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Synthesis and ^{1}H-NMR characterization of amphiphilic acrylate block copolymers to investigate the effect of chain rigidity on micelle formation in solution

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Synthesis and ^{1}H-NMR Characterization of Amphiphilic Acrylate Block Copolymers to Investigate the Effect of Chain Rigidity on Micelle Formation in Solution

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Abstract

Amphiphilic block copolymers are composed of distinct segments exhibiting different chemical properties. In solution, block copolymers may self-assemble to form micelles when triggered by a change in the environment. The effect of chain rigidity can be investigated in better detail if the molecular weights are controlled, as the polymer's ability to aggregate is also influenced by polymer size. Acrylate and methacrylate monomers were chosen for their similar chemical properties but their difference in reported glass transition temperature (T_g). Amphiphilic block copolymers were synthesized by a controlled free radical (RAFT) polymerization. ^{1}H-NMR methods were developed to measure molecular weight of poly(methyl methacrylate) and poly(*t*-butyl acrylate) homopolymers to corroborate size-exclusion chromatography measurements. Using qualitative measurements of peak broadening that occurs by the shortening of T_2 relaxation when polymers phase-separate from solution, it was found that polymers with a more rigid hydrophobic region tend to form micelles the most readily.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

1.1 - Polymer Overview

Polymers are large molecules that are made up of covalently linked repeat units consisting of monomers. In the simplest arrangement, they form linear molecular chains as the result of monomers adding onto the chain ends. Polymers always have significantly different physical properties from the monomers that make them up. For example, ubiquitous commercial plastics such as polystyrene, polyethylene, and poly(methyl methacrylate) are useful plastics, but their monomers are harmful liquids. The extreme differences between the two arise from the large molecular size of polymers, which may consist of thousands of monomers.

Synthetic polymers have a wide spectrum of uses from differences in their monomers, sizes, branching architectures, and molecular weight distributions. Homopolymers are the simplest classification of polymers and are made of a single species of repeat unit, the same as one species of monomer in the vinyl-based polymers in this research. Many of the industrial polymers are this type. Copolymers are polymers that are made up of two or more repeat units. These can be randomly assembled, perfectly alternating, a gradient composition along the length of the polymer, or arranged in distinct blocks. Depending on the monomer distribution, they can have the general properties of homopolymers or additional utility if the monomers are arranged in a more complex architecture.

1.2 - Molecular Weights of Polymers

When a batch of polymer is synthesized, there is always a distribution of molecular weight among the polymer chains. This is a natural result of the reaction, because not every polymer is formed from the same number of repeat units. Rather, a polymer population will have a Gaussian distribution around an average molecular weight. There are several methods to determine this, but the number average molecular weight (M_n) and the weight average molecular weight (M_w) are commonly used. They are defined as follows[1]:

$$M_n = \sum_i \frac{(N_i M_i)}{\sum_i N_i} \qquad M_w = \sum_i \frac{(N_i M_i^2)}{\sum_i N_i M_i}$$

Figure 1.1: Equations for the number average molecular weight (M_n) and the weight average molecular weight (M_w).

In the equations, *i* represents a fraction of the polymer distribution, N_i is the number of molecules in a fraction, and M_i is the molecular weight of a fraction. The two formulas give two different averages, and M_w is always larger than M_n. All ^{1}H-NMR measurements provide M_n, and size-exclusion chromatography (SEC) (Section 3.3) provides both M_n and M_w. The further these values diverge from each other, the wider the molecular weight distribution, defined as M_w/M_n, also called the polydispersity index (PDI). This distribution can be narrow or wide, depending on the control over the polymerization. A PDI equal to 1 indicates that all polymers are the same molecular weight, with a wider distribution having a larger PDI.

1.3 - Structure of Block Copolymers

Block copolymers consist of single polymer chains with distinct regions made up of different monomers that exhibit different physical or chemical properties. Such a monomer arrangement can allow for organized interaction and the formation of nanostructures. Many block copolymers are amphiphilic, where the difference in solubility in the same environment between the blocks is the salient feature of the polymer.

1.4 - Synthesis of Block Copolymers

Free radical polymerization is a technique to polymerize chain growth monomers that is mechanistically related to the other chain growth mechanisms, cationic and anionic polymerizations. It proceeds by the sequential addition of monomers onto a propagating free radical on the growing chain end. Carbon radicals are generated from a favorable homolytic cleavage of an initiator molecule. The initiating species then reacts with the double bond of a terminal alkene to form a new carbon-carbon bond and transfer the reactive radical. A chain reaction develops, with additional monomer adding sequentially to form polymer chains. Figure 1.2 shows this process with methyl methacrylate as the monomer and azobisisobutyronitrile (AIBN) as the initiator. Chain ends eventually terminate when they form a bond with another radical. Radical transfer and side reactions between chains are difficult to control in conventional polymerization and can lead to chain branching and wide molecular weight distributions.

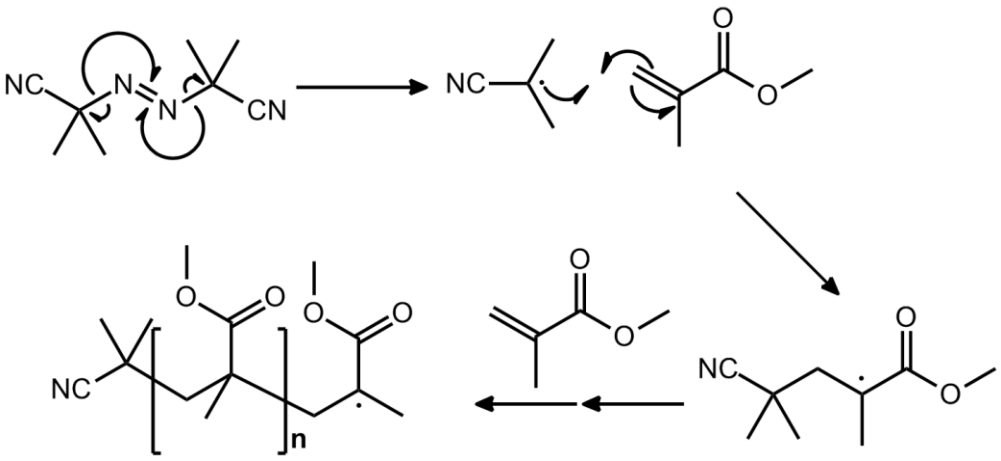


Figure 1.2: Free radical polymerization. The initiating species is formed by homolytic cleavage of the initiator, which then reacts with monomers in a chain reaction.

There are several types of free radical polymerization techniques that can be used to synthesize block copolymers of chain-growth monomers with a narrow polydispersity index (PDI). In all of them, an equilibrium of free radical transfer between chains leads to control over the PDI and the average molecular weight of a polymer population. Nitroxide-mediated polymerization (NMP), atom transfer radical polymerization (ATRP), organometallic-mediated radical polymerization (OMRP), reversible addition-fragmentation polymerization (RAFT), and the closely related macromolecular design via the exchange of xanthates (MADIX) are the leading methods of controlled radical polymerization.[2] Advantages of RAFT are that metal catalysts are not needed, a wide range of monomers and functional groups are tolerated, very extensive preparation of the reaction mixture (e.g. removal of water, oxygen) is not necessary, and the synthesis of RAFT chain transfer agents (CTAs) are relatively straightforward.[3] RAFT was first reported in 1998.[4] In RAFT polymerization, initiation and propagation begin as in conventional polymerization. At some point, a growing chain will come into contact with a CTA, which contains the functionality of either a dithioester or trithiocarbonate.

The growing chain reacts with the sulfur-carbon double bond to form a highly stabilized tertiary radical. A leaving group (in this example, a 2-cyano-2-propyl radical identical to the initiating species) from the CTA continues to propagate its own chain, effectively transferring the radical between chains (Figure 1.3). The original chain remains covalently linked to the CTA in a dormant state. Growing polymer chains can then add back to a CTA, and the previously dormant chains can leave to add more monomer. It is this effective shuffling of radicals between polymer chains that allows for narrow molecular weight distribution (PDI ≈ 1) (Figure 1.4). Additionally, polymers are capped with the functional groups of the CTA, allowing for further modification if desired. Perhaps more importantly, additional second monomer can be added to the dormant polymer to form a block copolymer, which is not possible with conventional free radical polymerization, as terminated chains are unreactive.

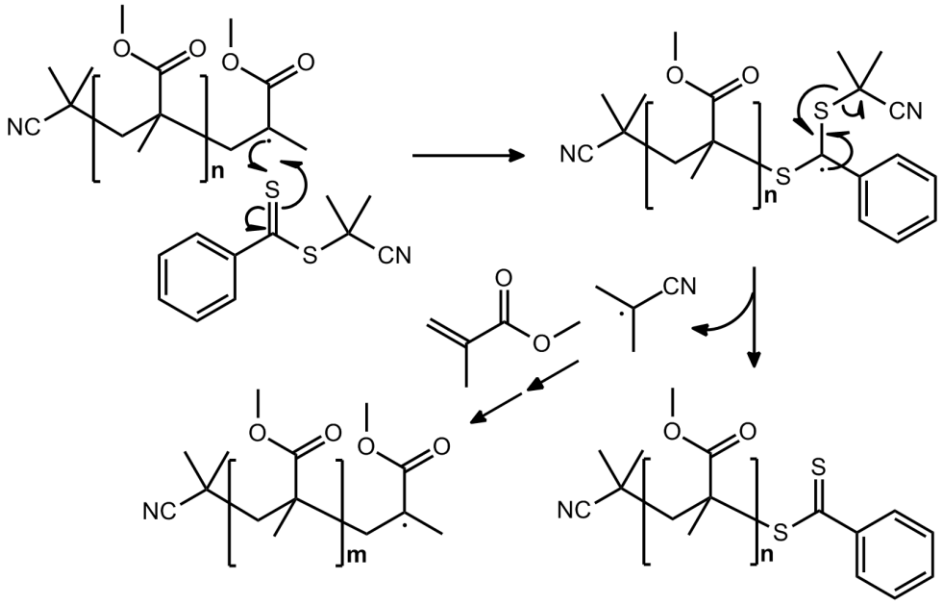


Figure 1.3: RAFT polymerization. A growing polymer chain interacts with a chain transfer agent to form a highly stabilized resting state. The chain transfer agent is designed to have a leaving group that can carry the radical and start a new polymer chain.

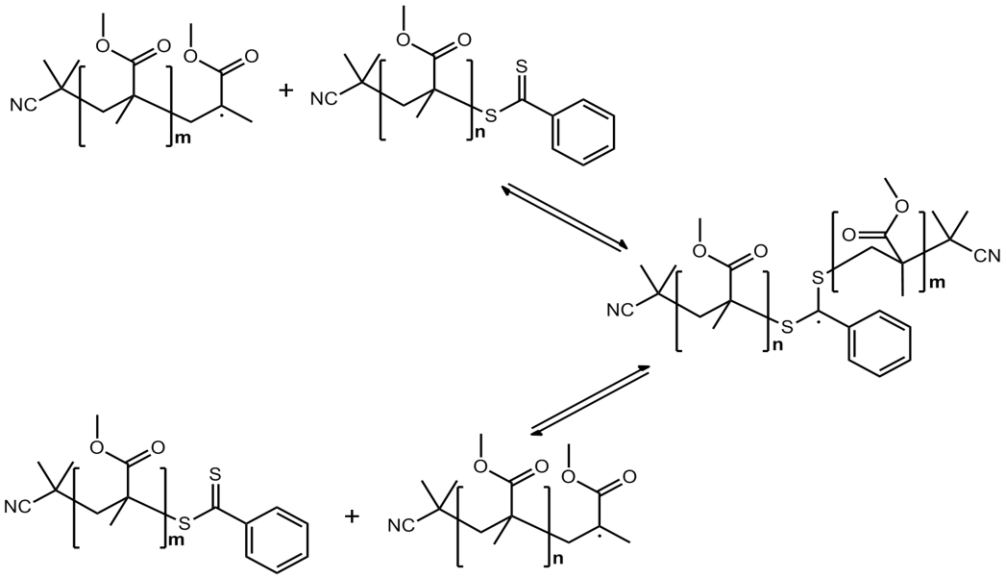


Figure 1.4: Transfer of free radicals between growing polymer chains through the CTA.

1.5 - Self-Assembly of Block Copolymers

The amphiphilic properties of many block copolymers allow them to self-assemble under certain conditions, often forming micelles. Under one set of conditions, both blocks can be freely soluble, but when there is an environmental change, one block will phase-separate from the environment to form the core of the micelle, leaving the other block freely soluble as the shell of the micelle. These are analogous to small molecule surfactants such as soaps and phospholipids, except they are significantly longer and have lower critical micelle concentrations, making them more stable in solution.[5,6] Likewise, polymer micelles can also disassociate as the result of an environmental change. The environment in which a polymer block might be soluble can be described by its solubility parameter.[7] Polymers that have a solubility parameter close to the solvent are more soluble in that solvent than to a solvent with a different solubility parameter. Thus, for a particular need the appropriate polymer could be predicted by knowing the solubility parameter of the environment it is to be in.

