Charles University

Faculty of Science

Studijní programme: Chemie

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Synthesis of stimuli-responsive copolymers via atom transfer radical polymerization

Syntéza kopolymerů reagujících na vnější podněty pomocí radikálové polymerizace s přenosem atomu

Bakalářská práce

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, dne 22.8. 2024

Eliška Müllerová

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Abstrakt

Bakalářská práce se věnuje syntéze termoresponzivních polymerů metodou radikálové polymerizace atomovým přenosem (ATRP), což je metoda kontrolované polymerizace, která umožňuje vysokou míru kontroly a molekulovou hmotností a strukturou výsledného polymeru. Pro přípravu termoresponzivního homopolymeru byly použity di(ethylen glykol) methyl ether akrylát a tri(ethylen glykol) methyl ether akrylát. Pro zlepšení funkcionality polymeru byl do jeho struktury zabudován pinakol ester 3-akrylamido fenylboronové kyseliny, který je zodpovědný za citlivost kopolymeru na pH a jeho schopnost vázat dioly a sacharidy. Optimalizace procesu ATRP bylo dosaženo úpravou různých složek, jako je katalyzátor, ligand, rozpouštědlo a jejich příslušné poměry. Syntetizované (ko)polymery byly charakterizovány pomocí nukleární magnetické resonance (^{1}H NMR) a gelové permeační chromatografie (GPC) k určení jejich struktury, průměrné molekulové hmotnosti a její distribuce. Termoresponzivní vlastnosti byly potvrzeny změřením teploty bodu zákalu. Pro přečištění získaných kopolymerů byla provedena dialýza proti acetonu. Vliv deprotekce na teplotu bodu zákalu byl také zjišťován. Předkládaná práce přináší nové poznatky pro syntézu termoresponzivních polymerů metodou ATRP a jejich možného využití.

Abstract

This thesis explores the synthesis of thermoresponsive polymers using atom transfer radical polymerization (ATRP), a controlled polymerization technique that allows for a high degree of control over molecular weight, dispersity, and polymer architecture. For the thermoresponsive homopolymer preparation, di(ethylene glycol) methyl ether acrylate and tri(ethylene glycol) methyl ether acrylate were used. To enhance the functionality of these homopolymers, a 3-acrylamidophenylboronic acid pinacol ester monomer was incorporated into the polymer structure, forming copolymers with thermoresponsive, pH-responsive and diol and saccharides binding properties. The optimization of the ATRP process was achieved by adjusting various components such as the catalyst, ligand, solvent, and their respective ratios. The synthesized (co)polymers were characterized using nuclear magnetic resonance (^{1}H NMR) spectroscopy and gel permeation chromatography (GPC) to determine their structure, number average molecular weight, and its distribution. To precisely purify the obtained copolymers, dialysis against acetone was performed. Thermoresponsive properties were confirmed by determining its cloud point temperature via light scattering. The effect of phenylboronic deprotection on the cloud point temperature was also investigated. Ultimately these new pieces of information bring insights into the synthesis of thermoresponsive (co)polymers via ATRP and their possible application.

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Abbreviations list

3-APBAE – 3-acrylamidophenylboronic acid pinacol ester

ATRP – atom transfer radical polymerization

A(R)GET – atom (re)generated by electron transfer

CRP – CRP-controlled radical polymerization

mDEGA - di(ethylene glycol) methyl ether acrylate

mTEGA - tri(ethylene glycol) methyl ether acrylate

DCM - dichloromethane

DMSO - dimethylsulfoxide

EBiB – ethyl-alpha-bromisobutirate

EBP – ethyl-2-bromopropionate

GPC – gel permeation chromatography

LCST - low critical solution temperature

Me_6TREN – tris[2-(dimethylamino)ethyl]amine

NMP – nitroxide-mediated radical polymerization

NMR – nuclear magnetic resonance

OEGA – oligo ethylene glycol acrylates

PBA – phenylboronic acid

PMDTA - *N, N, N', N'', N*''-pentamethyl-diethylenetriamine

RAFT – reversible addition-fragmentation chain transfer

TAE - triethylamine

THF - tetrahydrofuran

TPMA – tris(2-pyridyl methyl)amine

VPTT – volume phase transition temperature

1 Introduction

Thermoresponsive polymers have gained significant attention due to their ability to undergo reversible phase transitions in response to surrounding temperature. This makes them valuable in many fields such as drug delivery or smart coatings. Radical processes are the leading method used in industrial settings and therefore the development of controlled radical polymerization methods, such as ATRP, has many future applications. Preparing these polymers via ATRP allows to produce polymers with narrow molecular weight distributions and well-defined structures, which are essential for adjusting material properties for specific applications. ATRP is a type of controlled radical polymerization that employs a transition metal catalyst to mediate the growth of polymer chains. This process can be adjusted by different factors such as the type of catalyst, ligand, solvent, and their ratios.

This thesis focuses on the preparation of thermoresponsive homopolymers using di(ethylene glycol) methyl ether acrylate and tri(ethylene glycol) methyl ether acrylate as monomers. To enhance the functionality of these homopolymers, the study introduces 3-acrylamidophenylboronic pinacol ester monomer into the polymer structure, forming copolymers with pH responsiveness, diol, and saccharides binding properties as well as thermoresponsive properties.

2 Literature overview

2.1 ATRP polymerization

Atom Transfer Radical Polymerization is a method of controlled radical polymerization (CRP), which allows a synthesis of polymers with highly defined properties, such as composition, architecture, or functionalities. A general mechanism is shown in Figure 1. The process occurs with a constant rate of activation k_{act} and deactivation k_{deact}, which determine the degree of control and thus the dispersity of the produced polymer. The addition of monomers and the intermediate radicals (R·) created during the process occurs with the constant rate of propagation k_p. Termination also does occur, with the rate k_t, but only a small percentage of chins undergo this. For ATRP, the crucial step is the quick and reversible transfer of the halogen atom which generates persistent radicals (the oxidized metal complexes M_t^{n+1}-Y/ligand) which reduce the stationary concentration of growing radicals and therefore reduce termination. [1]

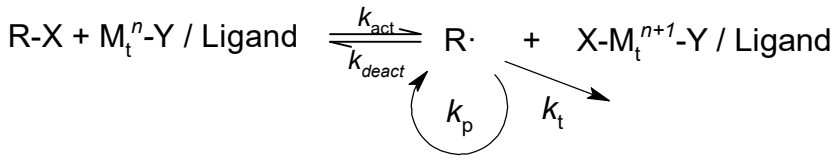

Figure 1: A plausible mechanism of transition-metal-catalyzed ATRP

2.1.1 Controlled/“living“ radical polymerization

During controlled/“living“ radical polymerization the termination rate of the process is negligible (should be less than 5 % of the growing chains) and the concentration of growing radicals remains constant as the method is based on the fast establishment of a dynamic equilibrium between a small number of growing radicals and a large number of dormant species. Initiation is faster than propagation and the polymer chain growth happens at a constant rate. Thus, polymer is created with low dispersity and predictable molar mass which increases linearly with monomer conversion. The polymerization follows pseudo-first-order kinetics. [2]

The rate of polymerization can be described by the equilibrium constant K_{ATRP} (if there are no side reactions) which is unique for every set of components and is the ratio of its rate of activation k_{act} and deactivation k_{deact}. [2]

The theoretical $M_{n,th}$ of the resulting polymer can be calculated via eq. 1, where $M_{n,th}$ is the theoretical number average molecular weight of the synthesized (co)polymer, $([M]_0/[RX]_0)$ is the molar ratio of monomer to the initiator, conversion is the percentage of monomer converted and M_{mn} is the molecular weight of a monomer unit.

$$M_{n,th} = ([M]_0/[RX]_0) \cdot \text{conversion} \cdot M_{mn}. \quad (1)$$

2.1.2 Components of the reaction system and their effects

2.1.2.1 Initiator

Organic halides are used as initiators. The organic group should be similar to the structure of propagating radicals. For the halogen species, brome is more reactive than chlorine, iodine is reactive but undergoes side reactions and fluoride is inactive because the C-F bond is too strong for the fluorine to migrate between the growing chain and the transition metal complex. The reactivity of the initiator should be similar to the monomer reactivity to get a narrow molecular weight distribution.

For the process to not be affected by termination and to achieve a narrow molecular weight distribution, the initial nonstationary stage needs to be as short as possible. This way all the chains start growing at the same time. This is the main task of the initiator – to induce fast initiation. It determines the number of growing polymer chains, which is constant and equal to the initial concentration of the initiator [1][3].

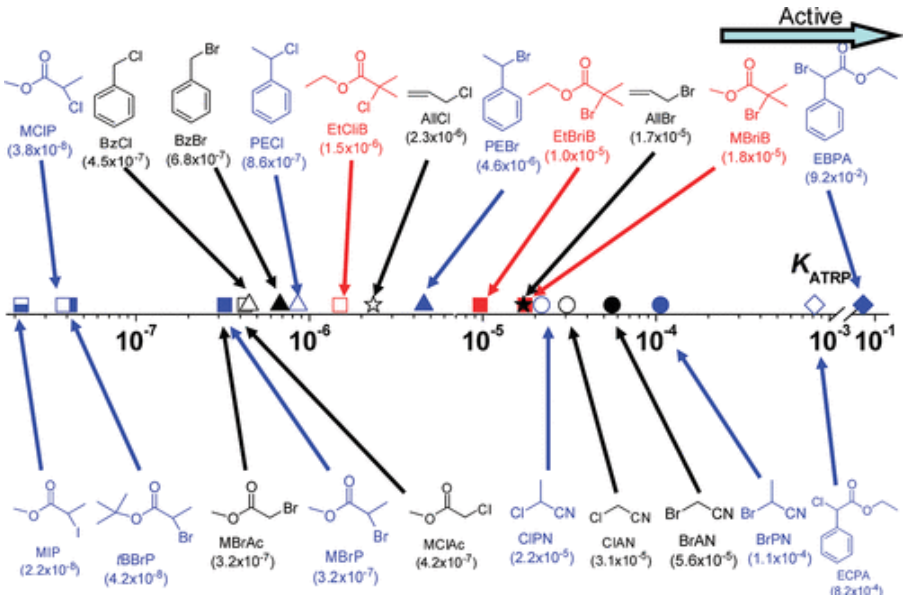

Figure 2: ATRP equilibrium constants K_{ATRP} for various initiators [4]

