking compared to the micelles with a longer PAMPS block (4:1 versus 3:1).

*Cationic Homopolymer Molecular Weight Effect on IPEC Formation*

Utilizing PDMAPA\textsubscript{995} (P\textsubscript{9}) and PDMAPA\textsubscript{200} (P\textsubscript{10}) the effect of the molecular weight of the cationic cross-linker was examined. Prior to IPEC formation, P(AMPS\textsubscript{225}-b-AAL\textsubscript{350}) (P\textsubscript{6}) block copolymer micelles (0.05 wt %) had hydrodynamic diameters of approximately 90 nm in a pH 1.0 solution. Cross-linking with either of the cationic homopolymers at a AMPS:DMAPA repeat ratio of 4:1 led to micelles with sizes of 95 nm with a unimodal distribution. Similar results were found for each block copolymer system, indicating that the molecular weight of the cationic cross-linker does not play a significant role in IPEC formation.

*Demonstration of pH-Reversibility of the IPECs*

The reversibility of this IPEC cross-linked micelle was studied utilizing \textsuperscript{1}H NMR\textsuperscript{29,128,232,233} under specified conditions as illustrated in Figure IV-16. Spectra (i) and (ii) are of molecularly dissolved PDMAEMA and P(AMPS\textsubscript{225}-b-AAL\textsubscript{350}) at pD 7.0. Upon decreasing the pD to 1.0 with DCl (iii), signals from the methine “c” and methyl “d” groups of PAAL are broadened relative to those associated with PAMPS. After cross-linking with PDMAEMA at pD 1.0 (iv), the methyl signal associated with PAMPS, peak “a”, decreases due to reduced mobility caused by the cross-linking. Also the
methine signal “b” shifts from 3.29 to 2.96 ppm due to the neutralization of the sulfonate
groups on the PAMPS. After increasing the pD to 7.0 using NaOD (v), an increase in the
intensity of the methine proton “c” associated with PAAL is observed due to the
solubility of the PAAL block at this pD. Also the methine proton “b” remains shifted
indicating that the micelles are still cross-linked. Further increasing the pD to 9.0 (vi)
results in dissociation of the IPEC, and all peaks associated with both the block
copolymer and the cationic polymer are observed.
Figure IV-16. $^1$H NMR spectra for PDMAEMA$_{160}$ (P11) complexation with the block copolymer P(AMPS$_{225}$-b-AAL$_{350}$): PDMAEMA at pD 7.0 (i), P(AMPS$_{225}$-b-AAL$_{350}$) (P6) at pD 7.0 (ii), P(AMPS$_{225}$-b-AAL$_{350}$) at pD 1.0 (iii), P(AMPS$_{225}$-b-AAL$_{350}$) after formation of interpolyelectrolyte complex with PDMAEMA$_{160}$ at pD 1.0 (iv), P(AMPS$_{225}$-b-AAL$_{350}$) after formation of interpolyelectrolyte complex with PDMAEMA$_{160}$ at pD 7.0 (v), and P(AMPS$_{225}$-b-AAL$_{350}$) and PDMAEMA$_{160}$ after increasing the pD to 9.0 (vi).

The reversibility of the cross-linking of these micelles was also followed by DLS under specified conditions. Figure IV-17 shows the aggregation behavior when PDMAPA$_{995}$ (P10) is used as the cationic cross-linker. At pH 7.0, P(AMPS$_{225}$-b-AAL$_{1000}$) (P8) copolymers exist as unimers with an apparent hydrodynamic diameter ($D_h$) of 25 nm (Figure IV-17, peak A). When the solution pH is lowered to 1.0, the polymers assemble into micelles with an average apparent $D_h$ of 150 nm (Figure IV-17,
peak B). The addition of PDMAPA leads to interpolyelectrolyte complexed micelles with an apparent Dₜ value of 190 nm (Figure IV-17, peak C). Similar results showing an increase in size of IPEC micelles has also been reported by Armes et al. 128 “Locking” of the nanoparticle is demonstrated by increasing the pH of the solution to 9.0 where uncross-linked P(AMPS₂₂₅-b-AAL₁₀₀₀) (P₈) would dissociate to unimers; however, under these conditions only micelles with an average apparent Dₜ of 300 nm are observed by DLS (Figure IV-17, peak D). This increase in diameter is due to the PAAL block becoming soluble leading to “swollen” micelle structures. Dissociation of the IPECs is induced by further increasing the solution pH to 10.0 fully deprotonating PDMAPA₉₉₅ (P₁₀). Upon removal of the cationic charge, the interpolyelectrolyte complex breaks down leading to dissociation into unimers of 28 nm (Figure IV-17, peak E).