A common means to trigger micelle formation is a change in temperature. Intuitively, polymer micelles may form at lower temperatures but become unstable when the temperature is increased due to increased molecular motion of the chains. Micelles of poly(styrene-*b*-*t*-butyl styrene) in *N*,*N*-dimethylacetamide have been shown to dissociate with increased temperature.[8] However, poly(ethylene glycol)-*b*-poly(N-isopropylacrylamide) and poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (PEO-PPO-PEO) form micelles with increased temperature.[9,10]

Polymers consisting of monomers with ionizable groups can be largely influenced by the pH of an aqueous environment. For example, poly(hexa-(ethylene glycol) methacrylate)-*b*-(2-(diethylamino)ethylmethacrylate) was found to be freely soluble under acidic conditions when the quaternary amino groups were protonated, but this block was found to aggregate at higher pH when the amino groups were neutral.[11] Conversely, poly(ethylene glycol)-*b*-poly(alkyl(meth)acrylate-*co*-methacrylic acid) polymers have been shown the opposite response, where at higher pH the polymers dissociated to release a loaded drug as the acids were deprotonated and more polar in the basic environment, making them more water soluble.[12]

The addition of other molecules can also cause micelle formation in some block copolymers. The self-assembly of PEO-PPO-PEO has been facilitated with increasing KCl concentration.[13] In another study, the addition of NaCl also facilitated the formation of micelles with PEO-PPO-PEO polymers, but micelle formation was inhibited with the addition of urea.[14] The addition of glucose has been found to dissociate polymers with boronic acid groups, offering the ability to release the contents contained in the core when under certain metabolic conditions.[15] Polymer micelles have been shown to

irreversibly break open when a photosensitive dye was esterified onto the backbone of a polymer, detaching upon exposure to light and liberating a free carboxylic acid.[16] While most studies focus on triggering micelle formation in aqueous systems, the addition of water to a polymer dissolved in organic solvents has been shown to cause the formation of micelles in poly(methyl methacrylate-*b*-acrylic acid), as the methyl methacrylate block is not soluble in water.[17]

These few examples demonstrate the wide range of triggers that may cause the formation or dissociation of block copolymer micelles. The many ways to control polymer self-assembly have several potential uses in biological and industrial applications.

1.6 - Applications of Block Copolymers

Like their small amphiphilic counterparts such as soaps, block copolymers can be used as surfactants. One area where they are widely used for this purpose is for emulsion polymerization. In one example, poly(styrenesulfonate)-*b*-poly(ethyl-ethylene) can help stabilize latex particles.[18] Poly(ethylene oxide)-*b*-(polyethylene imine) has been used to create gold nanoparticles when they are mixed with a solution of a gold salt, providing a method to predictably synthesize gold nanoparticles of a certain size.[19] They can also be useful as catalysts for organic reactions, such as the Heck reaction where stable palladium colloids formed in several solvents and were added to increase the rate of catalysis.[20]

Much current research has been dedicated to using block copolymers as a way to deliver hydrophobic drugs that would otherwise have a low bioavailability in the method they are administered to a biological system. The benefit is that hydrophobic compounds

can be loaded into the core of the micelle and be solubilized until they reach the desired destination for them to be released and act. In addition to the solubilization of the drug, loading them in the significantly larger micelles has implications for the pharmacokinetics of the drug. One major advantage of using micelles is that the excretion of the drug by the kidneys or breakdown in the liver is significantly reduced. The size of the micelles affects these factors and also the uptake by different tissues in the biological system.[21] Forming micelles from a mixture of different block copolymers can combine useful properties to modulate critical micelle concentration, drug loading capacity, bioavailability, and lower critical solution temperature.[22] Several examples show that polymers can be grafted onto biological molecules to increase their specificity of action. Monoclonal antibodies against tumor-associated glycoprotein were grafted by amination of antibody amino groups to aldehyde chain ends of poly(ethylene glycol-*b*-methacrylic acid).[23] Methoxy poly(ethylene glycol-*b*-caprolactone) has been conjugated to HER2 specific antibodies to bind with cells expressing the HER2 protein, as well as the peptide "nuclear localization signal" to target the cell nucleus once inside to release a loaded drug that makes the DNA sensitive to radiation of radioactive ^{111}In, also conjugated to the polymers.[24] Antibodies are not the only examples of targeting molecules, as any cell expressing a particular receptor could in theory be targeted by conjugating the polymer chains with the appropriate ligand. Paclitaxel was selectively delivered to cancer cells overexpressing the folate receptor using poly(alanine-*b*-ethylene glycol) linked to folate.[25] While liposomes crafted from naturally-occurring lipids are often used for purposes of drug delivery, block copolymers can be advantageous due to the different biological interactions possible from the different functional makeup of

monomers, reduced toxicity, and increased stability resulting from their lower critical micelle concentrations.[26,27]

In all applications that may use block copolymer micelles, control over when micelles form and dissociate is crucial to successful function, especially in the case of drug delivery systems where the solubilized compound must be selectively released by the micelle.

1.7 - ^{1}H-NMR Observation of Polymer Self-Assembly

When a block undergoes phase-separation to form the core of the micelle, the protons undergo significant changes in their nuclear magnetic resonance relaxation constants, T_1 (spin-lattice, longitudinal) and T_2 (spin-spin, transverse). T_1 relaxation times have been shown to decrease by the reduced molecular motion of the protons in the core of the micelle.[28] Both forms of relaxation are affected by the rate of tumbling of a molecule, and the T_2 relaxation decreases when polymer chains are aggregated into a micelle core. Smaller relaxation constants mean faster relaxation. Direct measurement of the T_1 and T_2 relaxation can be done with relatively straightforward two-dimensional NMR experiments. The T_1 relaxation can be done with an inverse-recovery experiment and the T_2 with a spin-echo experiment. While these experiments give quantitative measurements of the two relaxation constants, qualitative measurements of a change of T_2 relaxation can be seen by the phenomenon of peak broadening. Indeed, T_2 can be estimated by the peak width.[29]

Chapter 2: Research Overview

2.1 - Effect of Chain Flexibility

Much research has been put forth into controlling the precise conditions in which the self-assembly and dissociation of block copolymer micelles occur. The chemical nature of the monomer and the size of the polymer blocks are two variables that will affect this process. The characterization of polymer length in this research is to the end of exploring the effect of chain flexibility on the process. There are two hypotheses put forth with this in mind:

1) More rigid chains should be more prone to forming micelles compared with other chains of identical solubility and size in a certain chemical environment.

2) The self-assembly of amphiphilic block acrylate copolymers should be influenced by the flexibility of the portion of the polymer backbone that will phase-separate from the solvent. In this system, the solvent is polar and the non-polar chains are expected to self-assemble. The hydrophilic portion of the polymer that remains well-solvated should not affect the self-assembly of the polymers.

In order for the effect of chain flexibility to be investigated properly, three variables must be controlled: polymer solubility, monomer structure and size, and chain length. The first two are addressed by the choice of monomer, and the latter is by careful synthesis and characterization of the polymer length, the main focus of this research.

2.2 - Choice of Monomers

Polar/Non-Polar Pairs

Amphiphilic block copolymers are composed of what can be thought of as two covalently linked homopolymers with drastically different solubility profiles. To best isolate solubility as a variable, monomers should be similar in size and flexibility as the other, but differ greatly in solubility. To that end, carboxylic acid monomers and their corresponding methyl esters were chosen (Figure 2.1). Methyl methacrylate and methacrylic acid constituted one pair, and methyl acrylate and acrylic acid the other.

Rigid/Flexible Pairs

Based on reported glass transition temperatures (T_g) for each solid polymer,[30] the addition of a methyl group along the polymer backbone was hypothesized to decrease the flexibility of the chain in solution and was the chemical modification chosen for each pair of flexible/rigid polymer chains. This was the smallest addition to the polymer backbone possible without changing functional groups, and it was assumed that the solubility and other properties were not affected.

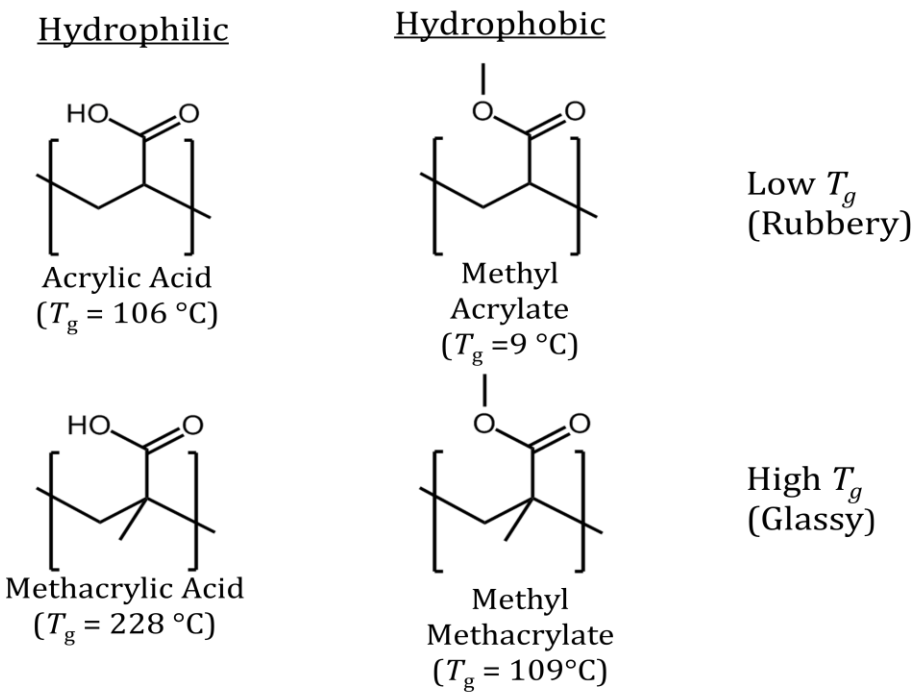


Figure 2.1: The four polymers used for amphiphilic block copolymers in this research.

To test the hypothesis fully, all four possible permutations were acquired and tested to demonstrate that only the flexibility of the hydrophobic esters had an effect on the onset of micellization under a certain set of solution conditions, and not the hydrophilic acids.

2.3 - Research Goals

Synthesize both Block Copolymers containing Poly(methacrylic acid)

Four block copolymers are possible with the monomers chosen, and all were obtained in order to fully investigate the effect of chain flexibility of the hydrophobic core on micelle formation. The two methacrylic acid containing polymers, poly(methyl methacrylate-*b*-methacrylic acid) and poly(methacrylic-*b*-methacrylic acid), were synthesized in this research, the others obtained from other sources.

Control Poly(methyl methacrylate) Length and PDI

Polymers of the same monomer composition vary in solubility depending on how long the chains are. With amphiphilic block copolymers, the hydrophobic core of a micelle phase-separates from a polar solution, and does so more easily with longer chains, all other things being equal. Therefore, chain length must be eliminated as a variable to compare polymer samples when investigating chain flexibility on polymer self-assembly. Poly(methyl methacrylate) synthesis was investigated in detail as a guide for other monomers. In addition, for the best resolution on observing when the self-assembly of polymers occurs, the PDI must be kept low. Meeting these goals was a main focus of this research.

Establish NMR Methods to Reliably Measure Polymer Length

^{1}H-NMR is a powerful tool to quantify the relative molar amounts of different protons. Proper integrations of peaks can translate to molar amounts of different monomers and polymer end groups. The absolute length of homopolymers and the absolute and relative lengths of block copolymers needed to be characterized. The methods for calculating this must be reliable and were compared with size-exclusion chromatography as corroborating data.

Monitor Micelle Formation by NMR

The hypothesis driving this research was tested after the required polymers were synthesized. The peak-broadening observation of polymers in micelles was adapted so that the effect of polymer chain flexibility of the hydrophobic chain could be tested.

Chapter 3: Methods

3.1 - Block Copolymer Synthesis

Chemicals

Methyl methacrylate (99%), methyl acrylate (99%), *t*-butyl methacrylate (98%), and methacrylic acid (98%) were all purchased from Sigma-Aldrich. Before polymerization, MEHQ inhibitor was removed by running the monomer through a small plug of neutral alumina for the esters or silica for methacrylic acid. 2,2′-Azobis(2-methylpropionitrile) (AIBN) (98%) was purchased from Sigma-Aldrich and recrystallized from methanol. Trifluoroacetic acid was from an unknown source but was a dark liquid suggesting the presence of impurities. Methanol (99.8%) was purchased from VWR. Hexane (99.9%) was purchased from Fischer Scientific. Benzene (>99.9%) and 2-cyanoprop-2-yl dithiobenzoate (CTA) (98%) were purchased from Sigma-Aldrich.