2.1.2.2 Transition metal catalyst as a carrier of halogen

Organic halides are used as initiators for the polymerization. For reversible redox processes a transition-metal catalyst acts as a carrier for halogen atom and it forms a complex (M_t^n-Y/ligand, where Y is either another ligand or the counterion) with a ligand. The complex then extracts the halogen atom from the dormant R-X species by undergoing one-electron oxidation, forming the oxidized X-M_t^{n+1}-Y/ligand species and the free radical R·. This step is reversible and determines the atom transfer equilibrium K_{ATRP}. This process is shown in Figure 3 with the reactions contributing to the equilibrium. Figure 4 shows the process after reaching the dynamic equilibrium. [1,2]

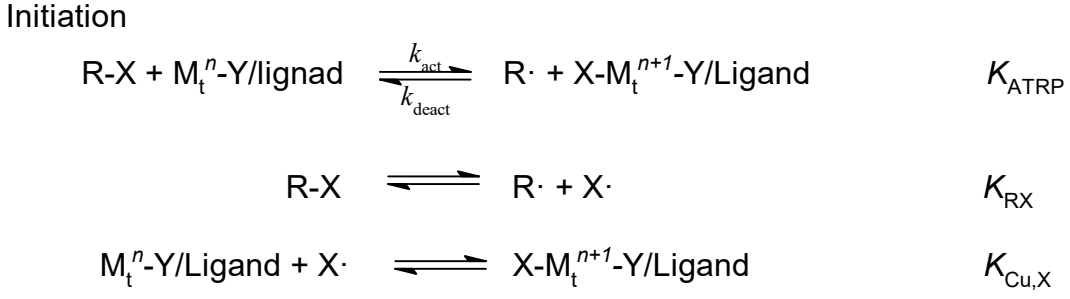

Figure 3: A plausible mechanism of the initiation process during ATRP [4]

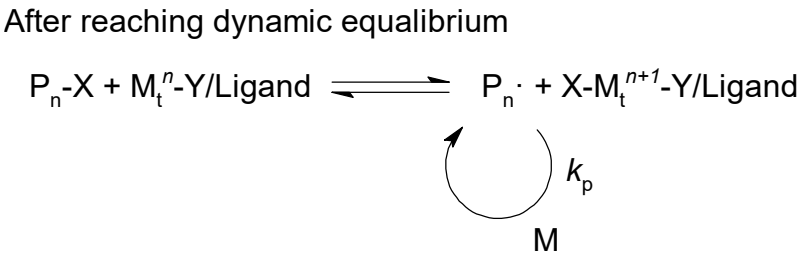

Figure 4: A plausible mechanism of the ATRP process after reaching the dynamic equilibrium [4]

The efficiency of a catalyst is determined by several factors. First, the transition metal centre must have at least two different oxidation states separated by one electron readily available. Second, the metal centre needs to have an affinity towards the halogen. Third, the coordination sphere needs to be expandable to accommodate the halogen. Fourth, the metal should complex the ligand relatively strongly. [1] Copper-based catalysts such as Cu(I)Br are

the most studied and are the most utilized for their effectiveness independently of the monomer used. For the proper function ligands are needed. [3]

Ideally, the equilibrium constant K_{ATRP} for the catalyst should be a large value so that the reaction can run at low concentrations of initiator and at the same time the deactivation rate k_{deact} should be a large value as well to provide good control and produce a polymer with low dispersity. [4]

2.1.2.3 Ligands

Ligands are needed to solubilize the transition metal salt in an organic solvent and to adjust the redox potential of the metal centre thus affecting its reactivity. For example, electron-withdrawing groups stabilize the reduced form while electron-donating stabilize the oxidized form. [5] The effectiveness of the ligand also depends on the solvent used. The complex formed by transition metal and ligand [M_t^{n+1}/L] acts as a deactivator for reversible deactivation to maintain low concentrations of radicals and thus minimize normal termination. [3]

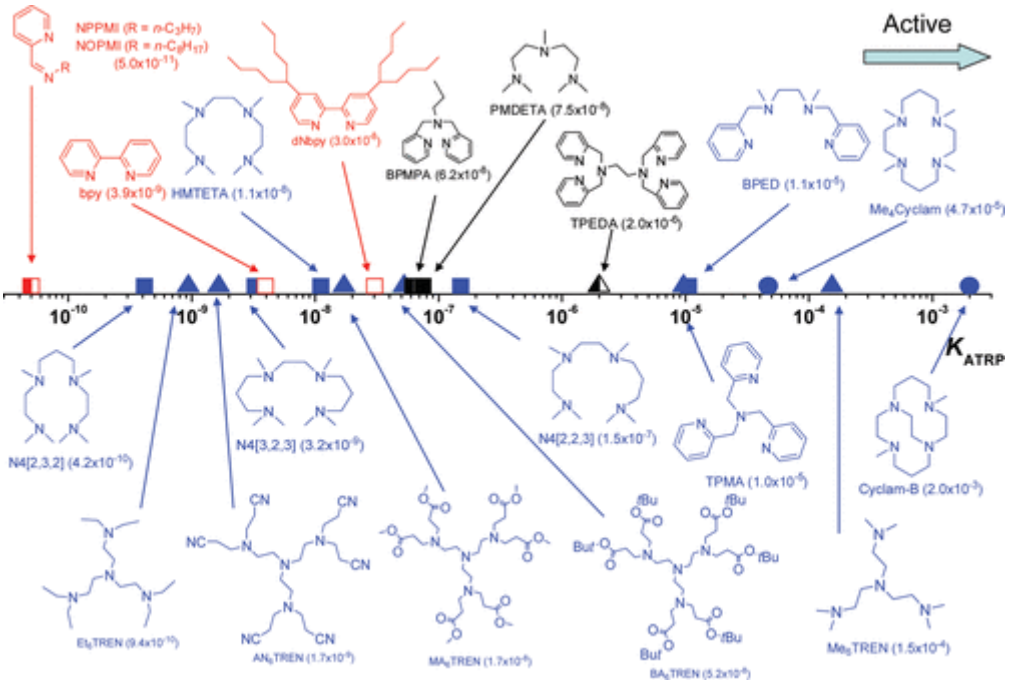

Figure *5: ATRP equilibrium constants K_{ATRP} for various N-based ligands [4]*

2.1.2.4 Monomers

A variety of monomers like (meth)acrylates, (meth)acrylamides or oligo ethylene glycol acrylates (OEGA) can be used for polymerization using ATRP. Typically, monomers

containing stabilizing substituents are used. For each monomer, a specific set of other components will yield the best results. By using different components, the ratio between active propagating radicals and dormant species can be adjusted. Monomers are added to the growing chains (intermediate radicals) $P_n\cdot$, with the constant rate of propagation k_p. [1]

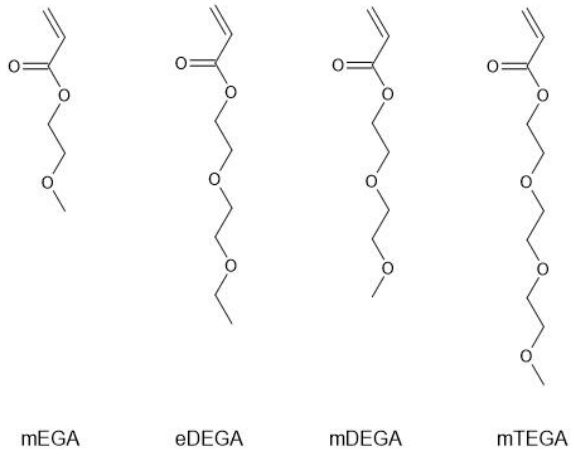

Figure 6: Examples of OEGA monomers used for polymerization in order of hydrophilicity with the least hydrophilic on the left [6]

2.1.2.5 Solvents

A solvent's polarity affects the activation rate of the reaction. The more polar the solvent is, the more active it is. The higher the polarity of the solvent, the faster is activation and the slower is deactivation. This results in a higher value of the equilibrium constant K_{ATRP}. The solvent can also hinder the polymerization if there is a transfer of chain to solvent or if there are side reactions of the catalyst with solvent or when catalyst poisoning occurs. [1]

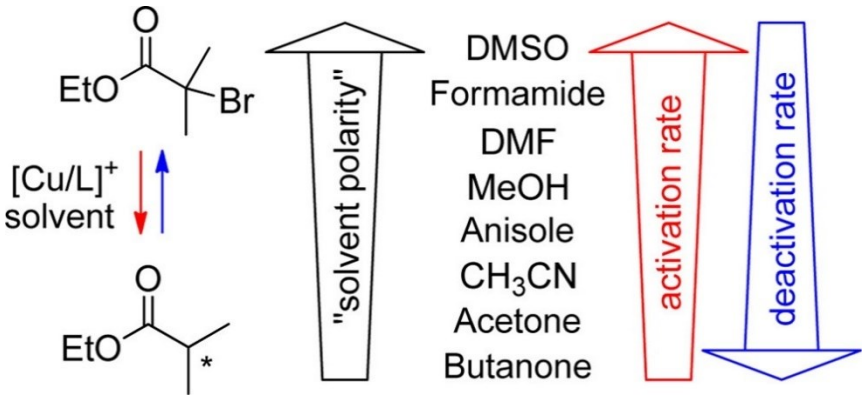

Figure 7: Schematic representation of the effect of solvent polarity on activation and deactivation rate [7]

2.1.2.6 Temperature and reaction time

With increasing temperature, the rate of polymerization increases due to the increase in radical propagation and the equilibrium constant. A negative effect of increased temperature is that any side reactions and chain transfers become more prominent. Another factor is better catalyst solubility with higher temperatures but also its decomposition may occur. [1]

Longer reaction time leads to a higher monomer conversion rate. At high conversion rates, the propagation slows down, but the rate of any side reactions doesn't change significantly. A conversion rate over 95% may cause the loss of end groups and thus destabilize the final polymer. [1]

2.2 Synthesis of Block copolymers and their properties

ATRP allows a controlled synthesis of said copolymers thus enabling control over the molecular weight and its distribution, copolymer composition, and architecture. [5] Their synthesis via free radical polymerization is usually not possible due to the insufficient degree of control necessary to have the desired function. [8] The synthesis can be performed either in bulk (by adding both monomers at the same time), using successive addition (by polymerizing one of the monomers first and then the other), or using a difunctional initiator [9] (which can be used to introduce functional group into the midpoint of the chain). [10]