**Figure IV-17.** Size distribution (measured by dynamic light scattering) of P(AMPS₂₂₅-b-AAL₁₀₀₀) (P₈): pH 7.0 (A), pH 1.0 (B), after formation of interpolyelectrolyte complex (IPEC) with PDMAPA₉₉₅ (P₁₀) at pH 1.0 (C), IPEC at pH 9.0 (D), dissociated IPEC at pH 10.0 (E).
In order to better demonstrate the reversibility of this cross-linked system, a plot of the hydrodynamic diameter as a function of pH is shown in Figure IV-18. At pH values below 2.5 the cross-linked micelles maintain their size of approximately 190 nm. At and above pH 2.5, the micelles swell to sizes of approximately 300 nm due to solvation of the PAAL block. At pH 9.5, the IPEC dissociates to unimers of approximately 28 nm, resulting from sufficient deprotonation of PDMAPA$_{995}$ (P10) to uncross-link the micelles.

![Graph](image)

**Figure IV-18.** Hydrodynamic diameter versus solution pH for P(AMPS$_{225}$-b-AAL$_{1000}$) (P8) cross-linked with PDMAPA$_{995}$ (P10).

In order to demonstrate the tunability of reversible shell cross-linking, PDMAEMA$_{160}$ (P11) (pK$_a$ = 7.3) was utilized as a cross-linker. Given the lower pK$_a$ of PDMAEMA than PDMAPA, disassembly should occur at a lower pH value. Figure 8 shows the size distributions at varying conditions. After cross-linking with PDMAEMA$_{160}$ (P11) an increase in the D$_h$ value to 190 nm was observed (Figure IV-19,
peak C). Upon increasing the pH to 7.0 after IPEC formation, the micelles remained intact with an apparent Dₚ value of 300 nm (Figure IV-19, peak D). Again the micelle sizes increase due to the PAAL block becoming soluble. By further increasing the pH to 9.0, the IPEC dissociates to unimers of 28 nm (Figure IV-19, peak E). With the cationic polymer PDMAPₐ₉₉₅ (P10), the complexes remain cross-linked at pH 9.0 because a significant number of cationic charges still remain, preventing dissociation. By contrast the pKₐ of PDMAEMA is significantly lower than that of PDMAPA, and thus there are insufficient charges to maintain the complexes at this pH. These results further evidence that the nature of the cationic cross-linker directly affects the pH-dependent dissociation of the IPECs.

![Figure IV-19](image)

**Figure IV-19.** Size distribution (measured by dynamic light scattering) of P(AMPS₂₂₅-b-AAL₁₀₀₀) (P8): pH 7.0 (A), pH 1.0 (B), after formation of interpolyelectrolyte complex (IPEC) with PDMEAMA₁₆₀ (P11) at pH 1.0 (C), IPEC at pH 7.0 (D), dissociated IPEC at pH 9.0 (E).
In order to better demonstrate the reversibility of this cross-linked system, a plot of the hydrodynamic diameter as a function of pH is shown in Figure IV-20. At pH values below 2.5 the cross-linked micelles maintain their size of approximately 190 nm. At and above pH 2.5, the micelles swell to sizes of approximately 300 nm due to solvation of the PAAL block. At pH 8.0, the IPEC dissociates to unimers of approximately 28 nm, resulting from sufficient deprotonation of PDMEAMA_{160} (P11) to uncross-link the micelles.