Poly(methyl methacrylate-*b*-methacrylic acid) Copolymer Synthesis

Poly(methyl methacrylate) Homopolymer

To an open Schlenk flask containing a stir bar, inhibitor-free methyl methacrylate was added by syringe. 2-cyanoprop-2-yl dithiobenzoate (CTA) was added either by weighing onto a weigh boat and washing into the flask with benzene or by adding a 200 mM solution of the chain transfer agent in benzene. AIBN was weighed separately and either washed off a weigh boat into the flask with benzene or added as a solution in benzene if less than approximately 5 mg. Benzene was added to reach a final monomer concentration of 2 M. The resulting solution was degassed by subjecting the solution to

three freeze/pump/thaw cycles using liquid nitrogen and a high vacuum system, and confirmed to be sufficiently low in dissolved gases by monitoring the pressure increase when the frozen solution was exposed to vacuum (<50 mTorr). The volume of the flask was then pressurized with N_2 and placed into an oil bath at 80 °C. A rubber septum was fitted onto the flask so that aliquots could be removed to monitor the progress of the polymerization.

After the reaction time, the flask was removed from heat and exposed to the atmosphere. The solution was precipitated into ~20 volumes hexane and stirred for several minutes. The solid was isolated by vacuum filtration and dried under vacuum for several hours at room temperature. The solid polymer was confirmed to be free of solvent and residual monomer by ^{1}H-NMR. A solid, fine light pink powder resulted. Reagent amounts varied, and the specifics of seven poly(methyl methacrylate) reactions can be seen in Table 3.1.

Polymer ID	mmol MMA	µmol CTA	µmol AIBN	Reaction Time (hr)	Yield (mg)
PMMA1	40	800	20	23	815
PMMA2	20	281	7	15	525
PMMA3	20	281	14	15	750
PMMA4	40	800	200	12	2370
PMMA5	40	800	400	13	1500
PMMA6	20	70	14	16	740
PMMA7	20	70	7	16	1070

Table 3.1: Reaction specifics for seven poly(methyl methacrylate) polymerizations.

Poly(methyl methacrylate-b-methacrylic acid) Copolymer

Purified CTA-terminated poly(methyl methacrylate) homopolymer (6.3 mmol methyl methacrylate units) was weighed into a Schlenk flask and a stir bar was added. Inhibitor-free methacrylic acid (6.3 mmol) was added to the open Schlenk flask by syringe. AIBN (0.14 mmol) was weighed onto a weigh boat and washed into the flask. Benzene (1.1 mmol) was added by syringe to be used as a reference NMR peak to quantify the degree of conversion of the monomer, giving relatively equal peak area of the two at the start of the reaction. *p*-Dioxane was added to reach a monomer concentration of 0.9 M, and the resulting solution was degassed as described above. The flask was then pressurized with N_2 and placed into an oil bath at 80 °C. A rubber septum was fitted onto the flask so that aliquots could be removed to monitor the progress of the polymerization.

After the polymerization, the solution was diluted with acetone and precipitated into ~20 volumes hexane, stirred for several minutes and placed in the freezer for at least a half hour. The solid was then isolated by vacuum filtration and dried under vacuum at room temperature. The polymer was confirmed to be free of solvent and residual monomer by ^{1}H-NMR. A light pink solid resulted.

Poly(methyl acrylate-*b*-methacrylic acid) Copolymer Synthesis

Poly(t-butyl methacrylate) Homopolymer

Poly(*t*-butyl methacrylate) homopolymers were prepared in the same manner as poly(methyl methacrylate), with the reactions performed in an oil bath or in a microwave reactor. For microwave reactions, monomer, chain transfer agent, and initiator were

prepared as described above in microwave reactor tubes. They were deoxygenated by gently bubbling N_2 through the solution on ice for approximately ten minutes. Tubes were placed in a CEM Discover microwave reactor at 80 °C with the following settings: solvent = toluene; power = 300 Watts; pressure max = 200 PSI; ramp time = 3 min. Reactions were stopped by exposure to atmosphere and immersion in ice. Homopolymer purification was the same as described in the above methods.

Methanol/water mixtures were used for precipitation instead of hexane. The solid polymer was confirmed to be free of solvent and residual monomer by ^{1}H-NMR. A fine pink powder resulted. Table 3.2 shows the specifics for seven poly (*t*-butyl methacrylate) reactions.

Polymer ID	mmol *t*BMA	µmol CTA	µmol AIBN	Reaction Time (hr)	Yield (mg)
P*t*BMA1	8	162	16	8.3	No data
P*t*BMA2	8	162	16	8.3	No data
P*t*BMA3	10	200	10	8.3	No data
P*t*BMA4	35	320	32	~12	2750
P*t*BMA5	10	67	10	8.3	No data
P*t*BMA6	10	50	10	8.3	285
P*t*BMA7	10	25	10	8.3	No data

Table 3.2: Reaction specifics for seven poly(*t*-butyl methacrylate) polymerizations.

Poly(t-butyl methacrylate-b-methyl acrylate) Copolymer

Purified CTA-terminated poly(*t*-butyl methacrylate) homopolymer (2.8 mmol *t*-butyl methacrylate units) was weighed into a Schlenk flask and a stir bar was added. Inhibitor-free methyl acrylate (2.8 mmol) was added to the open Schlenk flask by syringe. AIBN (2.8 µmol) was added as a 2 mg/ml solution in benzene. More benzene was added to reach a 2 M solution of monomer, and the resulting solution was degassed

by undergoing three freeze/pump/thaw cycles using liquid nitrogen and a high vacuum system and confirmed to be sufficiently low in dissolved gases (<50 mTorr) by monitoring the pressure change when the frozen solution was exposed to vacuum. The flask was then pressurized with N_2 and placed into an oil bath at 80 °C. A rubber septum was fitted onto the flask so that aliquots could be removed to monitor the progress of the polymerization.

After the polymerization, the solution was concentrated to dryness and redissolved in CH_2Cl_2. Five molar equivalents TFA to *t*-butyl methacrylate subunits were added, and the reaction was allowed to stir overnight at room temperature to remove the *t*-butyl groups from the poly(*t*-butyl methacrylate) block to liberate poly(methacrylic acid). This was concentrated to dryness again, diluted with acetone, and purified by dialysis. A membrane with a molecular weight cutoff of ~3,000 Da. was used to purify the copolymer. Acetone surrounding the membrane was exchanged three times until the small molecular weight impurities were diluted to negligible concentrations. The polymer solution was then evaporated to dryness.

3.2 - ^{1}H-NMR Acquisition

All ^{1}H-NMR spectra were obtained using a JEOL ECX 400MHz spectrometer and processed with Delta NMR Software. Deuterated chloroform, *p*-dioxane, and dimethyl sulfoxide were purchased from Cambridge Isotope Laboratories and used as received.

Reaction Mixtures and General Spectra

Experiments were performed without temperature control with 16 scans, 90° pulse width, 5 ppm offset, 2.18 second acquisition time, and a two-second relaxation delay. For reaction mixtures, care was taken to run the samples as soon as possible to avoid error introduced by the evaporation of volatile molecules.

Chain Length Estimation of Purified Polymers

Experiments were performed without temperature control with 320 scans, 90° pulse width, 5 ppm offset, 2.18 second acquisition time, and a five-second relaxation delay.

Micelle Formation Experiments

Stock solutions of samples were made up at 10 mg/ml in dioxane-d_8. They were then diluted to 2.5 mg/ml with appropriate volumes of dioxane-d_8 and D_2O to yield 0%, 25%, 50%, and 75% D_2O solutions. The samples were transferred to J. Young tubes and degassed with three freeze/pump/thaw cycles. Spectra were obtained at 25 °C, 50 °C, and 80 °C.

3.3 - Size-Exclusion Chromatography (SEC)

THF (99.2%) was distilled before use to remove BHT inhibitor. Polymer samples and poly(methyl methacrylate) standards were brought up at 4 mg/ml THF prior to analysis. A Shimazdu or Agilent 1100 series HPLC was used to inject samples on a Tosoh Bioscience LLC TSK-GEL G3000Hxl column, and polymers were detected by

monitoring absorption at 230 nm using distilled THF as the mobile phase at 1 ml/min. Cirrus software from Varian was used to construct the calibration curve of molecular weights as a function of retention times and to calculate PDI from measured M_n and M_w. For *t*-butyl methacrylate, Mark-Houwink parameters were used to translate the values of the poly(methyl methacrylate) standards to the samples.[31]

3.4 - Chain Transfer Agents

t-Butyl dodecyl carbonotrithioate was synthesized according to literature procedures.[32] 2-cyanoprop-2-yl dithiobenzoate was either synthesized following literature procedures[33] or was purchased from Sigma.

Chapter 4: Polymer Synthesis and Characterization of Polymer Length

4.1 - NMR Solvents

For poly(methyl methacrylate) and poly(*t*-butyl methacrylate) homopolymers, reactions in benzene were analyzed in $CDCl_3$. The solubility in DMSO-d_6 was much lower and generally required mild heating and a long wait for dissolution. DMSO-d_6 was important in the study of copolymers. It served as common solvent for both non-polar blocks and polar blocks and was used in quantitative assessment of molar ratios of the two blocks.

4.2 - ^{1}H-NMR Assignment of Homopolymers

The spectra of the polymers formed by the RAFT process used in this research were fairly easy to interpret. In all the homopolymers, there are three resonances that are expected as a result of the repeating monomer: 1) the protons from the methyl group directly off the polymer backbone in methyl methacrylate, *t*-butyl methacrylate and methacrylic acid, or the methine proton in the analogous position in methyl acrylate; 2) the methylene protons that are a direct part of the backbone in all polymers; and 3) the methoxy protons in the case of methyl methacrylate and methyl acrylate, the methyl groups of *t*-butyl methacrylate, or the carboxylic acid proton in the case of methacrylic acid. Assignments of the methoxy, *t*-butyl, and methyl protons off the backbone were made easily by comparing the spectra of the monomers and the polymers. Relative to monomers, all polymer resonances were shifted upfield slightly regardless of the NMR solvent. The chemical shift of the methylene group introduced by the polymerization was always in between the methoxy protons and the protons of the backbone methyl groups or

t-butyl groups. The shifts correlated with the relative expected values for the different types of protons. The vinyl protons were absent in the purified polymer, as they were converted to the methylene groups.

The spectra of the polymers are more complicated than the monomers because the peaks are wider, and they are split due to the stereocenters generated at every other carbon from achiral starting molecules. The protons from the methyl groups off the backbone are split into three distinct peaks, in a close 1:2:4 ratio (downfield to upfield). The methylene protons provided a complex series of peaks relative to the others in the polymer. The methoxy peak was a broad singlet, which did not appear to split significantly, likely because it is further removed from the chiral center of the polymer backbone. In between the obvious large backbone methyl peaks and methylene peaks, there were a few smaller, less obvious peaks to be assigned. In poly(methyl methacrylate), the methoxy peak was far removed from other resonances. Using it as a reference of three protons, the smaller peaks could be assigned by using careful integration. Information on the backbone methyl peak-splitting was then used to assist in assigning peaks in poly(*t*-butyl methacrylate). The spectra and assigned resonances for the two homopolymers are shown in Figures 4.1 and 4.2.

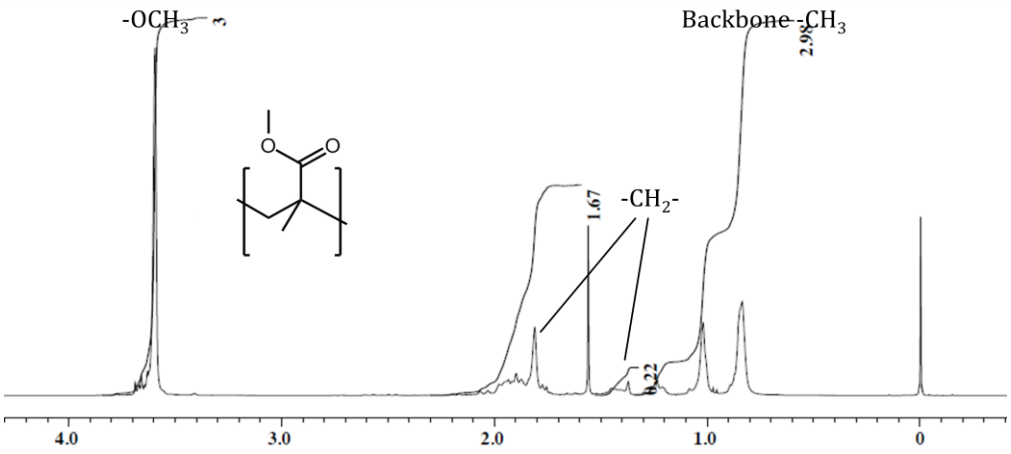


Figure 4.1: ^{1}H-NMR spectrum of poly(methyl methacrylate) in $CDCl_3$. All proton resonances are well-separated. The peaks are assigned by their relative areas. The H_2O peak at 1.55 ppm partially obscures the methylene protons.