Copolymers consisting of both hydrophobic and hydrophilic blocks have amphiphilic character leading to important applications such as drug delivery systems, substrates for cell culture, or enzyme activity control because of their aqueous solution properties like thermosensitivity or micellization. [11]

2.3 Self-assembly of particles

A process of spontaneous organization of nanoparticles, hydrogels, or other particles due to interactions with the environment to reach a thermodynamic equilibrium. This can even be observed in macroscopic structures. [12] For polymers, this results from the polymer's hydrogen bonding with the surrounding water and limited hydrogen bonding between monomer units. When the polymer is heated in an aqueous solution, the hydrogen bonds between polymer and water are broken, the inter and intramolecular hydrogen bonds become dominant and volume phase transition occurs. This is because the system becomes increasingly

disorganized (entropy increases), and the hydrophobic portion of the monomer units is no longer stable in the original arrangement of the molecule. The volume phase transition temperature (VPTT) can be regulated by copolymerization with either hydrophilic comonomers to increase the VPTT or hydrophobic comonomers to decrease the VPTT. [13] An advantage of this process is its reversibility. [14]

2.4 Stimuli-responsive (co)polymers

Polymers that respond to stimuli are also known as “smart” polymers. They can respond to several stimuli such as pH, temperature, UV light, diols and saccharide concentrations, or enzymatic activity. This characteristic can be used for various applications like temperature sensors or capsules for drug delivery. [16] This is partially due to the reversibility of stimuli-induced response which is more convenient than involving irreversible chemical bonds. [17]

Thermoresponsive polymers can be characterized by a lower critical solution temperature in solution [15] – meaning that the phase separation occurs when the temperature reaches LCST. The response is caused by the release of water molecules bonded to side groups of the polymer as the temperature increases which gives entropic gain to the molecule, allowing associative interactions [17]. When heated above the LCST, the polymer chains collapse and adopt a compact conformation. This polymer-polymer association is attributed to the hydrophobic effect. The chains form polymer aggregates which decrease the transparency of the solution which can be detected by measuring light scattering intensity. [17]

2.5 AGET and ARGET ATRP

AGET (ARGET) ATRP is an acronym for activator (re)generated by electron transfer. This electron transfer occurs without the involvement of organic radicals possibly causing a radical reaction during the activation of the catalyst complex and without the formation of intermediate products so as not to cause initiation of the ATRP process. This way, even highly active catalyst complexes can be added to the reaction before activation while maintaining a high degree of control. Transition metal complexes in an oxidatively stable state ($CuBr_2$/ligand, $CuCl_2$/ligand) are used as catalyst precursors. To activate these precursors, non-radical forming reducing agents such as tin-2-octanoate are used, which generate the activator from the stable oxidation state of the transition metal complex. [18]

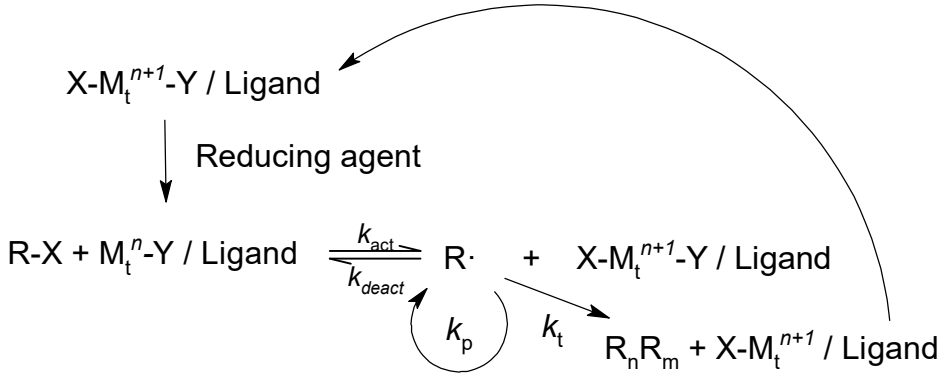

Figure 8: A plausible mechanism of ARGET ATRP using a reducing agent in comparison with standard ATRP

Some of the advantages of ARGET ATRP are the stability of reagents in the presence of oxygen and the possibility of controlling the rate of initiation by controlling the rate of addition of the reducing agent which determines the ratio of Cu^I (active species) and Cu^{II} (dormant species). [18,19] It also allows the concentration of the catalyst to be as low as 0.01 ppm vs. monomer, when Cu^I/L catalyst is used, which is beneficial both financially and environmentally. [19,20] For Cu^{II} catalysts, a higher concentration (10 ppm) is necessary in order to maintain the same degree of control. When the catalyst amounts are too low, the exchange between active and dormant species is not fast enough and therefore causes higher dispersity. These low concentrations of the Cu-containing catalyst could even allow the residual copper to be left in the final product. [20]

Control can also be increased by using ligands that bind to copper more strongly (TPMA binds more strongly than Me_6TREN and provides better results [20]). Another way of exerting control using ligands is by using an excess amount of ligand (up to 10 times the required amount). With the ligand concentration too low, it is possible for the metal catalyst to form a complex with the monomer which in turn results in a higher dispersity. [21] Side reactions, which may occur but at a slow rate, are during this relatively fast polymerization significantly reduced, particularly for acrylates [20, 22]. The method was also successfully used for the synthesis of block copolymers. [20]

2.6 Phenylboronic acid (PBA) and its derivatives as monomers

Derivates of phenylboronic acid can be used to synthesize various responsive copolymers such as thermoresponsive hydrogels. As mentioned above, the thermoresponsive properties of copolymers can be regulated by copolymerization with either hydrophilic or hydrophobic comonomers. Phenylboronic acid derivatives can exist as either one. When pH is below pK_a they are in the form of a trigonal neutral molecule, which is hydrophobic. When pH is above the pK_a of the solution or by complexation with glucose, they exist in their tetrahedral anionic form, which is hydrophilic. [23, 24, 25] This principle has been used for an ex situ self-regulating insulin release device where insulin was encapsulated within the gel and an increasing glucose concentration in the solution allowed for the diffusion of the insulin. [13]

They can also be used in the preparation of block copolymers which can form amphiphilic nanoparticles of various shapes via self-assembly. These particles also respond to changes in either change in pH by increasing/lowering pH above/below the pK_a of the acid units or saccharide concentration by forming reversible ester bonds with the sugar. [13]

The sensitivity of the different PBA derivatives in different pH conditions, for example, physiological conditions, depends on the pK_a of the particular derivative. Unsubstituted PBA with pK_a ~ 9 forms esters in basic environments. To decrease the pK_a, different substituents in the vicinity of the boron atom, such as nitrogen or oxygen were used. [26]

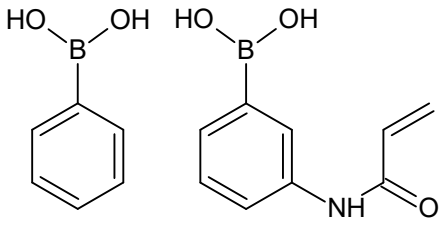

Figure 9: Structure of phenylboronic acid (left) and 3-acrylamide phenyl boronic acid, used in this work (right)

3 Aims of Thesis

The main aim of this thesis is to synthesize stimuli-responsive copolymers via the ATRP method. This is planned to be achieved via multiple steps:

1. Synthesis of homopolymers via the ATRP method – optimizing several variables of the process (solvent, temperature and catalytic system)
2. Synthesis of copolymers based on the initial screening (step 1)
3. Characterization and purification of prepared copolymers
4. Determination of the thermoresponsive properties (cloud point temperature of prepared homo- and copolymers)

4 Materials and Methods

4.1 Materials

The following chemicals were purchased from manufacturers and used as received. 3-aminophenylboronic acid ester (fluorchem, 98%), 2-bromo-2-methylpropionic acid (Sigma-Aldich, 98%), acetone (Lach-Ner, ≥99%), acryloyl chloride (Sigma-Aldrich, ≥97%), aluminum oxide (activated, basic, Brockmann I) (Sigma-Aldrich) anisole (Sigma-Aldrich, for synthesis), chloroform-d (Sigma-Aldrich, 99.8 atom% D), copper(I) bromide (Sigma-Aldrich, 98%), copper(II) chloride (Sigma-Aldrich, for synthesis), dichlormethane (DCM) (Lach-Ner, ≥99%), di(ethylene)glycol monomethyl ether (Sigma-Aldrich, for synthesis), dimethyl sulfoxide anhydrous (Sigma-Aldrich, ≥99.9%), ethyl acetate (Lachner, pa), ethyl-α-bromoisobutyrate (Sigma-Aldrich, 98%), ethyl-2-bromopropionate (Sigma-Aldrich, 99%), hexane pure (Lach-Ner), hydrochloric acid (35%, Lach-Ner), hydroquinone (Sigma-Aldrich, ReagentPlus®, ≥99%), N,N,N'.N",N"-Pentamethyl-diethylene-triamine (PMDTA) (Sigma-Aldrich, 99%), sodium chloride (Lach-Ner), sodium hydrogen carbonate (Lach-Ner), sodium sulfate anhydrous (Lach-Ner), tetrahydrofuran (THF) (Lach-Ner, ≥99%) tin(II) 2-ethylhexanoate (Sigma-Aldrich, 92.5 – 100%), triethylamine (TAE) (Sigma-Aldrich, ≥99%), tris[2-(dimethylamino)ethyl]amine (Me_6TREN) (Sigma-Aldrich, 97%)

4.1.2 Synthesis of methoxydi (ethylene glycol) acrylate (mDEGA)

mDEGA was synthesized according to an adapted procedure from literature [27]. To a Schlenk flask were added di(ethylene glycol) monomethyl ether (18.1 g, 0,13 mol), triethylamine (TAE) (18.7 g, 0.18 mol), and dichloromethane (DCM) (140 ml). The flask was then placed in an ice/water bath. A solution of acryloyl chloride (16.0 g, 0.18 mol) and DCM (30 ml) was prepared and subsequently added dropwise to the flask under an N_2 atmosphere. The flask was removed from the ice bath and the reaction mixture was stirred at room temperature overnight. The precipitate formed in the mixture was filtered out. The solution was treated with 100 ml of saturated aqueous solution of $NaHCO_3$, 200 ml of water, and 100 ml dichloromethane. Two layers were formed. The organic layer was treated with water (200 ml), and saturated aqueous solution of NaCl (200 ml) and water (200 ml) respectively. The aqueous layer was extracted with dichloromethane. The organic extracts were combined with the organic layer and dried over anhydrous Na_2SO_4. The drying agent was filtered out after

approximately 2 hours. A very small amount of hydroquinone was added in order to stabilize the product, and solvents were removed using rotavapor. The product was purified by vacuum distillation [27]. A general mechanism of this process is shown in Figure 10 and the ^{1}H NMR spectrum of the prepared mDEGA can be seen in Figure 11.