![Figure IV-20](image-url)  

**Figure IV-20.** Hydrodynamic diameter versus solution pH for P(AMPS_{225-b}-AAL_{1000}) (P8) cross-linked with PDMAPA_{160} (P11).
Section III. Stimuli-Responsive Layer-by-Layer Films Assembled Utilizing RAFT-Synthesized Homo- and Block Copolymers

Overview

In the previous section, the ability to cross-link P(AMPS-b-AAL) micelles utilizing their anionic corona via IPEC formation was discussed. These micelles should, therefore, be great candidates for incorporation into electrostatically driven layer-by-layer (LbL) film formation. The LbL incorporation of polyelectrolytes into thin films has attracted much attention over the past decade due to the simplicity and versatility of the technique. Such films have shown utility as biosensors, controlled drug release vehicles, antimicrobial coatings, and biocatalysts. Traditionally, LbL films are composed of oppositely charged polyelectrolytes; however, they have also been prepared utilizing hydrogen-bonding donors and acceptors. Although a wide selection of hydrogen-bonding polymers are available for utilization in LbL assembly, the resulting films are significantly less stable due to the weak hydrogen bonds rather than the strong electrostatic interactions of traditional LbL films. Recently, LbL films have been assembled by substituting one or both simple polyelectrolytes with other charged species including dendrimers, nanoparticles, proteins, and DNA. Also of great interest is the incorporation of micelles or vesicles in LbL films due to their potential in the drug delivery field. For example, Hammond and coworkers have made hydrogen-bonded LbL films containing linear-dendritic block copolymer micelles in which triclosan, a hydrophobic drug, was loaded within the micelles and shown to release from the films over an extended period of time. Her group has also demonstrated that the incorporation of biodegradable micelles into
hydrogen-bonded LbL films promotes drug delivery as the cores of the micelles degrade over time.\textsuperscript{246} Recently, this group covalently attached doxorubicin to a core-forming hydrophobic block via a carbamate linkage (cleavable under slightly acidic conditions) and subsequently incorporated these drug-loaded micelles into LbL films where drug release was shown to be pH sensitive.\textsuperscript{259} Biggs and coworkers have also performed numerous studies examining the incorporation of two different block copolymer micelles into LbL films.\textsuperscript{205,262,263} Micelle-only multilayer films on both planar and colloidal particulate substrates were reported. More recently, Addison and coworkers incorporated dye-loaded micelles into LbL films which were assembled on polystyrene latex particles.\textsuperscript{264}

Following our work discussed in the introduction of the first LbL films assembled from well-defined RAFT synthesized cationic and anionic block (co)polymers,\textsuperscript{197} we extended our studies with the intent of preparing films containing intact micelles from precisely designed, pH-responsive block copolymers. In this section, we report the assembly of LbL films containing well-defined, stimuli-responsive, anionically charged micelles of P(AMPS\textsubscript{x-b}-AAL\textsubscript{y}) and the well-defined cationic homopolymer PDMAPA onto a silicon substrate from aqueous solutions at pH 1 (Figure IV-21). At this pH, the AMPS repeat units remain negatively charged while the AAL units are protonated forming the micelle cores. The block copolymers in this study are synthesized \textit{via} aqueous RAFT polymerization with specific block lengths in order to vary the size of the resulting micelles. The effect of micelle size on the thickness of LbL films is monitored by ellipsometry while micelle morphology in the films is observed with AFM. Solutions of varying salt concentrations are also utilized to determine the effect of this variable on
film thickness and morphological characteristics. The stimuli-responsive behavior of the films is also examined by submersion of the films into solutions which trigger micelle dissociation. Finally, we demonstrate the pH-induced release of a model hydrophobic molecule, pyrene, loaded within the micelles incorporated into the LbL films to examine the potential of these films for biomedical coatings (Scheme IV-3).

**Scheme IV-3.** Schematic Representation of Micelle Formation, Loading, Layer-by-Layer Film Formation, and Stimuli-Responsive Release.