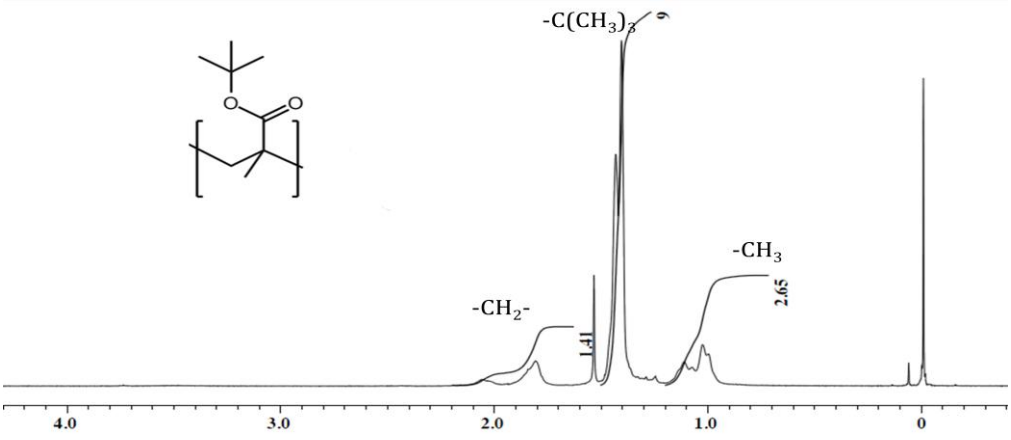


Figure 4.2: ^{1}H-NMR spectrum of poly(*t*-butyl methacrylate) in $CDCl_3$. The *t*-butyl protons overlap with the methylene and backbone methyl protons, as seen by the under-integrations of these peaks.

4.3 - CTA Selection

The efficiencies of CTAs vary widely with different monomers.[34] Two different CTAs (Figure 4.3) were investigated on their ability to incorporate into the polymer and act effectively on poly(methyl methacrylate) and poly(*t*-butyl methacrylate). Relatively short polymers were used to obtain a strong resonance from protons of the CTAs so that the presence or absence of CTA in the purified polymer would be easily observed.

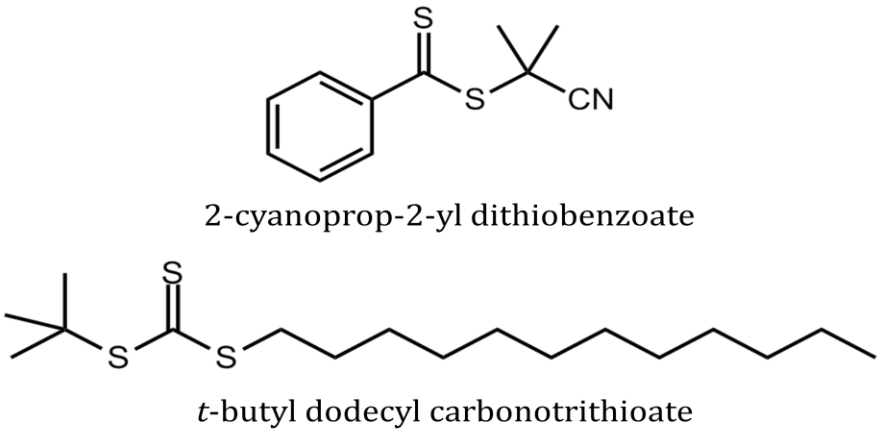


Figure 4.3: The two CTAs compared for the best polymer incorporation.

Poly(methyl methacrylate): To test *t*-butyl dodecyl carbonotrithioate, a 10:1 ratio of monomer:CTA was used. To test 2-cyanoprop-2-yl dithiobenzoate, a 25:1 ratio of monomer:CTA was used. Using *t*-butyl dodecyl carbonotrithioate, no observable CTA protons were visible in the spectrum of the purified polymer. With 2-cyanoprop-2-yl dithiobenzoate, approximately half of the CTA became attached at the chain end, based on the expected and observed monomer:CTA ratios.

Poly(*t*-butyl methacrylate): To test *t*-butyl dodecyl carbonotrithioate, a 10:1 ratio of monomer:CTA was used. To test 2-cyanoprop-2-yl dithiobenzoate, a 50:1 ratio of

monomer:CTA was used. Using *t*-butyl dodecyl carbonotrithioate, approximately half of the CTA became attached to the chain end, as seen by peak integration. Using 2-cyanoprop-2-yl dithiobenzoate, CTA was definitely present in the purified polymer, but the signal to noise was too low to estimate the efficiency of incorporation.

It was clear that not all CTA becomes incorporated into the polymer. After purification, the hexane layer was concentrated to dryness after several reactions and checked by NMR. Free CTA could be seen in the spectrum (along with unreacted monomer, reaction solvent, and unidentified byproducts). These results are qualitative, in that there was low signal to noise of the polymer, especially the CTA protons. Since most of the work to control polymer length was done on poly(methyl methacrylate), *t*-butyl dodecyl carbonotrithioate was abandoned and 2-cyanoprop-2-yl dithiobenzoate (referred simply as CTA from here unless otherwise noted) was used to carry out all subsequent polymerizations.

4.4 - ^{1}H-NMR of Reaction Mixtures – Monomer Conversion to Polymer

Monitoring Reaction Progress of Ester Monomers – Vinyl and Polymer Resonances

As the monomer is consumed, the vinyl protons are converted to methylene protons and the vinyl peaks become smaller. With *t*-butyl methacrylate and methyl methacrylate, the *t*-butyl or methyl groups remain as the monomer converts to polymer. Therefore, the degree of conversion can be estimated by comparing the integration of the vinyl protons to the sum of the *t*-butyl/methyl protons of the monomer and polymer. Ideally, this should be performed immediately after the reaction to avoid the problem of

monomer evaporation, which changes the proportions of the two. The formula is below, setting the integration area of each vinyl proton to 1:

% conversion = [1 – (number of protons of resonance / total integration area of monomer + polymer)] * 100%

It is worth noting that the T_1 values of the vinyl protons might be significantly different than the other protons. Thus, care should be taken in the measurements, and a sufficient relaxation delay should be used to make sure the integrations are accurate. Examples of these estimations are shown in Figures 4.4 and 4.5:

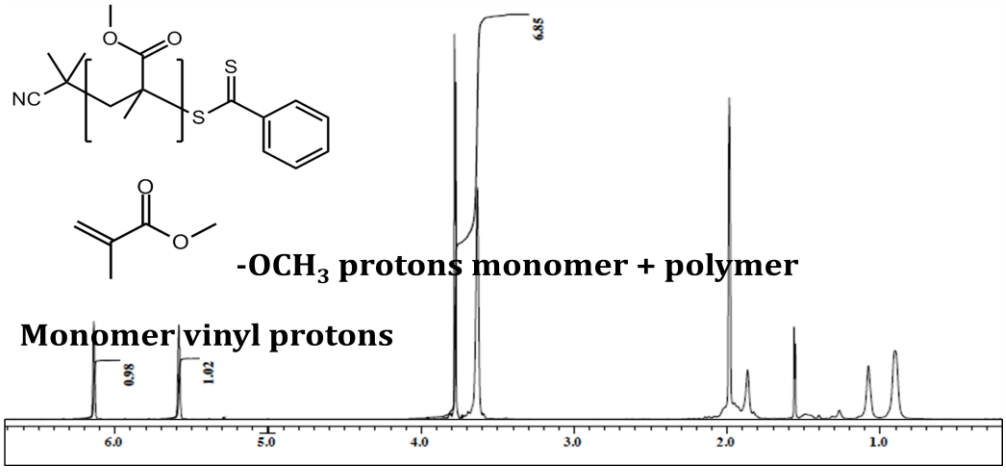


Figure 4.4: % conversion of methyl methacrylate = [1-(3/6.85)]*100% = 56%

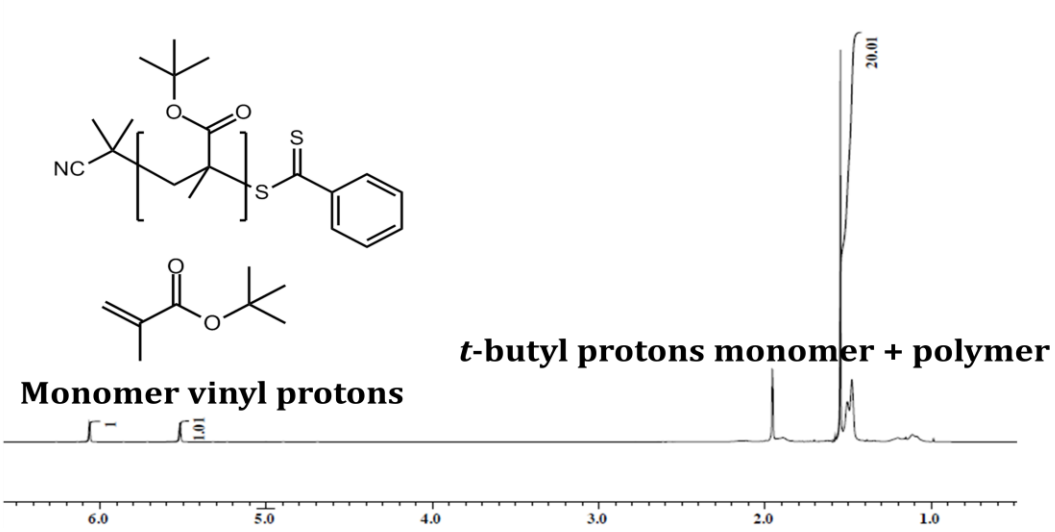


Figure 4.5: % conversion of *t*-butyl methacrylate = [1-(9/20.01)]*100% = 55%

Monitoring Reaction Progress of Acid Monomers - Vinyl and Benzene Resonances

Unlike the esters, acid monomers do not have convenient NMR resonances like the methyl or *t*-butyl groups. The best way to monitor reaction progress is to add a reference compound that is inert during the polymerization and integrate that compound's resonance against the diminishing vinyl protons as the reaction carries on. The chosen compound was benzene, and the conversion can be calculated by the following formula:

% Conversion = [1-(End vinyl:Benzene / Start vinyl:Benzene)] * 100%

Figure 4.6 shows an example of calculating the conversion of poly(methacrylic acid) onto a poly(methyl methacrylate) homopolymer.

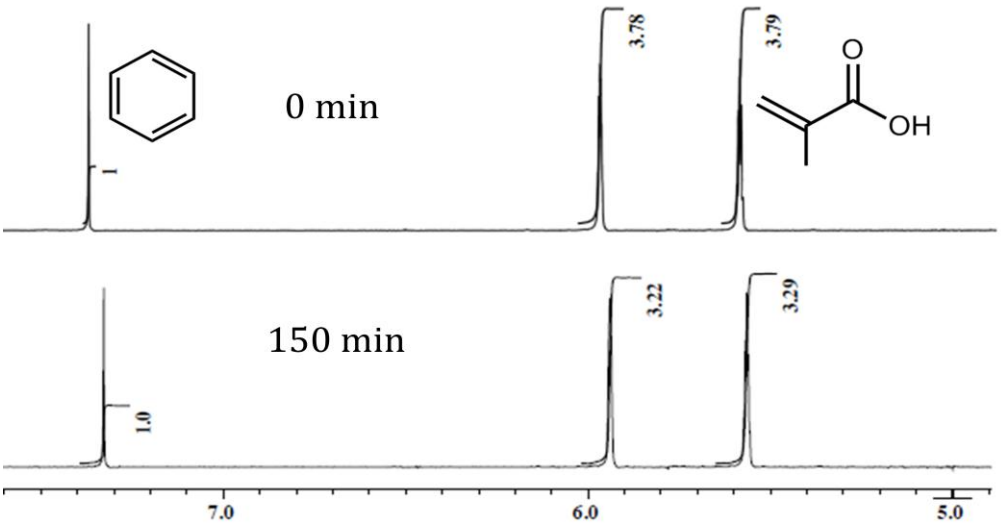


Figure 4.6: % conversion of methacrylic acid = [1-(3.255/3.785) * 100% = 14%

4.5 - ^{1}H-NMR of Reaction Mixtures – Monomer Conversion and Chain Length Estimation

The % conversion can be determined for polymerization reactions as described in the previous section. This value can be used to estimate the molecular weight of a polymer with two major assumptions: 1) Every CTA molecule gets incorporated into one polymer chain, and 2) every growing chain undergoes polymerization via the RAFT mechanism. The result of these assumptions is that there should be a direct correlation of chain length to monomer:CTA ratio, and this ratio multiplied by the fraction of converted monomer gives the degree of polymerization (DP). The formula is then:

Chain Length (DP) = (moles monomer / moles CTA) * % conversion

To test if it was reasonable to assume that % conversion is related to increased molecular weight, aliquots were removed from a poly(methyl methacrylate) polymerization. The % conversion was calculated by NMR and the samples were analyzed by SEC to obtain their M_n values. The results are plotted in Figure 4.7:

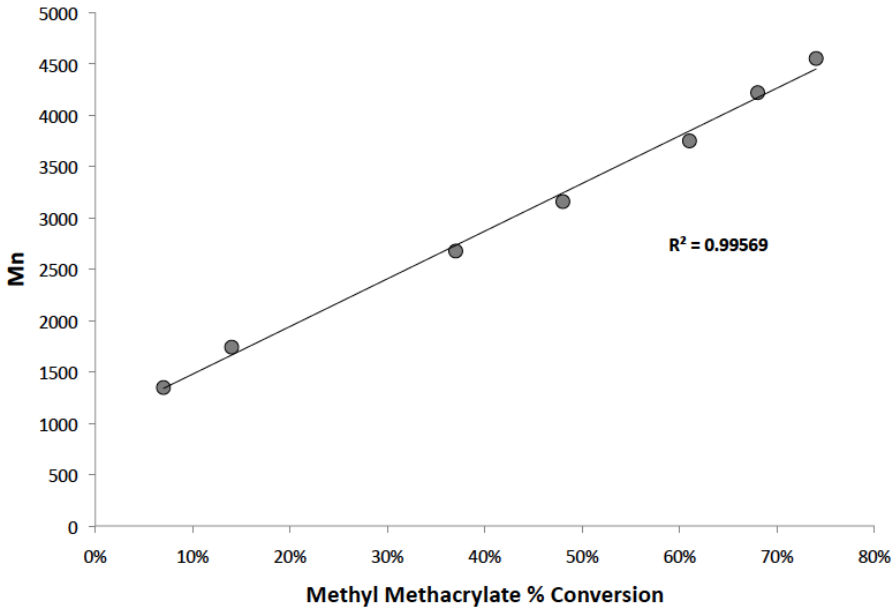


Figure 4.7: Increased conversion of methyl methacrylate leads to larger M_n, as measured by SEC.