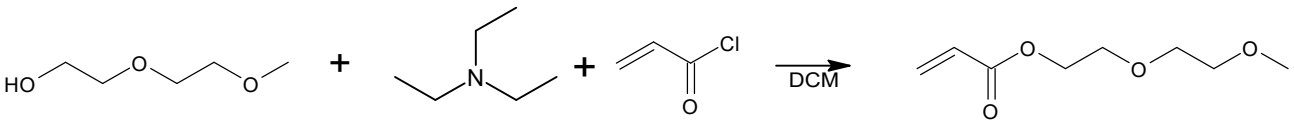

Figure 10: Synthesis of mDEGA for polymerization

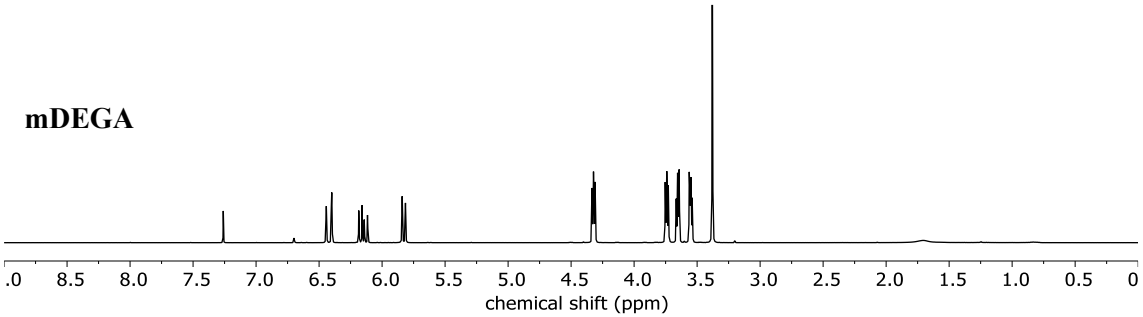

Figure 11: ^{1}H NMR spectrum of the prepared mDEGA

4.1.3 Synthesis of 3-Acrylamidophenylboronic Acid Pinacol Ester Monomer (3-APBAE)

3-acrylamidophenylboronic Acid Pinacol Ester Monomer (3-APBAE) was synthesized according to an adapted procedure from literature [28]. 3-aminophenyl boronic acid ester (3.6 g, 16.4 mmol) was dissolved in dry dichloromethane (60.5 ml) in a Schlenk flask, which was then placed in an ice/water bath and cooled to 0°C. TAE was added (2.10 g, 20.8 mmol) and the solution was stirred for 30 minutes. In a separate flask, acryloyl chloride (1.6 g, 17.7 mmol) was dissolved in dichloromethane (4.8 ml) and added dropwise for 2 h at 0°C. The reaction mixture was then warmed up to room temperature and the reaction was allowed to continue for 24 hours. The solvent was removed using rotavapor and the product was suspended in ethyl acetate (55 ml). The mixture was stirred for 30 minutes and subsequently filtered to remove all solid particles. The organic layer was extracted with water (2 x 100 ml), saturated aqueous sodium hydrogen carbonate (2 x 100 ml), water (2 x 100 ml) and brine (2 x 100 ml). The organic layer was then dried over anhydrous Na_2SO_4. The Na_2SO_4 was filtered out after approximately 2 hours. A very small amount of hydroquinone was added in order to stabilize the product, and solvents were removed using rotavapor. The product was

twice recrystallized from a mixture of toluene and hexane. A general mechanism of this process is shown in Figure 12 and the ^{1}H NMR spectrum of the prepared 3-APBAE can be seen in Figure 13.

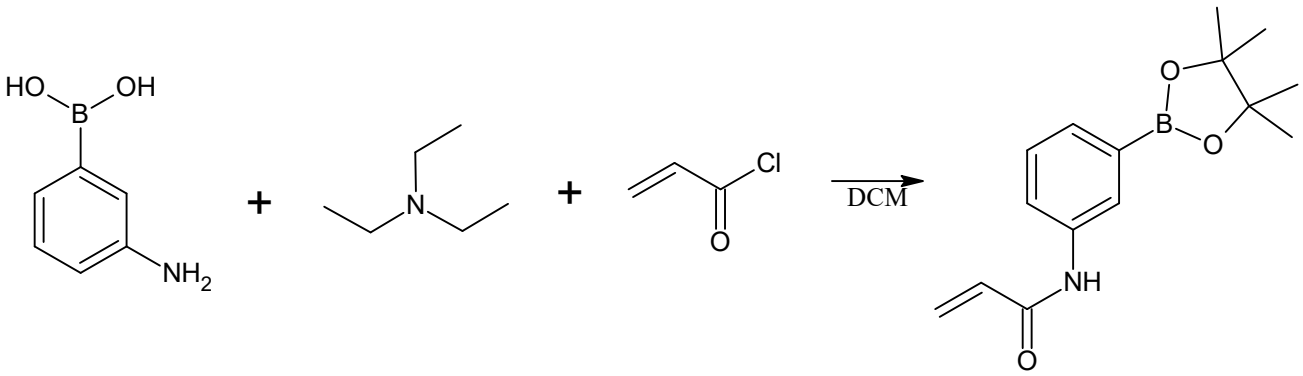

Figure 12: Synthesis of 3-Acrylamidophenylboronic Acid Pinacol Ester Monomer for polymerization

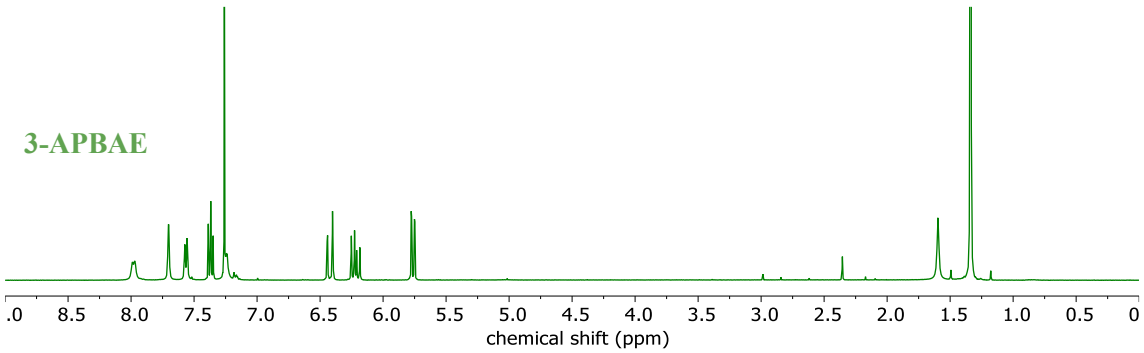

Figure 13: ^{1}H NMR spectrum of the prepared 3-APBAE

4.2 Experimental methods

4.2.1 NMR spectroscopy

NMR spectroscopy was used to determine the composition of the prepared (co)polymers and the conversion rate of individual monomers taking part in the (co)polymerization process. For ^{1}H NMR measurements, either the Bruker 400 MHz or Bruker Avance Neo 400 MHz spectrometer was used at room temperature with $CDCl_3$ as a solvent for the sample. For liquid sample preparation, a few drops of sample were transferred to the NMR tube and dissolved in 0.5 ml of $CDCl_3$. For solid samples, about 5 mg of the sample was dissolved in 0.5 ml of $CDCl_3$ directly in the NMR tube.

4.2.2 GPC

Gel permeation chromatography was used to determine the number average molecular weight (M_n) and dispersity (M_w/M_n). The analysis was performed using the OMNISEC RESOLVE system (degasser, pump, autosampler, and column oven) with multi-detector system OMNISEC REVEAL (refractive index, UV/Vis PDA, light scattering, and viscometer). The separation was conducted on two PLgel 5 µm mixed-D columns in a series at 55 °C in a solution of N, N-dimethylacetamide (DMA) containing 50 mM of LiCl at an elution rate of 0.5 ml/min. For sample preparation, 5 mg of (co)polymers was dissolved in 1 ml of GPC solvent and subsequently, the solution was pushed through a syringe filter (0.45 µm).

4.2.3 Determination of T_{cp} values by measuring light scattering intensity using a Fluorimetr

To measure T_{cp} values of prepared (co)polymers, Fluorlog-QM was used. A sample was prepared by dissolving 5.0 mg of (co)polymer in 5 ml of distilled water to obtain a 1 mg/ml concentration of the solution. The sample was then transferred to a 1 cm quartz glass cuvette with a magnetic stirrer. The spectra were measured at excitation and emission wavelength 600 nm and both excitation and emission slit were set to 0.5 nm. The temperature range was set from 20 °C to 90 °C with a temperature rate of 1°C/min, which was controlled by the Fluorlog-QM. Measured intensity data were normalized and plotted to an intensity/temperature plot. The steep part of the plot was fitted with a linear fit and the T_{cp} value was calculated as the intercept between the linear fit and zero (normalized value).

4.2.4 Homopolymerization

A reaction vial was cleaned with ethanol, ultrasonic bath, and acetone. 0,2 ml of mDEGA (0.2934 g, 1.68 mmol) was pushed into the cleaned cooled vial through a small column of basic Al_2O_3 to remove the hydroquinone used to stabilize the monomer. The column was then washed through with 0.6 ml of DMSO which was pushed through with nitrogen. CuBr (3.5 mg, 0.02 mmol) was added to the vial and any residue left on the walls was washed with 0.3 ml of DMSO. The vial was sealed with a septum cap and PMDTA (4.9 µl, 0.02 mmol) was added. Three rounds of freeze-pump-thaw were performed. After the last round the vial was filled with nitrogen and EBP (3.2 µl, 0.02 mmol) was added. The vial was placed in a heating block at 70°C for 1 hour. The polymerization was terminated by opening the vial to air, placing

it in an ice/water bath, and pushing air directly into the mixture via a syringe. The resulting green liquid was filtered through a column of neutral Al_2O_3 to remove copper residue. The column was washed with tetrahydrofuran (THF). The filtrate was then dripped into 300 ml of hexane and left to precipitate for 2 hours. The resulting polymer was dissolved in acetone and transferred into a vial. The acetone was partially evaporated by nitrogen flux and the rest was dried by vacuum overnight. The ^{1}H NMR spectrum of the prepared p(mDEGA) homopolymer can be seen in Figure 14.