**Figure IV-21.** Anionic block copolymers and cationic homopolymer used in the assembly of LbL films.
Layer-by-Layer Film Assembly

Table IV-3 shows the molecular characteristics of the block copolymers utilized in these studies along with the hydrodynamic radii of the micelles formed by these block copolymers at pH 1. These block copolymers were chosen for this study to determine the effect of micelle size on the morphology of LbL films formed from them and the cationic PDMAPA. Figure IV-22 shows film thickness (determined by ellipsometry) as a function of the number of bilayers deposited for LbL films prepared from solutions of different pH values. For films prepared from pH 1 polymer solutions, measured thickness appears equivalent for one and two bilayer films produced from each of the block copolymer systems. For films containing more than two bilayers, thickness depends on copolymer composition. Film thickness increases in the order P(AMPS\textsubscript{110}\textit{-}b\textit{-}AA\textsubscript{L490}) (P\textsubscript{5}) < P(AMPS\textsubscript{225}\textit{-}b\textit{-}AA\textsubscript{L350}) (P\textsubscript{6}) < P(AMPS\textsubscript{225}\textit{-}b\textit{-}AA\textsubscript{L660}) (P\textsubscript{7}) < P(AMPS\textsubscript{225}\textit{-}b\textit{-}AA\textsubscript{L1000}) (P\textsubscript{8}), corresponding to the increasing size of micelles formed from the copolymers in solution at pH 1 (Table IV-3). The LbL film is quite compact in comparison to the size of the individual micelles in solution. For example, P(AMPS\textsubscript{225}\textit{-}b\textit{-}AA\textsubscript{L1000}) (P\textsubscript{8}) with an $R_h$ value of 69.6 in the hydrated state yields a film thickness of $\approx$ 100 nm for a 5 bilayer film in the dry state. These results indicate loss of the hydration sphere around the anionically charged micelles during interpolyelectrolyte complexation with PDMAPA is substantial, consistent with previous literature reports. For instance, Hammond and coworkers reported that a 5 bilayer film containing PEO\textit{-}b\textit{-}PCL block copolymer micelles with hydrodynamic diameters of 71 nm had a film thickness of approximately 95 nm.\textsuperscript{246} Caruso and coworkers reported that an LbL film with 5 bilayers containing two different micelles of PS\textit{-}b\textit{-}P4VP (10 nm) and PS\textit{-}b\textit{-}PAA (7 nm) had a
thickness of 25 nm. In addition to the expected dimension changes due to interpolyelectrolyte complex formation, film thickness may also be diminished by some flattening of the micelles.

Table IV-3. Molecular Weight (M_n), Polydispersity (PDI), and Hydrodynamic Radius (R_h) for P(AMPS_x-b-AAL_y) Block Copolymers.

<table>
<thead>
<tr>
<th>Polymer^{a}</th>
<th>M_n (kDa)^{b}</th>
<th>PDI^{b}</th>
<th>R_h (nm)^{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(AMPS_{110}-b-AAL_{490}) (P5)</td>
<td>95.1</td>
<td>1.23</td>
<td>31.3</td>
</tr>
<tr>
<td>P(AMPS_{225}-b-AAL_{350}) (P6)</td>
<td>101.3</td>
<td>1.22</td>
<td>42.9</td>
</tr>
<tr>
<td>P(AMPS_{225}-b-AAL_{660}) (P7)</td>
<td>145.9</td>
<td>1.26</td>
<td>45.0</td>
</tr>
<tr>
<td>P(AMPS_{225}-b-AAL_{1000}) (P8)</td>
<td>198.4</td>
<td>1.29</td>
<td>69.6</td>
</tr>
</tbody>
</table>