Figure 4.7 shows that there is a linear relationship between the conversion of monomer to polymer and the measured M_n by SEC. This is convincing data that the monomer:CTA ratio can be reasonably used to estimate chain length based on % conversion. If the polymerization were not proceeding by the RAFT mechanism as expected, it could be that more chains would be created during the reaction, which could lead to a flattening of the curve despite increased % conversion. Since the M_n increases linearly, it suggests that monomer is adding onto a pre-existing number of chains determined by the number of CTA molecules rather than onto new chains. The amount

of initiator is kept low, typically one tenth of the CTA, to control the number of growing chains.

4.6 - ^{1}H-NMR of Purified Poly(methyl methacrylate) – Chain Length Estimation

Protons of the CTA can be integrated, assigned the proper value of protons per CTA resonance, and then integrated against a specific peak of the polymer to estimate chain length. For this technique, the best peak for poly(methyl methacrylate) is either of the two methyl peaks, and for poly(*t*-butyl methacrylate), the *t*-butyl peak. The chain length is estimated by the following formula:

Chain Length (DP) = Integral value of polymer peak / number of protons that peak represents in the unit structure of the monomer

This calculation still carries the assumption that there is one CTA for every chain in the purified sample but no longer that every CTA is involved in the polymerization reaction, so it is a more direct measurement. Figure 4.8 provides an example on using the above calculation for a purified poly(methyl methacrylate) sample. Both the methoxy protons and the methyl protons of the polymer were integrated and averaged to reduce error.

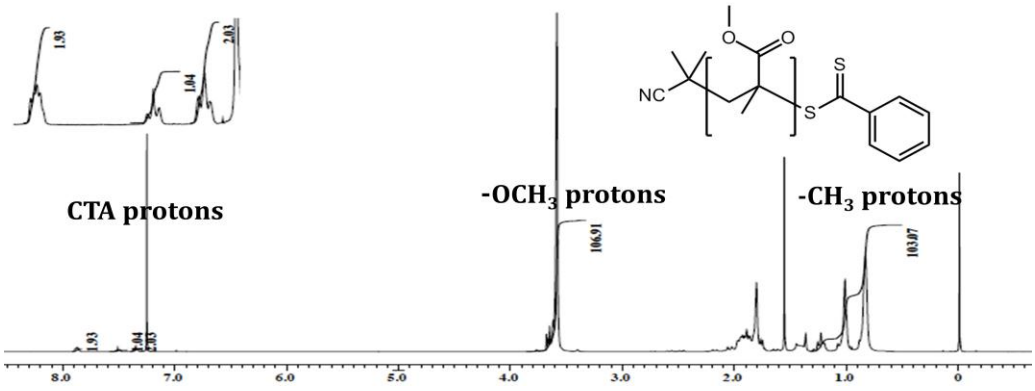


Figure 4.8: Estimating chain length in a poly(methyl methacrylate) sample.
DP = Integral Area/Protons in Peak = ((106.91+103.07) / 2) / 3 = 35

The purified polymer might represent a more accurate estimation of chain length than a polymerization mixture for several reasons. Mainly, it is possible that all CTA molecules are not incorporated into growing chains, which is an assumption that had to be made when estimating chain length of reaction mixtures. Indeed, this was qualitatively witnessed on several occasions. Also, the measured starting CTA:monomer ratio might be slightly different then the desired ratio, skewing the calculation for reaction mixtures. Estimations with purified polymers only involve CTA that is incorporated into polymer. Using this method, comparing only one CTA functional group with the repeating monomer, integrating the CTA becomes difficult if the degree of polymerization gets much above 100 (this depends on the peak intensity of the monomer used, e.g., methyl methacrylate can be more reliably integrated than *t*-butyl methacrylate because of the larger *t*-butyl area).

4.7 - NMR Estimations of Lengths of Block Copolymers

The spectra of the block copolymers were essentially a summation of the two homopolymer spectra. No major changes were seen in the chemical shifts of any of the peaks for the copolymers studied.

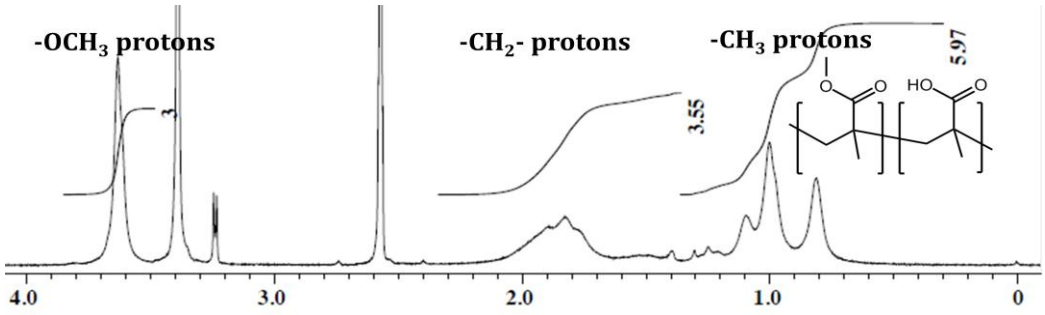
In looking at the length of an added second block, integration of specific resonances in the first and second blocks can be used. Rather than a CTA being used to synthesize the second, the first block itself is the CTA, and the estimated number of these "macro-CTAs" present in the mixture heavily depends on a proper estimation of the M_n of the first block. So even though the ratios of the two monomers can be easily obtained by direct NMR integration of identifying peaks for each block, calculating the chain length of the second relies completely on knowing the chain length of the first.

The chosen method for the best estimation of diblocks is as follows. SEC data are used with NMR data to corroborate the M_n of the first (non-polar) block. This is converted into the chain length (DP) by dividing the calculated M_n by the molecular weight of the monomer. Converting M_n to DP provides a way to compare molar ratios of the blocks by NMR. From there, integration of the two blocks is used. Three block copolymers were prepared in this study, and formulas of their characterization are as follows:

- **Poly(methacrylic acid) : Poly(methyl methacrylate) = (Integral of both overlapping backbone methyl groups – Integral of poly(methyl methacrylate) methoxy) / Integral of poly(methyl methacrylate) methoxy**

- **Poly(methacrylic acid) : Poly(methyl acrylate) = poly(methacrylic acid) methyl group integral / poly(methyl acrylate) methoxy integral**
- **Poly(*t*-butyl methacrylate : Poly(methyl acrylate) = (Integral of *t*-butyl protons / 3) / Integral of methyl acrylate methoxy protons**

These ratios are equal to the molar ratios of the monomers in the two blocks. Figure 4.9 shows an example to calculate the ratio in a poly(methyl methacrylate-*b*-methacrylic acid) copolymer.



5.97 $-CH_3$ protons from both polymer blocks. 3 protons from the methyl methacrylate block and 2.97 from the methacrylic acid block.
methyl methacrylate:methacrylic acid = 1.0 : 0.99

The methylene groups can also be used:
3.55 $-CH_2-$ protons from both polymer blocks. 2 protons from the methyl methacrylate block and 1.55 from the methacrylic acid block.
methyl methacrylate:methacrylic acid = 1.0 : 0.78

Figure 4.9: Calculation of molar ratios of a poly(methyl methacrylate-*b*-methacrylic acid) sample.

4.8 - Size-Exclusion Chromatography (SEC)

SEC in the Analysis of Homopolymers

The NMR techniques for estimating the chain length are subject to some major assumptions that must be clearly acknowledged. For one, it is assumed that the polymer molecules have all incorporated into chains containing CTA end groups, where the number of CTA molecules is equal to the number of chains. This is an assumption based on the theory of RAFT that has been used previously.[35,36] NMR is incapable of providing evidence to support this assumption. It is possible that only a small fraction of the polymer interacts with the CTA in the manner assumed, and NMR does not easily distinguish between free and polymer-bound CTA. SEC provides information about the molecular weight distribution (PDI) of the polymer population, which, if sufficiently high, can be a sign that the CTA is not interacting as assumed. Using SEC, it would be clear if there was significant polymer formation without RAFT in addition to some RAFT polymerization because two different peaks would be seen for polymers with different molecular weight averages. SEC provides M_n based on polymer standards. Standards with narrow molecular weight distributions are used to construct a calibration curve. The method is direct unlike the NMR methods, which estimate M_n by assuming there is one CTA per polymer. The NMR methods that compare the CTA and polymer protons are sensitive to small differences in the integration of the CTA peaks in large polymers, making these measurements prone to error. Finally, SEC separates any unincorporated CTA from the polymer during analysis, making the UV/VIS spectrum for the CTA coeluting with the polymer as evidence for its incorporation into the polymer.

SEC in the Analysis of Block Copolymers

SEC is a more challenging method to calculate molecular weight of copolymers compared with homopolymers. This arises from challenges in detection and also in finding an appropriate standard that has the same ratio of the two blocks. Accurate SEC data from block copolymers can be obtained, but it requires a light scattering detector that was not available with the HPLCs used in this study.

Theoretically, amphiphilic polymers could also aggregate in the mobile phase (THF) and largely increase the size of the measured species. As SEC separates species (not necessarily individual polymer chains) based on hydrodynamic volume, aggregation of polymers into micelles would be expected to give an erroneously large M_n, as several aggregated polymers would behave as a single separated species and elute more quickly. Due to the reasons above, for block copolymers, SEC was primarily used to confirm a single peak, which indicated a single population of block copolymer, rather than two or more, which may have indicated a mixture of block copolymers and homopolymers.

4.9 - Optimization of NMR Methods for Purified Poly(methyl methacrylate)

Poly(methyl methacrylate) samples were synthesized as described in Section 3.1. Table 4.1 shows the details for each reaction, demonstrating the range of reaction conditions used to obtain samples. All reactions were performed at 80 °C.

Polymer ID	Monomer:CTA	CTA:AIBN	Monomer:AIBN	Reaction Time (hr)
PMMA1	50	40	2000	23
PMMA2	71	40	2860	15
PMMA3	71	20	1430	15
PMMA4	50	4	200	12
PMMA5	50	2	100	13
PMMA6	284	5	1430	16
PMMA7	284	10	2860	16

Polymer ID	MMA Used (mmol)	[MMA]	Approx. Pressure (mTorr)*
PMMA1	40	4 M	50
PMMA2	20	2 M	50
PMMA3	20	2 M	50
PMMA4	40	4 M	20
PMMA5	40	2 M	40
PMMA6	20	2 M	50
PMMA7	20	2 M	50

Table 4.1: Reaction details for purified poly(methyl methacrylate) polymers used in this research. *Air pressure in reaction vessel after the frozen solution was allowed to thaw and release dissolved gases. Reagent ratios are molar equivalents.

There are five aromatic protons on the polymer ends from the CTA that can be integrated against the protons in the repeat unit in the polymer chain (Figure 4.8). These can be used to estimate the length of the polymer by ^{1}H-NMR (Section 4.6). All the protons, one, or several can be integrated against the monomer to calculate chain length. These options were compared to determine which method gave values closer to SEC.

The protons of the CTA that were used in these calculations are labeled in Figure 4.10.

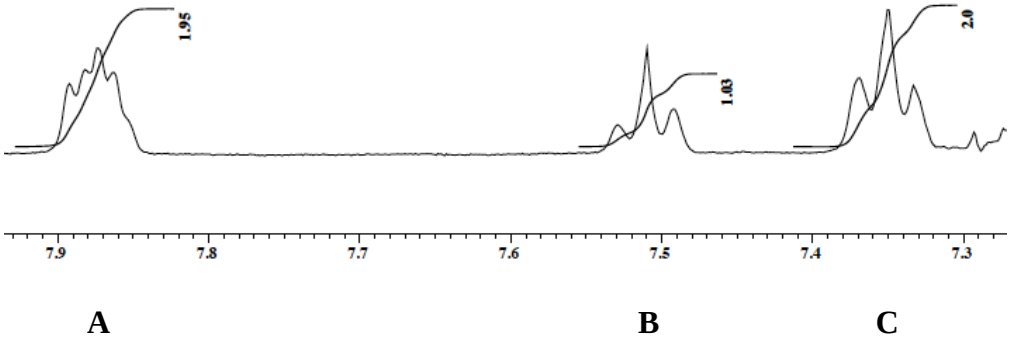


Figure 4.10: The aromatic protons from the CTA used to estimate polymer length.