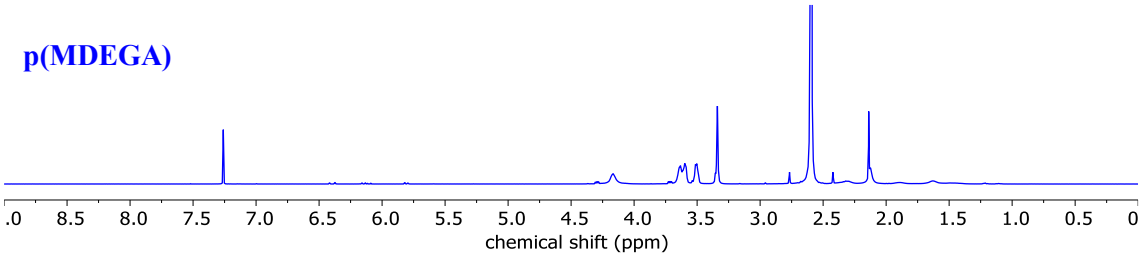

Figure 14: ^{1}H NMR spectrum of the prepared p(mDEGA) homopolymer

4.2.5 Freeze-pump-thaw

The reaction vial was sealed by a septum cap and placed in a dry ice/ethanol bath. The reaction mixture was allowed to freeze completely. All gas present in the vial was removed by a syringe connected to a vacuum pump which was allowed to run for approximately 3 minutes. Afterwards, the vial was removed from the dry ice/ethanol bath and was placed in a warm water bath and the mixture was allowed to thaw completely.

4.2.6 Copolymerization

A reaction vial was cleaned with ethanol, ultrasonic bath, and acetone. 0.2 ml (0.2624 g, 1.51 mmol) mDEGA was pushed into the cleaned cooled vial through a small column of Al_2O_3 to remove the hydroquinone used to stabilize the monomer. The column was then washed through with 0.6 ml of DMSO, which was pushed through with nitrogen. CuBr (12.0 mg, 0.08 mmol) and 3-APBAE (51 mg, 0.2 mmol) were added to the vial and any residue left on the walls was washed with 0.2 ml of DMSO. The vial was sealed with a septum cap and PMDTA (8.3 µl, 0.04 mmol) was added. Three rounds of freeze-pump-thaw were performed. After the last round the vial was filled with nitrogen and EBiB (6.1 µl, 0.04 mmol) was added. The vial was placed in a heating block at 70°C for 2 hours. The polymerization was terminated by opening the vial to air, placing it in an ice/water bath, and pushing air directly into the

mixture via a syringe. The resulting green liquid was filtered through a column of neutral Al_2O_3. The column was washed with THF. The filtrate was then dripped into 300 ml of hexane and left to precipitate for 2 hours. The resulting polymer was dissolved in acetone and transferred into a vial. The acetone was partially evaporated by nitrogen and the rest was dried by vacuum overnight. The ^{1}H NMR spectrum of the prepared mDEGA/3-APBAE copolymer can be seen in Figure 15.

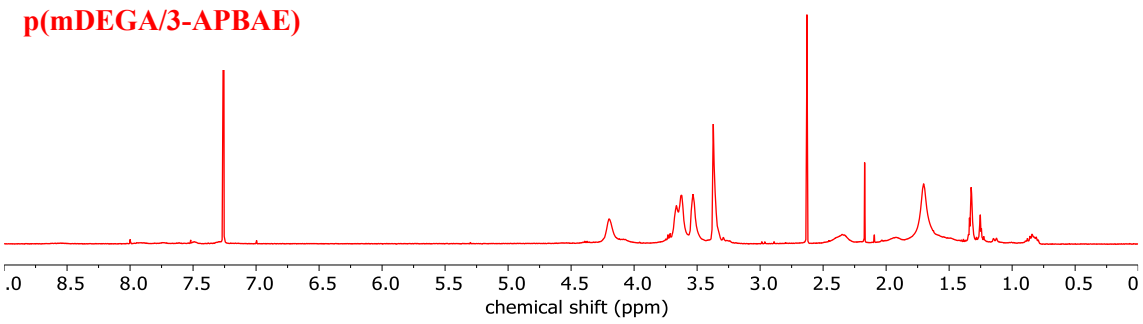

Figure 15: ^{1}H NMR spectrum of the prepared mDEGA/3-APBAE copolymer

4.2.7 Kinetic measurement

Kinetic measurements were done via a method similar to that of homopolymer preparation. In this case, 1 ml (1.107 g, 6.36 mmol) mDEGA, 3.4 ml DMSO, 16.26 mg (0.11 mmol) CuBr, 22.7 µl (0.011 mmol) PMDTA, 14.7 µl (0.11 mmol) EBP was used. The reaction mixture was then divided into the desired number of vials which were left to run at chosen polymerization conditions, being terminated by the method described above in selected intervals.

4.2.8 ARGET ATRP

A reaction vial was cleaned with ethanol, ultrasonic bath, and acetone. 0.2 ml (0.2945 g, 1.69 mmol) mDEGA was pushed into the cleaned cooled vial through a small column of Al_2O_3 to remove the hydroquinone used to stabilize the monomer. The column was then washed through with 0.6 ml of DMSO, which was pushed through with nitrogen. 3 - APBAE (51.3 mg, 0.2 mmol) was added, and the vial was sealed with a septum cap. Three rounds of freeze-pump-thaw were performed. A solution of $CuCl_2$ (0.11 mg/ml) and PMDTA (1.77 µl/ml) in DMSO was prepared and three rounds of freeze-pump-thaw were performed with the solution. The $CuCl_2$ solution (0.1 ml) was added to the reaction vial under a protective atmosphere of nitrogen. A solution of $SnOct_2$ in toluene was prepared and 0.1 ml of this solution

was added to the reaction vial. EBP (3.2 µl, 0.2 mmol) was added and the vial was placed in a heating block at 70°C for 2 hours. The polymerization was terminated by opening the vial to air, placing it in an ice/water bath, and pushing air directly into the mixture via a syringe. The resulting clear liquid was diluted with THF and dripped into 300 ml of hexane and left to precipitate for 2 hours. The resulting polymer was dissolved in acetone and transferred into a vial. The acetone was partially evaporated by nitrogen and the rest was dried by vacuum overnight.

4.2.9 Dialysis

The copolymer was dissolved in 5 ml of acetone. The solution was placed in a dialysis membrane and dialyzed against acetone for 26 hours. The acetone bath was changed 4 times during the dialysis. The dialyzed solution was transferred back into a vial.

4.2.10 Deprotection

Copolymer (26.4 mg) was dissolved in 5 ml of acetone and 5 ml of distilled water. The solution was placed in a dialysis membrane and dialyzed against 0.3% hydrochloric acid solution in distilled water. The dialysis was left to run for 30 hours, and the dialysis bath was changed twice during this time. The solution was then dialyzed against distilled water for 42 hours, changing the bath three times. The dialyzed solution was transferred back into a vial and used for light scattering measurements.

5 Results and discussion

5.1 Synthesis of homopolymers

5.1.1 Synthesis of mDEGA homopolymers

Different reaction conditions were studied during the preparation of mDEGA homopolymers. The variables used were the solvent (anisole, DMSO), time (from 1 to 22 hours), and temperature (70°C and 90°C) in combination with PMDTA as a ligand and EBP as initiator. These conditions were chosen based on results in previously published literature. [4, 7] Results are summarized in Table 1.

Table 1: Results of mDEGA homopolymerization catalyzed by CuBr, PMDTA as ligand, and EBP as initiator

exp.	*solvent*	*component ratio* [1]	*time* [h]	*temperature* [°C]	M_n [2] [g·mol^{-1}]	M_w/M_n [2]	*conv.* [3] [%]	$M_{n,\,teor}$ [4] [g·mol^{-1}]
EM 1	anisole	30/1/1/1	2.5	90	2 000	1.07	20	1 000
EM 3	anisole	40/1/1/1	22	90	3 400	1.11	54	3 600
EM 7	DMSO	70/1/1/1	4	90	7 800	1.35	100	12 000
EM 12	DMSO	40/1/1/1	1	70	2 700	1.16	37	2 300

[1] molar ratio of monomer/catalyst/ligand/initiator; [2] determined by GPC analysis; [3] determined by ^{1}H NMR; [4] theoretical number average molecular weight, calculated based on stoichiometry and conversion $M_{n,th} = ([M]_0/[RX]_0) \cdot \text{conversion} \cdot M_{mn}$

As seen in Table 1, entry 1, the first experiment was performed with a lower ratio of monomer to initiator to catalyst was used (30/1/1). The polymerization was left to run at 90°C for 2.5 hours, producing a narrow molar mass distribution (M_w/M_n = 1.07) but a very low conversion (20 %) and related to that a low molar mass (M_n = 2 000 g·mol^{-1}).

To achieve a higher conversion and thus a larger molar mass, a higher ratio of monomer to initiator was used (40/1), and the polymerization was left to run for 22 hours at 90°C (Table 1, entry 2). A higher conversion was achieved (54 %, M_n = 3 400 g·mol^{-1}) with molar mass distribution kept low (M_w/M_n = 1.11).

For the next set of experiments (Table 1, entries 3 and 4), DMSO was used as a more polar solvent with an activation rate almost twenty times higher than anisole in order to achieve a higher conversion but still maintain control over the polymerization [7]. For the third

experiment (entry 3), the polymerization was run at 90°C for 4 hours with the monomer to initiator ratio at 70/1. A complete conversion was achieved with the number average molar mass of M_n = 7 800 g·mol^{-1} and the highest molar mass distribution (M_w/M_n = 1.35) of this set of experiments.

Based on the screening set of experiments (Table 1), DMSO was chosen as the most promising solvent and the kinetic analysis was performed to evaluate the progress of polymerization.