^{a} Subscripts represent the degree of polymerization (DP) for the respective blocks. ^{b} As determined by aqueous size exclusion chromatography (ASEC). ^{c} As determined by dynamic light scattering (DLS) at pH 1
Figure IV-22. Average thickness as a function of bilayer number for films containing anionically charged micelles of P(AMPS<sub>110</sub>-b-AAL<sub>490</sub>) (P<sub>5</sub>) ( ), P(AMPS<sub>225</sub>-b-AAL<sub>350</sub>) (P<sub>6</sub>) ( ◆ ), P(AMPS<sub>225</sub>-b-AAL<sub>660</sub>) (P<sub>7</sub>) ( ▲ ), or P(AMPS<sub>225</sub>-b-AAL<sub>1000</sub>) (P<sub>8</sub>) ( × ) deposited at pH 1 and P(AMPS<sub>225</sub>-b-AAL<sub>660</sub>) (P<sub>7</sub>) ( × ) unimers deposited at pH 7. The inset shows a magnified plot of the film made using P(AMPS<sub>225</sub>-b-AAL<sub>660</sub>) (P<sub>7</sub>) ( × ) unimers deposited at pH 7. The narrow PDI homopolymer, PDMA<sub>995</sub> (P<sub>10</sub>), used in bilayer formation remains cationically charged over the pH range of these studies. Error bars represent one standard deviation.

The insert in Figure IV-22 shows film thickness as a function of bilayer number for a film prepared from P(AMPS<sub>225</sub>-b-AAL<sub>660</sub>) (P<sub>7</sub>) where the dipping and rinse solutions were maintained at pH 7. Under these conditions, the block copolymers are highly charged. They exist as extended individual polymer chains that are expected to deposit in a similarly extended conformation, producing thin films. Thus the films produced from pH 7 solutions should have lower thickness than those assembled under conditions where micelles are present. The film made at pH 7 has a 5 bilayer thickness of ≈ 7.5 nm while that of the film assembled at pH 1 is ≈ 75 nm. This 10-fold increase is consistent with the interpretation of unimer incorporation at pH 7 and micelle incorporation at pH 1.
AFM analysis was performed to further evaluate the incorporation of micelles into the LbL films. Figure IV-23 shows AFM height images of LbL films composed of P(AMPS<sub>225</sub>-b-AAL<sub>660</sub>) (P<sub>7</sub>) and PDMAPA<sub>990</sub> (P<sub>10</sub>) assembled at pH 1 with one, three, and five bilayers. Micelles are observed as spherical features in the images, which increase in density with increasing number of bilayers. The measured root mean square (RMS) roughness also increases with bilayer thickness (1.1 nm for the one bilayer film, 6.0 nm for the three bilayer film, and 6.8 nm for the five bilayer film). Figure IV-24 shows AFM images of a 5 bilayer film made utilizing P(AMPS<sub>225</sub>-b-AAL<sub>660</sub>) (P<sub>7</sub>) and PDMAPA<sub>995</sub> (P<sub>10</sub>) assembled utilizing pH 7 solutions. Although these films exhibit spherical morphologies, the shapes are not as regular as those observed for films assembled under micellar conditions. Previous research has shown that incomplete surface coverage at initial stages leads to the morphology observed in Figure IV-24. The film dimensions and smoothness (RMS value of 1.4 nm) are consistent with assembly from unimers rather than micelles.
Figure IV-23. AFM height images of one (A & D), three (B & E), and five (C & F) bilayer films produced from P(AMPS\(_{225}\)-b-AAL\(_{660}\)) (P7) and PDMAPA\(_{995}\) (P10) at pH 1.0 with no salt. Upper row figures are 25 \(\mu\text{m}^2\) images, lower row figures are 1 \(\mu\text{m}^2\) images.

Figure IV-24. AFM height images of a 5 bilayer film made from P(AMPS\(_{225}\)-b-AAL\(_{660}\)) (P7) and PDMAPA\(_{995}\) (P10) at pH 7.0 with no salt. Figure on left is 25 \(\mu\text{m}^2\) image, figure on right is 1 \(\mu\text{m}^2\) image.