The integrations from peaks A (2 protons), B (1 proton), and C (2 protons) or using all three (A+B+C, 5 protons, as in Figure 4.8) were compared to the average integration from the two monomer methyl groups to estimate M_n. SEC values were considered accurate, and the NMR values were compared with these. The results are shown in Table 4.2. The variance between the methods for each polymer is shown in Table 4.3. Table 4.4 shows the NMR/SEC M_n for each NMR integration method, averaged for all seven polymers.

Polymer ID	M_n (Integrals A+B+C)	M_n (Integral A)	M_n (Integral B)	M_n (Integral C)	Avg. M_n (NMR)	M_n (SEC)
PMMA1	3120	3197	3085	3064	3116	2691
PMMA2	3531	3939	3502	3444	3529	3365
PMMA3	4760	4864	4601	4740	4741	4130
PMMA4	7588	8196	7252	7220	7564	5624
PMMA5	11123	11111	11262	11065	11140	5454
PMMA6	16056	19410	11220	16772	15865	18512
PMMA7	24491	28208	21555	23025	24320	23133

Table 4.2: M_n estimations using the different CTA integrals and the corresponding SEC values.

Polymer ID	**Average M_n (NMR)**	**Standard Deviation**	**Deviation/Average (%CV)**
PMMA1	3116	58.44	1.88
PMMA2	3529	81.89	2.32
PMMA3	4741	107.92	2.28
PMMA4	7564	453.09	5.99
PMMA5	11140	85.18	0.76
PMMA6	15865	3415.54	21.53
PMMA7	24320	2855.70	11.74

Table 4.3: Variance between the values obtained between the four NMR methods for poly(methyl methacrylate) samples.

Integration Method	Average NMR/SEC M_n
A + B + C	1.24
A	1.32
B	1.17
C	1.22

Table 4.4: Comparison of SEC and NMR estimations of polymer M_n

From these data, the best agreement between SEC measurements and NMR estimation was obtained by using resonance B only (Table 4.4). Whichever integration method was used, the M_n was overestimated by NMR relative to SEC values. There are a few reasons that could explain this. During the polymer purification process, it is possible that some CTA end groups were being removed from the polymer. The CTA is a good leaving group, but the absence of any suitable nucleophiles makes this unlikely, as hexane was used in the precipitation and the polymer was dried under vacuum. It is also possible that a significant number of polymer chains were not capped with the CTA functionality because of the nature of the RAFT mechanism. Since the CTA only transfers radicals formed from the initiator, there should theoretically be chains equal to the number of initiating radicals without CTA end groups. It is reported that the CTA:initiator ratio should be kept low to minimize the number of chains that are not capped with CTA end groups.[37] As an example, a typical reaction with a 10:1 CTA:initiator ratio leads to twelve chains instead of ten if each of the two radicals from the initiator successfully form chains, which may not be the case, as newly formed initiating species can react with each other irreversibly before they react with monomers. If this number were significant, two SEC peaks should have been seen after the synthesis of a block copolymer: a peak corresponding to the newly formed population of block copolymers and a peak eluting later corresponding to the homopolymers without CTA end groups that were unable to attach the second block. This was not observed, either because a significant number of initiator molecules did not make polymers, or the homopolymers that did not form block copolymers did not precipitate easily and were lost during the purification. Ideally, the CTA:initiator ratio is held high enough so the

number of chains without CTA is negligible. In this research, relatively lower ratios (~10:1) were needed to get reasonable rates of polymerization.

Another explanation for the molecular weight overestimation is that the T_1 values of the aromatic protons are significantly longer than the repeating protons of the polymer, and their integrations were lower because they do not have enough time to return to the ground state before the next Rf pulse. A five-second relaxation delay between pulses was used with this in mind, but it is possible that a longer relaxation delay or a lower pulse angle is needed give closer agreement with SEC.

Any of the methods may be used to obtain reasonable estimations close to SEC, as the variation between the different NMR methods was relatively low (Table 4.3). However, more variance was seen when measuring polymers with higher M_n, which suggests more error in the integrations in longer polymers. This is likely due to the increased noise in the NMR spectrum as the CTA end groups constitute less of the polymer and contribute less signal to the overall area.

4.10 - Comparison of SEC with NMR methods for Reaction Mixtures vs. Purified Poly(methyl methacrylate)

The data in the previous section demonstrate a reasonable agreement of M_n between ^{1}H-NMR and SEC with purified poly(methyl methacrylate), especially for PMMA1-PMMA5, although the NMR method for purified polymers overestimates the M_n relative to SEC. The M_n values for purified polymers (using all three CTA resonances), SEC, and reaction mixtures were compared for the same set of polymers. The values are compared in Figure 4.11.

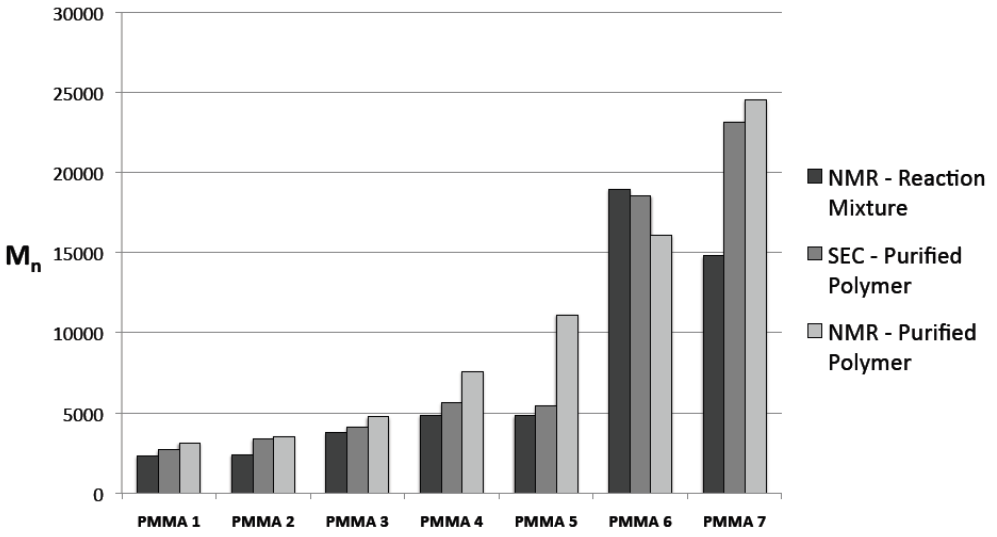


Figure 4.11: M_n values obtained by SEC compared with the estimates obtained from reaction mixtures and purified polymers.

It is interesting that while the purified polymer integration method overestimated polymer M_n (Section 4.9) in almost all cases, the reaction mixture method underestimated M_n relative to SEC values, which were assumed to be more accurate measurements (Section 4.8). The most likely explanation for the underestimation is that not all of the CTA gets incorporated into the polymer. Since the calculation to estimate polymer length from % conversion uses the monomer:CTA ratio as a theoretical chain length, the polymer length should be longer than estimated if not all CTA is taking part in the polymerization. This is what was seen. When the hexane layer from the polymer precipitation was evaporated and analyzed by NMR, unreacted CTA was indeed present.

4.11 - Comparison of M_n measurements of SEC vs. NMR of *t*-butyl Methacrylate Reaction Mixtures

Seven poly(*t*-butyl methacrylate) polymers were synthesized as described in Section 3.1. All reactions were performed with a monomer concentration of 2 M and a temperature of 80 °C. Table 4.5 shows the details for each reaction.

Polymer ID	Monomer:CTA	CTA:AIBN	Monomer:AIBN	Reaction Time (hr)
P*t*BMA1	50	10	500	8.3
P*t*BMA2	50	10	500	8.3
P*t*BMA3	50	20	1000	8.3
P*t*BMA4	110	10	1100	~12
P*t*BMA5	150	6.7	1000	8.3
P*t*BMA6	200	5	1000	8.3
P*t*BMA7	400	2.5	1000	8.3

Polymer ID	*t*BMA Used (mmol)	Heating Method	Conversion
P*t*BMA1	8	M	41%
P*t*BMA2	8	M	35%
P*t*BMA3	10	M	13%
P*t*BMA4	35	O	58%
P*t*BMA5	10	M	16%
P*t*BMA6	10	M	37%
P*t*BMA7	10	M	32%

Table 4.5: Details for poly(*t*-butyl methacrylate) reactions used in this research. Microwave Heating (M); Oil Bath Heating (O). Reagent ratios are molar equivalents.

The M_n of each polymer in the crude reaction mixture was calculated from the % conversion and compared with SEC values calculated for each polymer. The values obtained for the two methods are plotted in Figure 4.12.

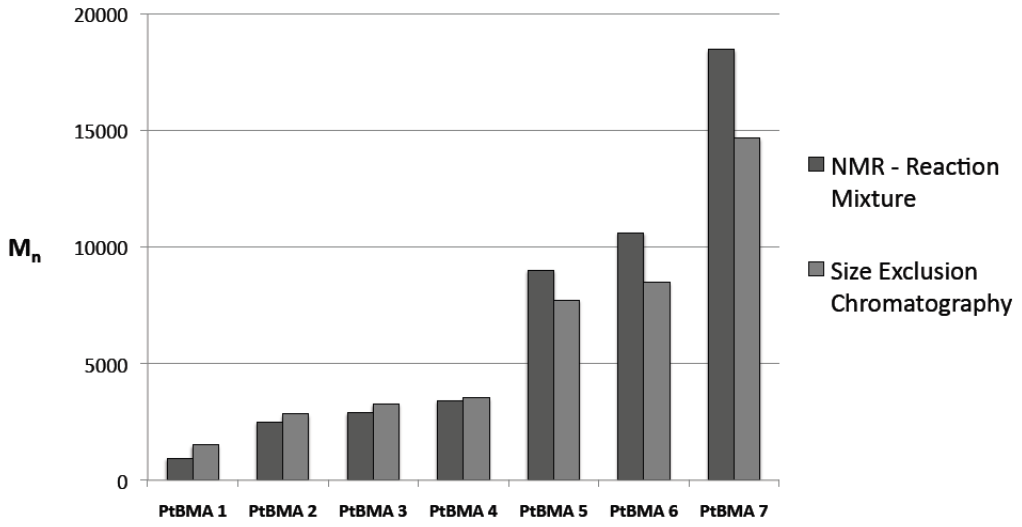


Figure 4.12: Comparison of calculated M_n of a set of poly(*t*-butyl methacrylate) samples by SEC and ^{1}H-NMR.

With P*t*BMA 1-4, the NMR method underestimated polymer length relative to SEC. This is the same as seen with poly(methyl methacrylate) samples. However, with P*t*BMA 5-7, the reverse was seen, and the NMR method overestimated the SEC values. Underestimation that likely resulted from unincorporated CTA is logically explained (Section 4.10), but it is difficult to explain how this NMR method could overestimate. There was some overlap of the methylene and methyl groups in the poly(*t*-butyl methacrylate) NMR spectrum, which could give falsely high results when integrating the *t*-butyl protons, but this does not seem significant enough. A likely explanation is that the Mark-Houwink parameters that translate poly(methyl methacrylate) standards to poly(*t*-butyl methacrylate) samples with SEC were not providing accurate values. The molecular weights for P*t*BMA 1-4 are below the reliable range of the SEC column used, which may explain why P*t*BMA 1-4 underestimated values relative to SEC and P*t*BMA 5-7 overestimated. Caution should be taken in the future when using poly(methyl methacrylate) standards for different types of polymer samples.

Unlike the poly(methyl methacrylate) samples described in Section 4.6, these samples were not purified to compare the NMR methods for the purified polymer vs. reaction mixtures. This should be investigated to see if the purified polymer NMR method can show a better trend with SEC values.

4.12 - Synthesizing Predictable Polymer Lengths by Altering Monomer:CTA Ratios

The average DP of a polymer population is theoretically calculated by the number of monomers in the reaction divided by the number of chain transfer molecules. Thus, a predictable polymer length should be synthesized by altering the proportions of these in the polymerization reaction and keeping all other factors constant. While variables such as temperature and polymerization time are obvious constants between reactions, the amount of initiator is not, as the rate of polymerization depends on rates of initiation, chain transfer, and chain termination. In a series of polymerizations, CTA:AIBN or monomer:AIBN ratios could be held constant. The AIBN and monomer concentrations were held constant in this experiment to keep the rate of polymerization the same to obtain a similar % conversion over the same time period.

Three target DP of 50, 100, and 200 were attempted in three separate conventional heating reactions. The monomer concentration was 2 M, and all reactions were held at 80 °C for approximately 15 hours. Monomer:AIBN ratios were held constant at 1420:1, and the concentration of CTA was changed to affect polymer size. The characteristics of each reaction are listed in Table 4.6.