Kinetic analyses

The kinetic analyses were performed to monitor the reaction rate and evolution of the molar mass and its distribution during the polymerization process. The first kinetic analysis was performed at 90°C for 10 - 80 minutes with DMSO as a solvent, CuBr as a catalyst, PMDTA as a ligand, and EBP as an initiator. The results showed that at this temperature, the reaction runs very fast, reaching the equilibrium after 20 minutes of reaction (Figure 16, left), causing the uncontrolled character of polymerization as visible from the semilogarithmic plot (Figure 16, right).

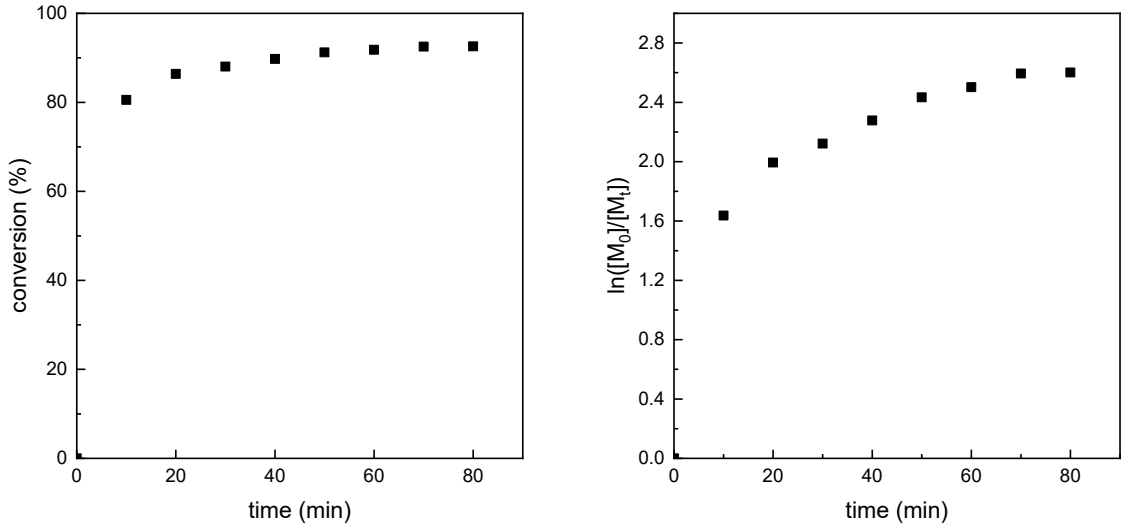

Figure 16: Kinetic plots of mDEGA homopolymerization (in DMSO with PMDTA, catalyzed by CuBr and initiated by EBP) at 90°C showing conversion rate (left) and ln([M_0/M_t]) plotted vs. time (left)

To combat this, the polymerization was performed at 70°C for 1 hour with the molar ratio of monomer to initiator lowered back to 40/1. This resulted in a polymer with M_n = 2 700 g·mol^{-1} and M_w/M_n = 1.16. From this, a higher degree of control was concluded,

which was confirmed by performing a second kinetic analysis at 70°C. The kinetic analysis was performed, using the same reaction conditions (monomer, solvent, catalyst, ligand, and initiator) as during the first kinetic measurement except for the lower polymerization temperature, which was 70°C. It was run for 0.5 – 3 h and resulted in controlled polymerization of mDEGA as displayed in Figure 17.

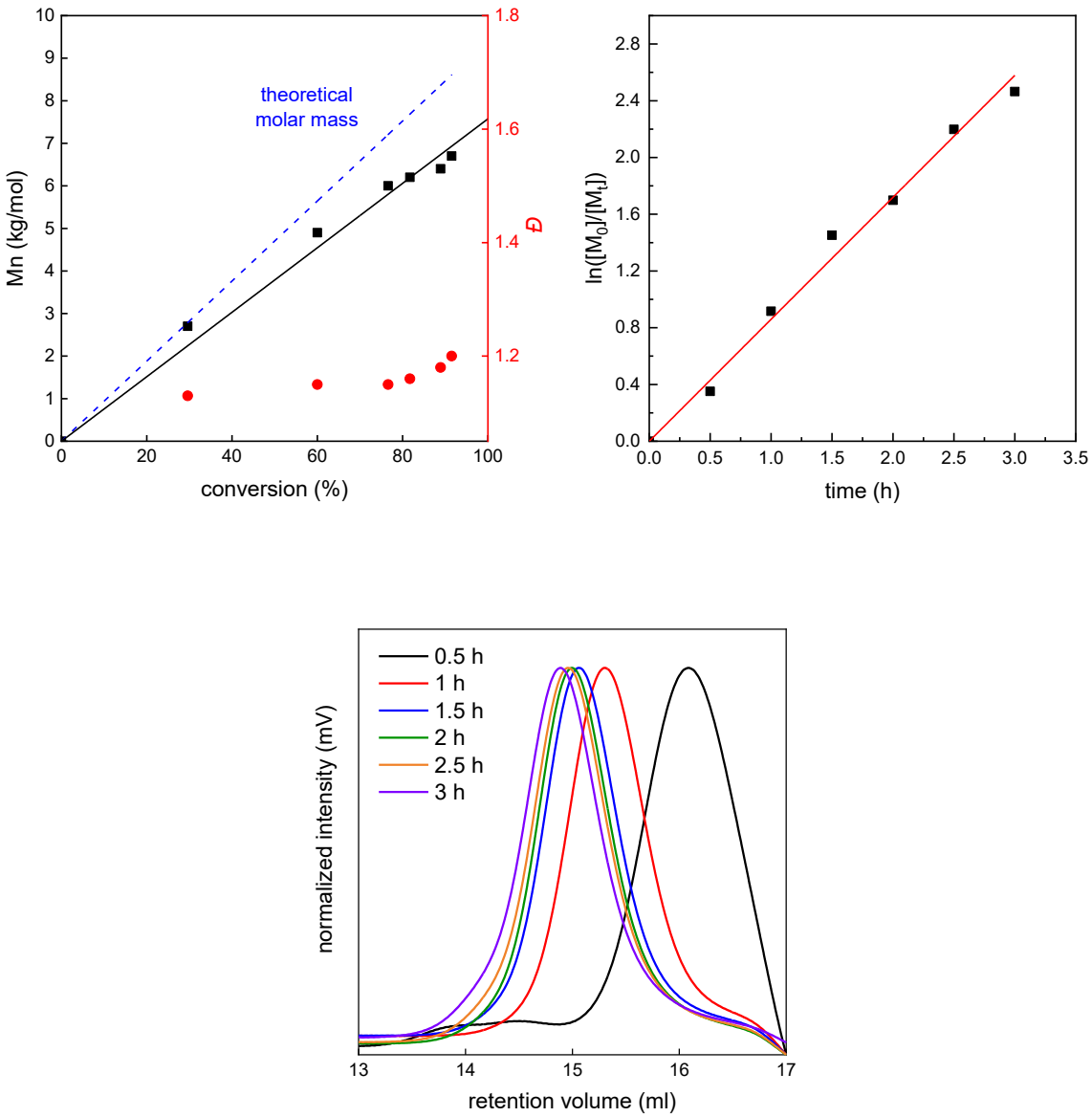

Figure 17: Kinetic plots of mDEGA homopolymerization (in DMSO with PMDTA, catalyzed by CuBr and initiated by EBP) at 70°C. The apparent number- average molecular weights (M_n) and dispersities (Đ) plotted vs. monomer conversion (top left), ln([M_0]/[M_t]) plotted vs. time (top right), chromatograms corresponding to different reaction times (bottom).

5.1.2 Synthesis of mTEGA homopolymers

During the preparation of mTEGA homopolymers, these reaction conditions were studied: the initiator (EBP, EBiB) and the ligand (PMDTA, Me_6TREN) in DMSO with CuBr as catalyst. These conditions were chosen based on our previous results of mDEGA homopolymerization, and also taking into account previously published literature. [27] Results are summarized in Table 2.

Table 2: Results of mTEGA homopolymerization in DMSO, catalyzed by CuBr, at 70°C for 2h

exp.	*initiator*	*ligand*	M_n (1) [g·mol^{-1}]	M_w/M_n (1)	*conv.* (2) [%]	$M_{n,\,teor}$ (3) [g·mol^{-1}]
RM 142	EBP	PMDTA	3300	1.15	60	7100
RM 143	EBP	Me_6TREN	4300	1.24	54	6400
EM29_2h	EBiB	PMDTA	3500	1.14	64	7600

[1] determined by GPC analysis; [2] determined by ^{1}H NMR; [3] theoretical number average molecular weight, calculated based on stoichiometry and conversion $M_{n,th} = ([M]_0/[RX]_0) \cdot \text{conversion} \cdot M_{mn}$

Using conditions that yielded the best results for mDEGA homopolymerization (combination of PMDTA and EBP) at 70°C for 2h, a conversion of 60 % and molecular weight of M_n = 3 300 g·mol^{-1} was obtained with dispersity kept low at M_w/M_n = 1.15.

Based on published literature [4], the experiment was repeated with the same conditions, except for the ligand. In this instance, Me_6TREN was used as a more active ligand (Figure 5), with a larger K_{ATRP} value and smaller k_{deact} value [4]. This yielded the expected results of a slightly higher number average molecular weight at M_n = 4 300 g·mol^{-1} but also a higher dispersity at M_w/M_n = 1.24 (Table 2, entry 2).

Based on these findings (Table 2), PMDTA was chosen as the ligand used in the kinetic analysis, which was performed twice to assess the differences in the progress of polymerization for the different initiators used.

Kinetic analysis

The kinetic analyses were performed to assess the progress of the polymerization process. The first kinetic analysis was performed at 70°C for 0.5 – 3 hours with DMSO as a solvent, CuBr as a catalyst, PMDTA as a ligand, and EBiB as an initiator. The results seen in Figure 18 showed a narrow dispersity through the entire analysis as well as a linear progression of molar mass but not reaching a full conversion (72% after 3 hours).