Salt Effects on LbL Film Assembly

Reports by Schlenoff and Dubas\(^{172,267}\) indicated that small molecule salt concentration in solutions used to prepare LbL films is a critical factor affecting polyelectrolyte multilayer film thickness. They reported that LbL films prepared utilizing 0.5 M salt solutions showed a 20-fold increase in thickness compared to those made with no salt. Although this phenomenon is generally observed on addition of salt to
polyelectrolyte solutions, the ultimate increase in film thickness depends on the structure of the polyelectrolyte used. Typically, the addition of salt results in partial shielding of charges along the polymer backbone, enabling the polymer chain to adopt a random coil rather than extended chain conformation, which results in production of thicker films. Figure IV-25 shows film thickness as a function of bilayer number for P(AMPS_{225-b}-AAL_{660}) (P7)/PDMAPA_{995} (P10) LbL films prepared from pH 1 solutions containing 0, 0.25, and 0.50 M NaCl. Film thickness increases at all bilayer numbers with increasing salt concentration. A six-fold increase in thickness is observed for 5 bilayer films produced with 0.5 M salt (thickness – 75 nm for no salt, 310 nm for 0.5 M salt). The relatively lower increase in thickness in comparison to that observed by Schlenoff and Dubas is attributed to the nature of the micellar anionic layer. While the anionic blocks in the shell of the micelle develop a more random coil conformation due to small molecule salt effects, the hydrophobic core is largely unaffected.

AFM images of 1, 3, and 5 bilayer films formed from P(AMPS_{225-b}-AAL_{660}) (P7)/PDMAPA_{995} (P10) at pH 1 and 0.25 M salt solutions are shown in Figure IV-26. One bilayer films (Figure IV-26 A & D) have micelles embedded along with larger entities that are thought to be salt crystals. The larger features are more apparent in the three and five bilayer films (Figure IV-26 B & C). The micellar structures appear more regular in the films produced from 0.25 M salt solutions than in those produced from solutions with no added salt. We speculate that the high salt concentration which leads to the observed crystallization may facilitate the “locking” of the micelle, thereby preventing the collapse of assemblies. Figure IV-27 shows an AFM image of a 5 bilayer P(AMPS_{225-b}-AAL_{660}) (P7)/PDMAPA_{995} (P10) film prepared with pH 1 and 0.50 M salt
solutions. As with the film prepared at 0.25 M salt, salt crystals are observed; however, as might be expected from the increased salt concentration, salt crystals dominate the surface of the films. An image of the top of one of these salt crystals (Figure IV-27) shows micellar structures maintained either within or on the crystals. Increasing salt concentration results in increased incorporation of salt in the films and higher RMS roughness values. Films made with 0, 0.25, and 0.50 M salt have respective roughness values of 7, 50, and 70 nm.

**Figure IV-25.** Average thickness versus bilayer number of films assembled utilizing P(AMPS$_{225}$-b-AAL$_{660}$) (P7) and PDMAPA$_{995}$ (P10) under varying conditions: at pH 1.0 with no salt (■), at pH 1.0 with 0.25 M salt ( ), or at pH 1.0 with 0.50 M salt (▲).
Figure IV-26. AFM height images showing one (A & D), three (B & E), and five (C & F) bilayer films made from P(AMPS$_{225}$-b-AAL$_{660}$) (P7) and PDMAPA$_{995}$ (P10) at pH 1.0 with 0.25 M salt. Upper row figures are 25 $\mu$m$^2$ images, lower row figures are 1 $\mu$m$^2$ images.

Figure IV-27. AFM height images of a five bilayer LbL film made from P(AMPS$_{225}$-b-AAL$_{660}$) (P7) and PDMAPA$_{995}$ (P10) at pH 1.0 with 0.5 M salt. Figure on left is 25 $\mu$m$^2$ image, figure on right is 1 $\mu$m$^2$ image.