Desired DP	Actual DP (NMR)	Actual DP (SEC)	Monomer:CTA
50	48	41	71
100	107	104	142
200	161	185	284

Table 4.6: Comparison of target DP and actual DP for three poly(methyl methacrylate) polymers.

Preliminary NMR data when aiming for a DP of 100 suggested that under these conditions, the purified polymer has a M_n about 70% of what would be expected from monomer:CTA ratios. Taking this into account, the target DPs were reasonably met when using a conversion of 1.42 (1/0.7) and starting with an excess of monomer. The results show that simply altering the monomer:CTA ratio does allow one to come reasonably close to synthesizing polymers of a predetermined length. However, these results could benefit from replicate experiments to rule out experimental error and more data points to investigate whether a linear relationship exists.

4.13 - Effect of Increased Monomer Conversion on PDI

It was worth investigating how the PDI of a polymer population is affected by the conversion of monomer to polymer. For the observation of micelle formation by NMR, a low PDI is crucial to determine when the micellization point occurs. Greater resolution of the effect will be obtained if the polymers in a sample are near the same size. If the polymer length varies greatly in a sample, a wider range of conditions will trigger micelle formation of differently sized polymers in the population.

A reaction of 40 mmol methyl methacrylate, 0.8 mmol CTA, and 0.4 mmol AIBN (50:1:0.5) was performed by conventional heating at 80 °C. Aliquots of the reactions were analyzed by SEC to determine PDI. The % conversion by NMR was also recorded to corroborate reaction progress. The results are shown in Table 4.7.

Time (min)	% Conversion (NMR)	M_n (SEC)	M_w/M_n
20	7%	1349	1.13
30	14%	1742	1.16
60	37%	2678	1.23
75	48%	3160	1.23
105	61%	3751	1.25
135	68%	4221	1.26
165	74%	4554	1.27

Table 4.7: PDI as a function of % conversion for a single methyl methacrylate polymerization.

It can be seen that as % conversion increased, the overall trend was that PDI did as well, although the increase was rather small.

PDI as a function of % conversion was also examined over a sample of independent *t*-butyl methacrylate reactions rather than a single reaction's progress (Table 4.8).

Polymer ID	% Conversion (NMR)	PDI
P*t*BMA3	13 %	1.12
P*t*BMA5	16 %	1.30
P*t*BMA7	32 %	1.14
P*t*BMA2	35 %	1.16
P*t*BMA6	37 %	1.18
P*t*BMA1	41 %	1.12
P*t*BMA4	58%	1.11

Table 4.8: PDI as a function of % conversion for several poly(*t*-butyl methacrylate) reactions.

Unlike the data of several time points for a single reaction, when several different reactions are compared, there is no clear correlation between % conversion and PDI. The different poly(*t*-butyl methacrylate) samples were performed under different conditions (Table 4.5). It is possible that altering these other variables affects the PDI more than the degree of conversion of a reaction. Since other factors besides monomer conversion affect PDI more significantly, no limitation on conversion was considered necessary for polymerization reactions.

4.14 - Effect of the Length of the First Block on the Polymerization Rate of the Second

It seemed possible that the polymerization rate of the second block could be affected by the length of the first block, the macro-CTA. Three poly(methyl methacrylate-*b*-methacrylic acid) copolymers were synthesized using homopolymers of different lengths. For each reaction, 2 mmol poly(methyl methacrylate) homopolymer, 3 mmol methacrylic acid monomer, and 3 μmol AIBN were dissolved in benzene with a monomer concentration of 2 M. The reactions were prepared and performed simultaneously in the same oil bath at 80 °C. NMR was performed on each aliquot three times and the % conversion averaged. The ratio of each vinyl proton/benzene was averaged to calculate the conversion as described in Section 4.4. The % conversion for all three reactions over time is plotted in Figure 4.13.

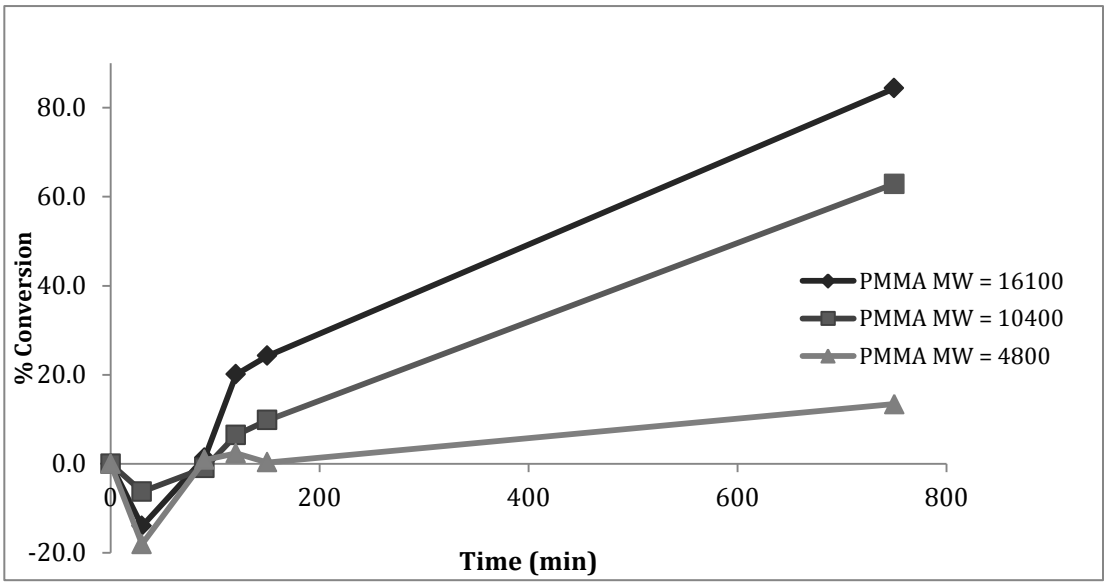


Figure 4.13: Polymerization rate of methacrylic acid onto poly(methyl methacrylate) homopolymers of different molecular weight.

The data suggest that the rate of polymerization of the second block does depend on the length of the first block. The experiment could be improved with more data points between 150 and 750 min, but there is a clear trend that the longer the poly(methyl methacrylate) homopolymer, the faster the rate of methacrylic acid polymerization. While the concentration of methacrylic acid and number of methyl methacrylate subunits is the same in all cases, the concentration of CTA is lower in reactions using long poly(methyl methacrylate) homopolymers, which is likely the cause for this result. In the reaction with longer homopolymers, fewer of the radicals are in the stable resting state, and the polymerization goes more quickly. Controlled synthesis of the second block is thus more difficult than the first, and this should be noted in future research. The % conversion is calculated to be negative at the first two measured time points and should be addressed. This is likely the result of the added benzene reference escaping into the gas phase as the reaction is heated. After freeze/pump/thawing, there is a very low vapor pressure in the reaction flask.

4.15 - Microwave Heating vs. Oil Bath Heating

Benefits of performing reactions with microwave heating have been reported for these monomer types.[38] Increased polymerization rates are a notable reason. However, microwave assisted polymerizations were inferior to oil bath heating in this study for two reasons. One was the variability in reaction rates between identically prepared reaction mixtures. If the goal is to make predictable polymer lengths, then the method that is most reproducible under a set of conditions is preferred. Heating with an oil bath was much more reproducible as seen by similar conversion rates with reactions performed in

tandem or replicated. One reason for this might be related to the observation that reaction volumes are significantly reduced during the deoxygenation process of microwave reactions. The bubbling of nitrogen through the solution seems to facilitate the evaporation of solvent and monomer, and it was difficult to control the flow of the stream of nitrogen used for the process. The freeze/pump/thaw process of the oil bath reaction did not seem to exhibit this variability. The other reason that microwave polymerizations were inferior in this study is the observation that reactions with methyl methacrylate, but not *t*-butyl methacrylate, would inexplicably stop polymerization abruptly after rapidly reaching a certain degree of conversion. For these reasons, oil bath heating was ultimately used for the polymerization of methyl methacrylate.

4.16 - Remaining Issues with Poly(methyl acrylate-*b*-methacrylic acid) Copolymer Synthesis

Incomplete Deprotection of *t*-Butyl Esters

Removal of *t*-butyl esters to form free carboxylic acids with the use of TFA is a well-documented, standard deprotection reaction. Reactions commonly go to completion. However, in this research the removal rarely went to completion. For example, two block copolymers made with the same *t*-butyl methacrylate block but with different methyl acrylate lengths showed 80% and 96% *t*-butyl removal, with the more successful reaction on a shorter methyl acrylate chain. After the reactions, the polymer precipitated as the acid groups are less soluble in dichloromethane. A further reaction in a methanol:dichloromethane mixture that fully solvated the polymer was performed. No additional removal of *t*-butyl groups was observed despite the increased solubility.

Furthermore, attempting to remove *t*-butyl groups from a poly(*t*-butyl methacrylate) homopolymer yielded poor conversion of only 55%, which was not improved by increasing solubility.

A possible explanation for the poor conversion might be polymer self-assembly. As the acids are formed, they may aggregate together, much like the formation of a micelle, with the non-polar methyl acrylate chains remaining well-solvated. Remaining adjacent *t*-butyl groups could be drawn into the core, inhibiting interaction with TFA. This agrees with an observation that more *t*-butyl removal was seen in a copolymer with a shorter methyl acrylate chain. However, the lower *t*-butyl removal of the poly(*t*-butyl methacrylate) homopolymer is difficult to explain, since aggregation should be less likely to occur.

Homopolymer Synthesis with Methacrylic Acid

Avoiding the deprotection step entirely could be a useful way to mitigate the problem of incomplete removal of the *t*-butyl groups. This would involve direct synthesis of the poly(methacrylic acid) homopolymer as the first block. Synthesis of poly(methacrylic acid) homopolymer was investigated, but it presented some problems that would need to be resolved before it becomes a viable method. For one, a polar solvent such as methanol must be used to dissolve the poly(methacrylic acid) homopolymer. The polymer is insoluble in dioxane, THF, benzene, and toluene. Poly(methyl acrylate) and poly(methyl methacrylate) have limited solubility in methanol, so phase-separation may occur when adding the second block. Perhaps the largest hurdle lies in homopolymer characterization by SEC. The polymer is insoluble in the THF mobile phase so a different solvent, column, and set of standards would have to be used

to obtain SEC information. These materials were unavailable at the time of this research. Since the NMR methods to quantify polymer length need to be validated by correlating with SEC, this obstacle would need to be overcome before the free acid homopolymer synthesis can be confidently investigated.

Reverse-Order Copolymer Synthesis

Block copolymers are ideally synthesized by making the more substituted polymer first. Methacrylic acid is more substituted than methyl acrylate and was chosen to be the second block to be added. However, it may be possible to synthesize the copolymer in reversed order. The poly(methyl acrylate) block can be prepared first and then have the second block added on as poly(methacrylic acid), analogous to the procedure for preparing poly(methyl methacrylate-*b*-methacrylic acid). This method was not investigated in this research, but should be considering the difficulties synthesizing this copolymer.

Chapter 5: Observation of Micelle Formation by ^{1}H-NMR

5.1 - Polymers Used for Micelle Formation Experiments

Poly(methyl methacrylate-*b*-methacrylic acid) and poly(methyl acrylate-*b*-methacrylic acid) were synthesized in this research. Poly(methyl acrylate-*b*-acrylic acid) was synthesized by Kevin Kawchak, a member of the Wilmes lab. Poly(methyl methacrylate-*b*-acrylic acid) was purchased from PolymerSource, Inc. The four polymers were allowed to incubate at varying D_2O/dioxane-d_8 ratios and at different temperatures. ^{1}H-NMR was performed to check for line-broadening, a sign that self-assembly had occurred.[39] The qualitative results are discussed here. As the polymer chains come out of solution and aggregate together in close proximity, the T_2 relaxation time gets significantly shorter, which creates the broadening effect. Table 5.1 lists the lengths of each block in the four polymers.

Polymer	Hydrophobic DP	Hydrophilic D	PDI
P(MA-*b*-AA)	66	60	1.10
P(MA-*b*-MAA)	40	77	1.27
P(MMA-*b*-AA)	55	69	1.15
P(MMA-*b*-MAA)	104	84	1.27

Table 5.1: Block lengths and PDI of the four copolymers used in this experiment.