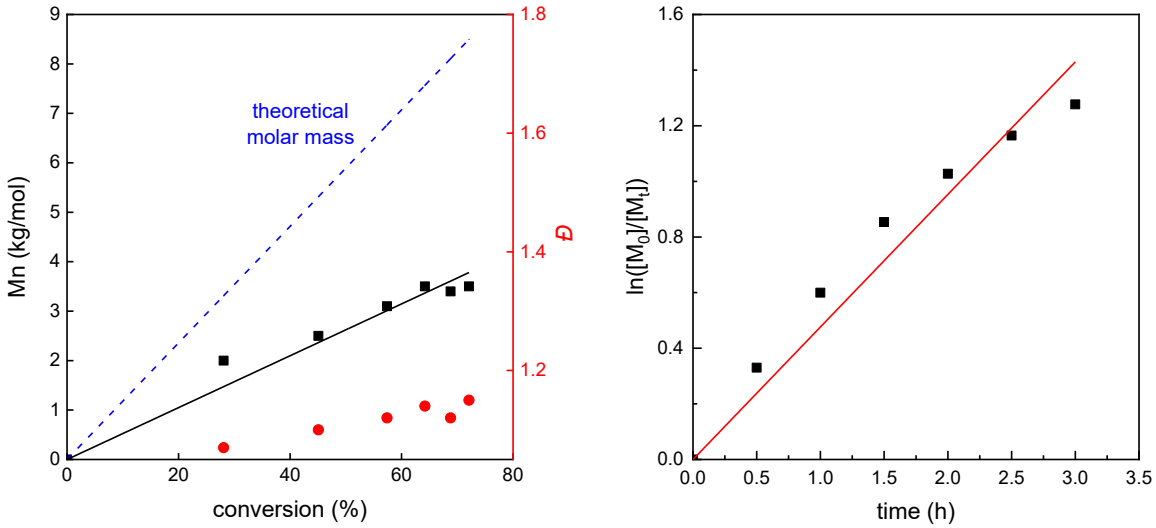

Figure 18: Kinetic plots of mTEGA homopolymerization (in DMSO with PMDTA, catalyzed by CuBr and initiated by EBiB) at 70°C. The apparent number- average molecular weights (M_n) and dispersities (Đ) plotted vs. monomer conversion (left), ln([M_0]/[M_t]) plotted vs. time (right).

The analysis was performed again under the same conditions, except for EBP being used as initiator instead of EBiB. EBP is the less active initiator as expected from Figure 5. The results of this are summarized in Figure 19. This analysis had a very similar progression to the first, again exhibiting a linear progression of number average molecular weight, a narrow dispersity throughout the polymerization time but also never reaching full conversion (60% after 3 hours).

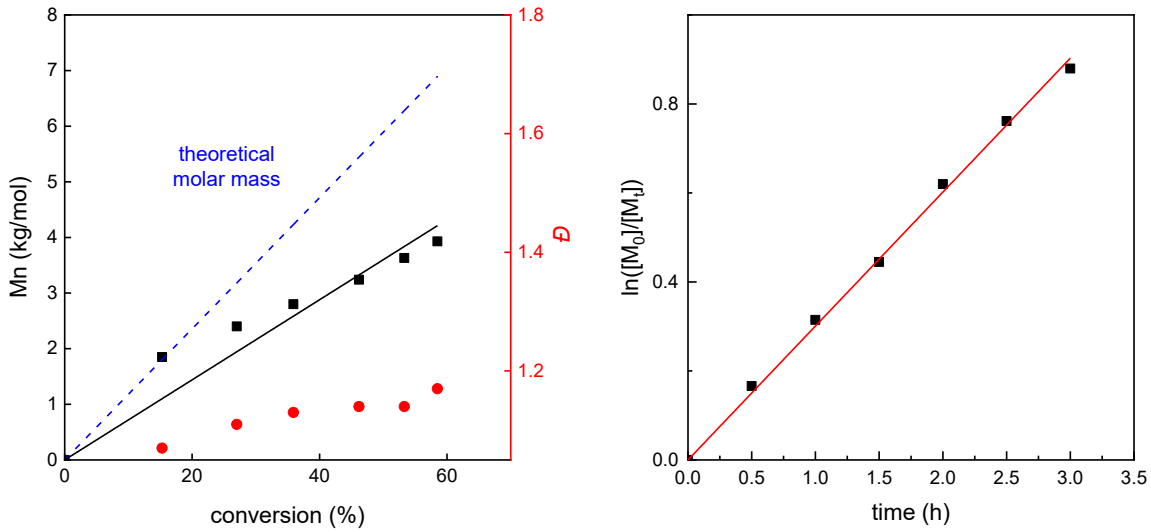

Figure 19: Kinetic plots of mTEGA homopolymerization (in DMSO with PMDTA, catalyzed by CuBr and initiated by EBP) at 70°C. The apparent number- average molecular weights (M_n) and dispersities (Đ) plotted vs. monomer conversion (left), ln([M_0]/[M_t]) plotted vs. time (right).

Overall, mDEGA showed a faster polymerization rate accompanied by higher number average molecular weights of the corresponding homopolymer produced when similar experiments are compared (Figure 18, Figure 19). This can be explained by the fact that mTEGA has an extra unit in the side chain (Figure 20) making it a larger molecule and slowing down the polymerization process.

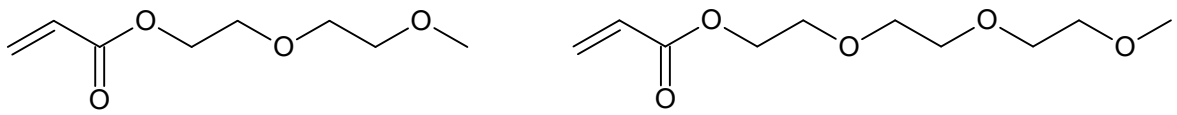

Figure 20: Structure of mDEGA (left) and mTEGA (right)

5.2 Synthesis of copolymers

5.2.1 Synthesis of mDEGA/3-APBAE copolymers via ATRP method

Following the set of homopolymerization experiments, similar conditions were tested for the copolymerization of mDEGA and 3-APBAE. The variables used were the ligand (PMDTA, Me_6TREN) initiator (EBP, EBiB) and time (from 2 to 24 hours) in combination with CuBr as a catalyst and DMSO as solvent. Results are summarized in Table 3.

Table 3: Results of copolymerization of mDEGA with 3-APBAE in DMSO, catalyzed by CuBr, at 70°C

exp.	*ligand*	*ini.*	x_{APBAE} (1) [%]	*time* [h]	M_n (2) [g·mol^{-1}]	M_w/M_n (2)	*conv. Acrylate* (3) [%]	*conv. APBAE* (3) [%]	$M_{n,teor}$ (4) [g·mol^{-1}]
EM 16	PMDTA	EBP	10	24	5 600	1.29	30	49	3 900
EM 17	Me_6TREN	EBP	10	2	2 800	1.99	13	-	-
EM 20	PMDTA	EBiB	10	2	7 800	1.38	52	63	4 100
EM 30	PMDTA	EBiB	5	2	5 800	1.49	60	46	4 100

[1] molar percentage of 3-APBAE in feed; [2] determined by GPC analysis; [3] determined by ^{1}H NMR; [4] theoretical number average molecular weight, calculated based on stoichiometry and conversion $M_{n,th} = ([M]_0/[RX]_0) \cdot \text{conversion} \cdot M_{mn}$

The route of using Me_6TREN as a ligand was abandoned after the experiment yielded dispersity of M_w/M_n = 1.99 with the conversion of acrylate only 13 % after 2 hours. The incorporation of 3-APBAE into the copolymer was not observed on the ^{1}H NMR spectra so the theoretical molar mass of the copolymer was not calculated (EM 17, Table 3, entry 2). Therefore, PMDTA was further explored with EBiB as a more active initiator [1]. Different comonomer ratios were tested and it was concluded that while the lower molar ratio of comonomer 3-APBAE did produce a higher conversion rate (60 % and 46 %) it also showed a higher dispersity (M_w/M_n = 1.49) and lower number average molecular weight (M_n = 5 800 g·mol^{-1}, Table 3, entry 5). Experiment EM 20 (Table 3, entry 4) showed much more promising results (M_n = 7 800 g·mol^{-1} and M_w/M_n = 1.3).

Another set of experiments was therefore performed, where the catalyst to ligand to initiator ratios were adjusted, the initiator always being 1 as the reference point. For these experiments, the mDEGA/initiator ratio was kept at around 40/1, and a combination of reactants and reaction conditions determined by previous experiments to yield the best results was used (CuBr, PMDTA, EBiB at 70°C in DMSO). Results are summarized in Table 4.

Table 4: Results of copolymerization of mDEGA with 3-APBAE with CuBr, PMDTA, and EBiB ratios adjusted at 70°C for 2 h in DMSO

exp.	$n_{CuBr}/n_{inic.}$ [1]	n_{PMDATA}/n_{inic} [1]	x_{APBAE} [2] [%]	M_n [3] [g·mol^{-1}]	M_w/M_n [3]	*conv. Acryl.* [4] [%]	*conv. APBAE* [4] [%]	$M_{n,teor}$ [5] [g·mol^{-1}]
EM 20	2	1	11	7 800	1.38	52	63	4 100
EM 21	2	2	10	2 300	2.37	30	45	3 000
EM 30	2	1	5	5 800	1.49	60	4 100	4 100

[1] ratio of the amount of given substance and initiator; [2] percentage of 3-APBAE in feed; [3] determined by GPC analysis; [4] determined by ^{1}H NMR; [5] theoretical number average molecular weight, calculated based on stoichiometry and conversion

$$M_{n,th} = ([M]_0/[RX]_0) \cdot \text{conversion} \cdot M_{mn}$$

A higher ratio of catalyst to initiator (2/1) was tested and yielded promising results. A number of different combinations with ligand and comonomer ratios were tried. The best results showed the ratio of catalyst/ligand/initiator being 2/1/1, with the molar ratio of comonomer at 11 % and the reaction time 2 h. This produced a copolymer with number average molecular weight M_n = 7 800 g·mol^{-1} and dispersity M_w/M_n = 1.38. This sample was therefore chosen for further analysis of the cloud point temperature of the thermoresponsive copolymer.

5.2.2 Synthesis of mDEGA/3-APBAE copolymers via ARGET ATRP method

To gain a higher degree of control over the polymerization process, copolymerization via the ARGET ATRP method described above was investigated. For this method, previously established reaction conditions were used (mDEGA/initiator ratio 70/1, 3-APBAE molar ratio 10 %, PMDTA for 2 h at 70°C in DMSO). For this method, the transition-metal-catalyst was used in the form of an oxidatively stable state (in our case $CuBr_2$/ligand, $CuCl_2$/ligand) as catalyst precursors. Additionally, a solution of $SnOct_2$ in toluene was used as a stabilizer to activate these precursors. To attempt to maintain control over the polymerization, both more and less active initiators (EBiB and EBP) were tested. Results can be seen in Table 5.