**Stimuli-Responsive Film Behavior**

The stimuli-responsive behavior of these block copolymers in solution has been previously demonstrated. At low pH, the block copolymers form micelles through hydrophobic interactions of the protonated carboxylate groups in the PAAL block. As the pH is increased, deprotonation of the carboxylic acid groups occurs rendering the PAAL segment hydrophilic, and the micelles dissociate. In order to demonstrate the stimuli-
responsive behavior of micelle-incorporated films, films formed at low pH were dried and subsequently exposed to aqueous pH 7 solution. Changes in film thickness and morphology were analyzed by ellipsometry and AFM measurements. Table IV-4 shows comparative thickness values for 5 bilayer films assembled utilizing dipping solutions at pH 1 before and after submersion for 5 minutes in a pH 7 aqueous solution. A dramatic decrease in film thickness is observed with thicknesses ranging from 17 to 10% of their original values. As the pH increases, the PAAL blocks become deprotonated, leading to a transition from a hydrophobic to hydrophilic state. This transition results in the dissociation of micelles within the film. Figure IV-28 shows AFM images of a 5 bilayer film assembled utilizing P(AMPS_{225}-b-AAL_{660}) (P7)/PDMAPA_{990} (P10) before and after submersion in a pH 7 solution. Prior to submersion, the micelle features are robust on the film surface; however, after submersion the micelles are no longer visible. Interestingly, the film appears to have holes or indentations where micelles previously existed. The AMPS shells of the micelles are “locked” in place due to electrostatic interactions with the PDMAPA layer; therefore, when the PAAL becomes deprotonated, it can only interact with the PDMAPA layer in the surrounding area which leads to the morphology seen in the AFM image. It is also interesting to note that the RMS roughness observed before and after submersion is very similar, with values of 6.8 and 6.4 nm, respectively. For a 5 bilayer film made from pH 7 solution, the block copolymers are expected to deposit in unimeric film, and no micelles are formed. When this film is immersed in pH 7 water, there is very little measured change in the thickness or the RMS roughness in comparison to the dry state. This is expected, as no dissociation or change in conformation is expected for films produced at the same pH level.
Table IV-4. Block Copolymers Incorporated within the Layer-by-Layer Film, 5 Bilayer Film Thickness Before Submersion, and Film Thickness After Submersion in pH 7 Solution.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Initial Film Thickness (nm)</th>
<th>Final Film Thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(AMPS$<em>{110}$-b-AAL$</em>{490}$) ($P_5$)</td>
<td>45.6</td>
<td>7.7</td>
</tr>
<tr>
<td>P(AMPS$<em>{225}$-b-AAL$</em>{350}$) ($P_6$)</td>
<td>65.9</td>
<td>8.4</td>
</tr>
<tr>
<td>P(AMPS$<em>{225}$-b-AAL$</em>{660}$) ($P_7$)</td>
<td>75.2</td>
<td>9.2</td>
</tr>
<tr>
<td>P(AMPS$<em>{225}$-b-AAL$</em>{1000}$) ($P_8$)</td>
<td>105.3</td>
<td>10.3</td>
</tr>
</tbody>
</table>

Figure IV-28. AFM height images showing a 5 bilayer film made from P(AMPS$_{225}$-b-AAL$_{660}$) ($P_7$) and PDMAPA$_{995}$ ($P_{10}$) at pH 1.0 A) before submersion and B) 5 minutes after submersion in a pH 7 solution.

Stimuli-Responsive Pyrene Release

Finally, to further examine the stimuli-responsive behavior of these micelle-containing LbL films, we encapsulated a model hydrophobic compound, pyrene, within the P(AMPS$_{110}$-b-AAL$_{490}$) ($P_5$) micelles which were subsequently utilized for the formation of LbL films as outlined in Scheme IV-3. These films were assembled on quartz slides in order to monitor the pyrene remaining in the films using fluorescence
spectroscopy. Once made, the films were subjected to submersion in solutions at selected pH values ranging from 1 to 7. Figure IV-29 shows the percent of pyrene remaining in the films as a function of submersion time into pH 1, 2, 3, 5, and 7 solutions. As expected, the release of pyrene from the films could be tailored by adjusting the pH of the solution in which the films were submersed. Figure IV-29 shows that the release profiles for the pH 1, 2, and 3 solutions are very similar due to the stability of the micelles under these conditions with 80 to 90 % of the pyrene being released after 30 minutes. In this case, release of pyrene from the films is controlled by diffusion of the pyrene into solution. When the pH is raised to 5, the micelles collapse leading to faster release from the film. At pH 7, micelle dissociation becomes almost instantaneous, resulting in a burst release effect in which approximately 97 % of the pyrene is released from the film after 2 minutes of submersion time. These results indicate that the release of the payload contained within the micelles can be triggered at specified pH values and should be applicable to other such stimuli-responsive micelles incorporated within LbL films.
Figure IV-29. Pyrene remaining in a 5 bilayer film made from $\text{P(AMPS}_{110-b-AAL_{490}})$ (P5) and PDMAPA$_{905}$ (P10) at pH 1.0 with no salt as a function of submersion time using varying solution pH values: pH 7 (×), pH 5 (○), pH 3 (△), pH 2 (■), and pH 1 (◇).
CHAPTER V
CONCLUSIONS