5.2 - Poly(methyl methacrylate-*b*-acrylic acid)

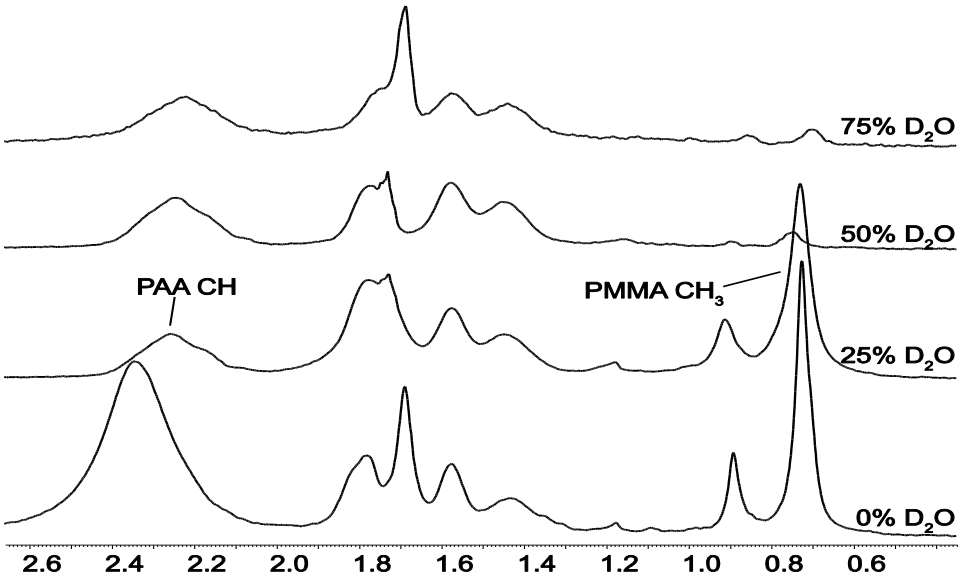


Figure 5.1: ^{1}H-NMR spectra of poly(methyl methacrylate-*b*-acrylic acid) at 25 °C.

The spectra of poly(methyl methacrylate-*b*-acrylic acid) at 25 °C at different D_2O concentrations can be seen in Figure 5.1. The large peak at ~2.4 ppm is likely water on the polymer backbone that disappears via exchange when D_2O is added. With increased D_2O, a sudden broadening of the backbone methyl of poly(methyl methacrylate) is observed with 50% D_2O. The methine proton of acrylic acid does not appear to significantly broaden between 25%-75% D_2O. At 0% D_2O, the methine peak appears large, but is likely again obscured by water on the polymer, which can be seen to have a different downfield chemical shift. These results are consistent with the expectation that the hydrophobic poly(methyl methacrylate) block should form the core of the micelle while the hydrophilic poly(acrylic acid) block remains on the exterior of the micelle and well solvated. Figure 5.2 shows this polymer at different temperatures.

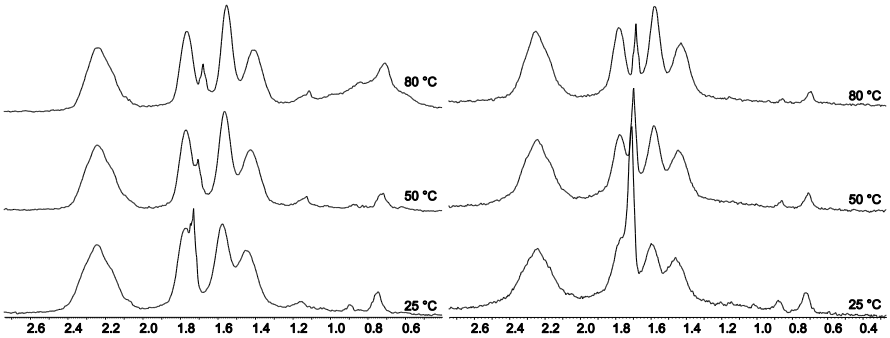


Figure 5.2: ^{1}H-NMR spectra of poly(methyl methacrylate-*b*-acrylic acid) at 50% D_2O (left) and 75% D_2O (right).

At 80 °C in 50% D_2O, the methyl methacrylate methyl groups show a slight sharpening, which may be interpreted as disruption of the micelle from increased molecular motion at high temperature. At 75% D_2O, no sharpening was observed, as the increased water content may lead to stronger hydrophobic interactions that cannot be disrupted even at high temperatures. This copolymer had the clearest positive result of micelle formation.

5.3 - Poly(methyl methacrylate-*b*-methacrylic acid)

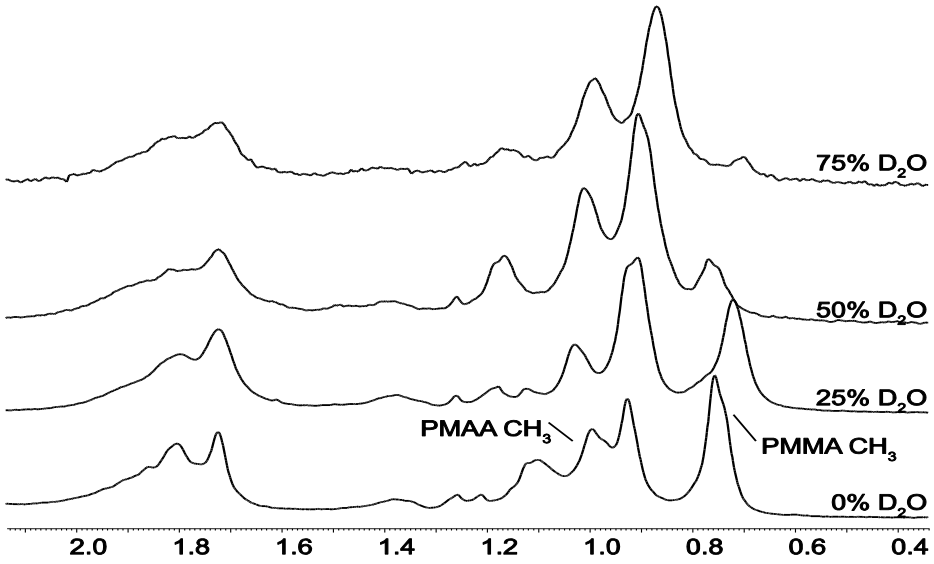


Figure 5.3: ^{1}H-NMR spectra of poly(methyl methacrylate-*b*-methacrylic acid) at 25 °C.

The spectra of poly(methyl methacrylate-*b*-methacrylic acid) at 25 °C at different D_2O concentrations can be seen in Figure 5.3. There is overlap between the two methyl groups for each polymer, which makes the results difficult to interpret. At 25% D_2O, the upfield peak from the methacrylic acid overlaps with the downfield peak of the methyl methacrylate. The upfield peak from methyl methacrylate seems to slightly broaden. At 50% D_2O, the upfield methyl methacrylate peak is definitely broadened. The downfield methyl methacrylate peak may be completely obscured by the more upfield methacrylic acid peak. However, since the middle resonances (one from each block) seem to shift at 25% D_2O, it is possible that the most downfield peak at 1.2 ppm is the other methyl methacrylate peak, which has exhibited a chemical shift change due to the different chemical environment. At 75% D_2O, the large peaks are likely from the methacrylic acid with a slightly different chemical shift, where the methyl methacrylate peaks are significantly smaller and broadened.

5.4 - Poly(methyl acrylate-*b*-acrylic acid)

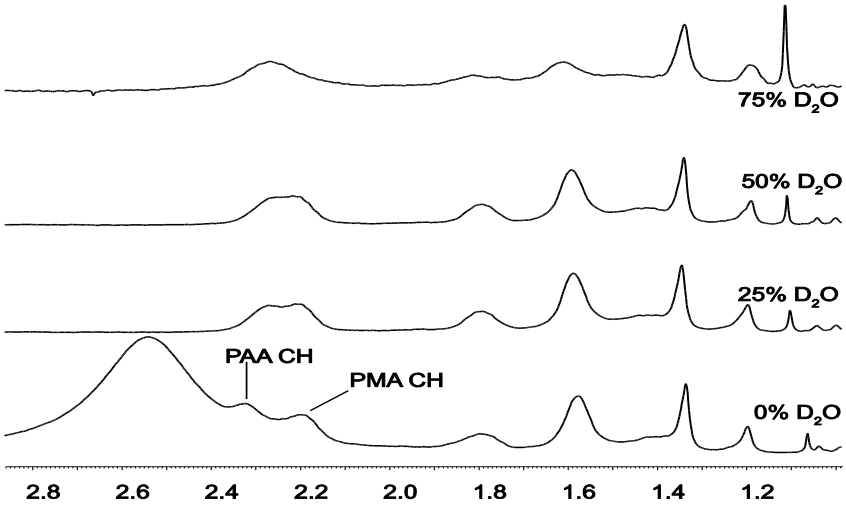


Figure 5.4: ^{1}H-NMR spectra of poly(methyl acrylate-*b*-acrylic acid) at 25 °C.

The spectra of poly(methyl acrylate-*b*-acrylic acid) at 25 °C at different D_2O concentrations are arrayed in Figure 5.4. Whether or not the formation of micelles occurred is difficult to determine. The methine protons from each polymer have slightly different chemical shifts between 2.2 and 2.4 ppm. At 25% D_2O, these are seen to overlap more, and this continues further at 50% and 75% D_2O. Due to the overlap, it is not clear whether or not there was significant peak broadening of one resonance or simply chemical shift overlap. The large peak around 2.6 ppm again is likely water on the polymer that disappears by proton exchange with the addition of D_2O.

5.5 - Poly(methyl acrylate-*b*-methacrylic acid)

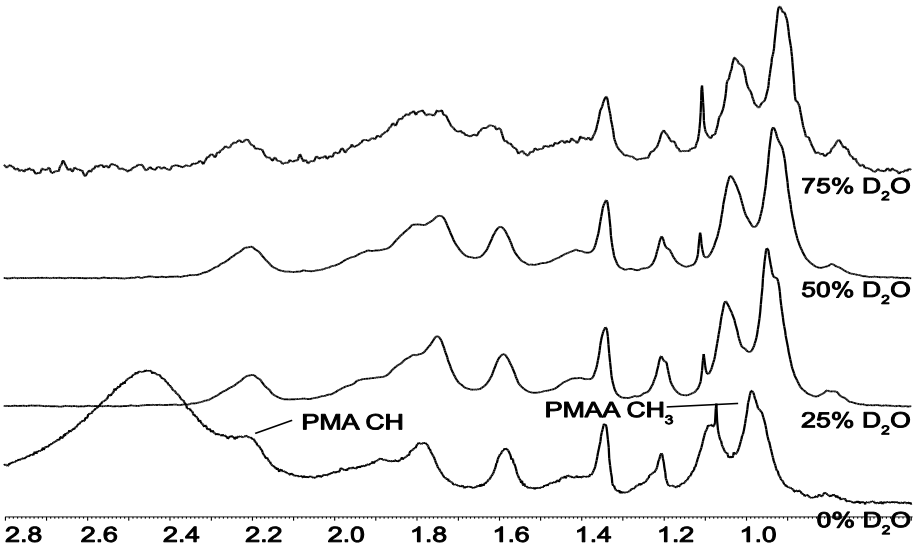


Figure 5.5: ^{1}H-NMR spectra of poly(methyl acrylate-*b*-methacrylic acid) at 25 °C.

The spectra of poly(methyl acrylate-*b*-methacrylic acid) at 25 °C at different D_2O concentrations are arrayed in Figure 5.5. With increased D_2O, neither the methine proton of methyl acrylate nor the backbone methyl protons of the methacrylic acid broaden with the addition of D_2O. The results suggest that micelle formation does not occur with this copolymer. Again, the broad peak at 2.5 ppm is believed to be water on the polymer that disappears via exchange when D_2O is added.

5.6 - Results

These data show that poly(methyl methacrylate-*b*-methacrylic acid) and poly(methyl methacrylate-*b*-acrylic acid) exhibited line broadening for methyl methacrylate resonances with an increased aqueous environment. Poly(methyl acrylate-*b*-methacrylic acid) showed no difference with the addition of D_2O. The results of poly(methyl acrylate-*b*-acrylic acid) are not very clear due to overlapping signal but do not suggest micelle formation. These observations are consistent with the hypothesis that the more rigid poly(methyl methacrylate) will be more prone to self-assembly versus the more flexible poly(methyl methacrylate).

Chapter 6: Conclusions and Future Work

Poly(methyl methacrylate) polymer size measurements by NMR reasonably agreed with SEC, and polymer molecular weights were confidently assigned. The NMR integration method for reaction mixtures tended to estimate a lower molecular weight relative to SEC measurements and the method for purified polymers tended to overestimate molecular weight. In the future, altering pulse angle and relaxation delay should be investigated to see if the values for purified polymers can come closer to SEC values. SEC data showed that there was a linear relationship between conversion and molecular weight and also confirmed that a single polymer population was created. Polymers close to a predetermined chain length can be synthesized by simply altering the monomer:CTA ratio, keeping other things constant. The methods investigated for poly(methyl methacrylate) can be used as a model for other homopolymers. Estimating poly(*t*-butyl methacrylate) polymer size by NMR was done with less confidence, as there was less of a trend relative to SEC values, and the reliability of SEC values themselves, as they are translated from poly(methyl methacrylate) standards, needs to be investigated.

The data from micelle formation experiments for all four polymer permutations strengthened the hypothesis that more rigid hydrophobic chains should be more prone to forming micelles compared with more flexible ones under the same set of conditions. Polymers that had the more rigid poly(methyl methacrylate) as the hydrophobic block formed micelles, while polymers containing the more flexible poly(methyl acrylate) did not. In future experiments, chain length should be controlled for all polymer blocks to better isolate the effects of chain rigidity on the formation of micelles, as polymer size also has an effect that should be controlled.

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