Section I. Development of Novel pH/Salt-Responsive ARAFT-Synthesized P(AMPS-b-AAL) Block Copolymers

A series of block copolymers composed of AMPS (M16) and AAL (M18) with varying block lengths has been synthesized. The weight percent of amphiphilic AAL in the block copolymers was varied and the effect on pH-responsive assembly was observed. The self-assembly behavior of these copolymers was determined over a range of pH values and salt concentrations. By plotting the critical salt concentration necessary for micelle formation versus solution pH, we observed that increasing the AAL block length leads to a lower CSC which is attributed to an overall higher percent of hydrophobicity within the block copolymer. DLS and SLS studies were utilized to determine the size and shape of the aggregates formed by the block copolymers. Although theory predicts vesicular formation for block copolymers with f < 55 %, only micellar structure were observed in our studies. TEM and AFM were also used to confirm the shape and sizes of self-assembled micelles.
Section II. Reversible IPEC Shell Cross-linking of Micelles Derived from Stimuli-Responsive Diblock Copolymers

The cationic homopolymers PDMAPA and PDMAEMA were employed for the \textit{in situ} formation of corona cross-linked micelles \textit{via} interpolyelectrolyte complexation. DLS studies showed that the molecular weight of the cationic homopolymer used for cross-linking did not affect IPEC formation; however, the ratio of charges used for cross-linking provided a crucial role in preventing aggregation and premature dissolution of the IPECs. DLS and $^1$H NMR spectroscopy indicated that the micelles were stable, yet reversible to changes in pH. The ionically cross-linked micelles were shown to dissociate at pH values above the pK$_a$ of the cationic polymer. This system may have utility over traditional covalent methodologies based on the ease of synthesis and cross-linking, the interpolyelectrolyte cross-linked micelle reversibility, and the tunable control over the pH at which these cross-linked micelles dissociate. Additionally, application of these systems for use in controlled release could lead to advancements in drug delivery.
Section III. Stimuli-Responsive Layer-by-Layer Films Assembled Utilizing RAFT-Synthesized Homo- and Block Copolymers

In this work we have demonstrated the release of hydrophobic molecules from stimuli-responsive micelles incorporated within LbL films. The assembly of these films utilizes electrostatic interactions between amphiphilic block copolymer micelles of P(AMPS-b-AAL) and PDMAPA. We were able to incorporate micelles of varying sizes within the films which directly affected the overall film thickness. We also studied the effect of salt concentration on film thickness and morphology showing that salt increases the film thickness but also drastically increases the RMS roughness due to salt crystallization within the film. Moreover, by taking advantage of the pH-responsive micelles incorporated within the films, we were able to show pH-responsive film behavior where film thicknesses decrease up to 90% when the film is exposed to pH 7 solutions due to collapse of the micelles within the films. Finally, micelles loaded with pyrene were incorporated within LbL films which were shown to have different release profiles depending on the pH of the solution in which the films were submersed. Overall, we anticipate that the incorporation of stimuli-responsive micelles within electrostatically assembled LbL films should provide many opportunities to deliver therapeutics via surface coatings with controllable release properties.
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