Table 5: Results of mDEGA and 3-APBAE copolymerization via the ARGET ATRP method with PMDTA in DMSO at 70°C for 2 h.

exp.	*initiator*	*catalyst*	M_n [1] [g·mol^{-1}]	M_w/M_n [1]	*conv. Acrylate* [2] [%]	*conv. APBAE* [2] [%]	$M_{n,teor}$ [3] [g·mol^{-1}]
EM 31	EBiB	$CuCl_2$	17 000	2.23	40	45	5 800
EM 32	EBP	$CuCl_2$	22 000	2.10	100	81	13 600
EM 33	EBP	$CuBr_2$	31 600	2.00	30	27	4 200

[1] determined by GPC analysis; [2] determined by ^{1}H NMR; [3] theoretical number average molecular weight, calculated based on stoichiometry and conversion

$$M_{n,th} = ([M]_0/[RX]_0) \cdot \text{conversion} \cdot M_{mn}$$

According to research, the ARGET ATRP method should have reduced the occurrence of side reactions, particularly for acrylates [20,22]. In our case, this method unfortunately did not provide a higher degree of control. On the contrary, with the dispersities being $M_w/M_n \geq 2.00$ for all catalyst and initiator combinations. This route of research was therefore abandoned, and samples of copolymers produced via this method were not used for the cloud point temperature analysis.

5.2.3 Synthesis of mTEGA/3-APBAE copolymers

Synthesis of mTEGA/3-APBAE copolymer was performed similarly to the mDEGA/3-APBAE copolymer synthesis. Based on the results of previously conducted experiments, the variables tested were the initiator (EBiB and EBP) and the molar percentage of 3-APBAE in feed The results of mTEGA/3-APBAE copolymerization are summarised in Table 6.

Table 6: Results of copolymerizations of mTEGA with 3-APBAE in DMSO, catalyzed by CuBr, at 70°C for 2 h

exp.	*ini.*	*ligand*	x_{APBAE} [1] [%]	M_n [2] [g·mol^{-1}]	M_w/M_n [2]	*conv.* [3] [%]	*conv. APBAE* [3] [%]	$M_{n,teor}$ [4] [g·mol^{-1}]
EM 28	EBiB	PMDTA	13	3 400	1.48	31	44	1 900
RM 145	EBP	PMDTA	5	3 800	1.33	49	27	5 500

[1] percentage of 3-APBAE in feed; [2] determined by GPC analysis; [3] determined by ^{1}H NMR; [4] theoretical number average molecular weight, calculated based on stoichiometry and conversion $M_{n,th} = ([M]_0/[RX]_0) \cdot \text{conversion} \cdot M_{mn}$

The experiment using EBiB, the more active initiator, showed a lower degree of control as evidenced by the dispersity value M_w/M_n = 1.48. The less active initiator EBP was therefore used and showed more promising results of M_w/M_n = 1.33 with a lower conversion of 49 % and 27 % of the respective monomers and lower number average molecular mass of M_n = 3 800, which was improved by performing dialysis (Table 7). Sample RM_145 was therefore chosen for further cloud point temperature analysis.

5.2.4 Summary of mDEGA/3-APBAE synthesis

An overall scheme showing the conditions of p(mDEGA) homopolymer and mDEGA/3-APBAE copolymer synthesis is shown in *Figure* 21. ^{1}H NMR spectra of monomers, homopolymer, and copolymer are shown in Figure 23 for comparison.

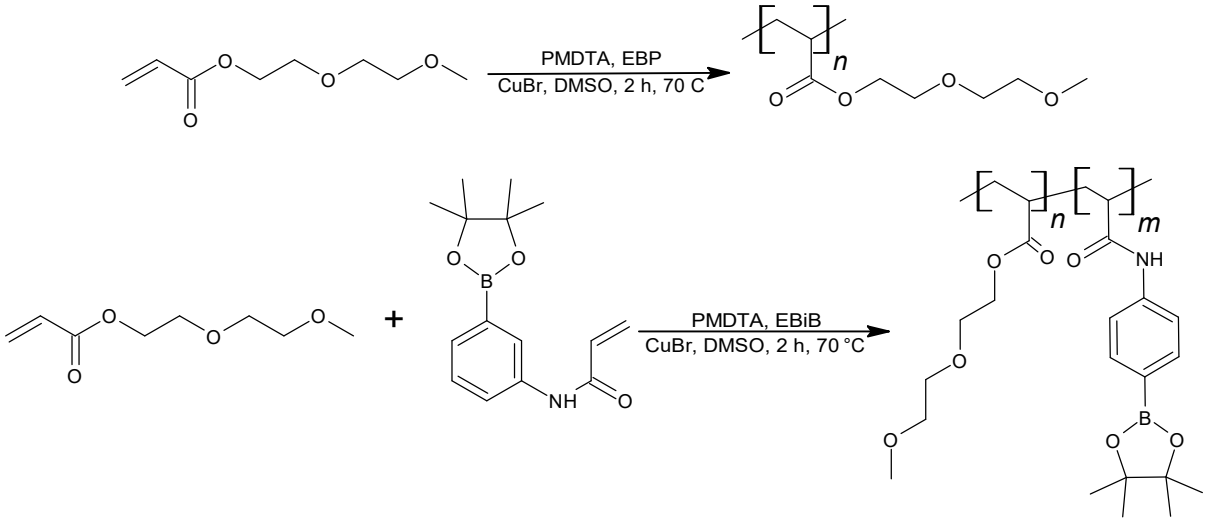

Figure 21: Scheme showing the conditions of p(mDEGA) homopolymerization (top) and mDEGA/3-APBAE copolymerization (bottom)

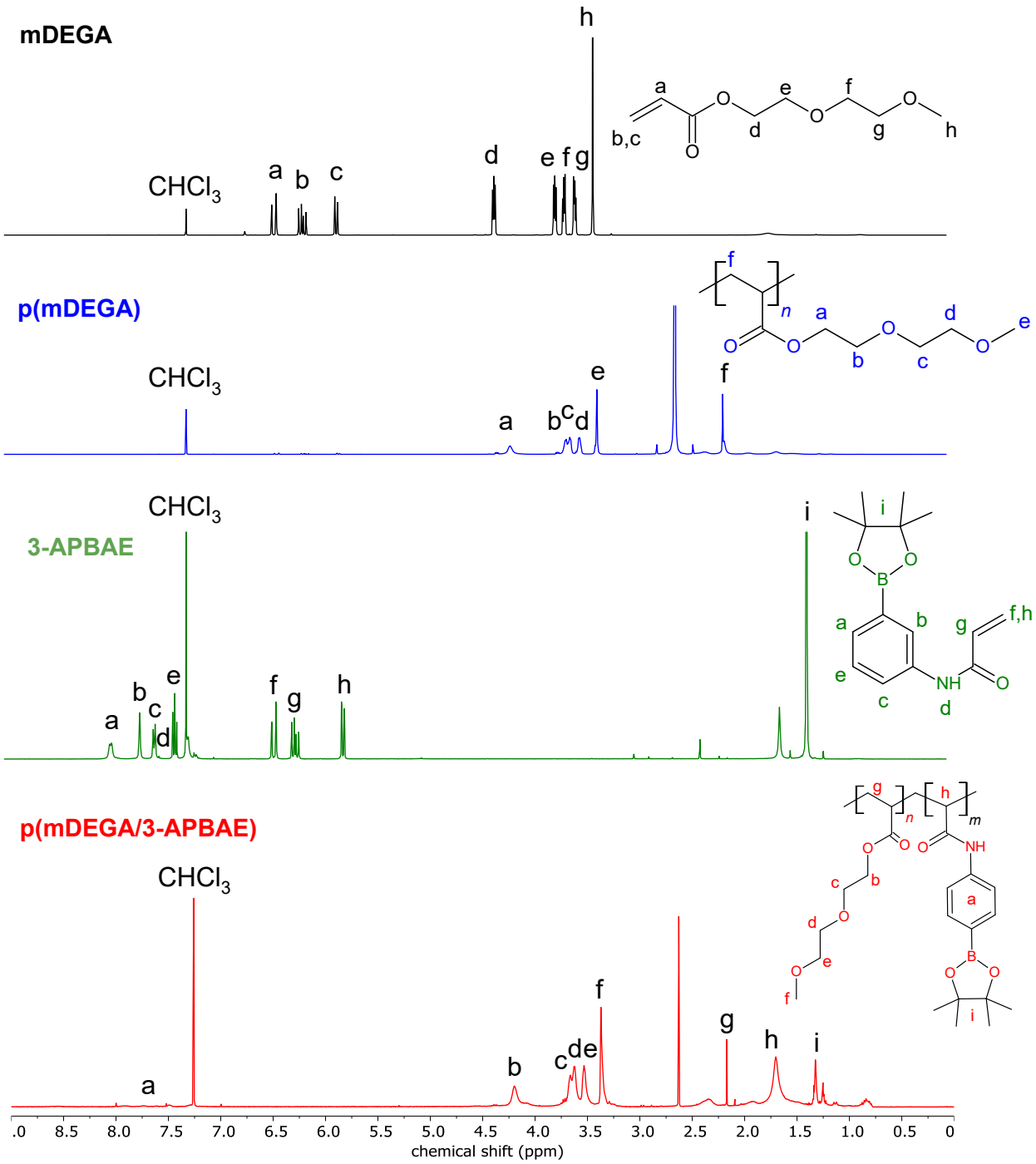

Figure 23: [1]H NMR spectra comparing mDEGA, p(mDEGA), 3-APBAE and p(mDEGA/3-APBAE)

5.2.4 Dialysis of copolymers

Some of the samples (Table 7) were purified via dialysis in a process described above to eliminate lower molar mass peaks revealed during GPC analysis. A subsequent chromatography was performed to confirm the success of this process. Results summarized in Table 7 show slightly lower dispersity values for the dialyzed copolymers.

Table 7: The values of the number average molecular weight and dispersity before and after dialysis

	before dialysis		*after dialysis*	
experiment	M_n [1] [g·mol^{-1}]	M_w/M_n [1]	M_n [1] [g·mol^{-1}]	M_w/M_n [1]
EM_20	7 800	1.38	7 200	1.35
RM_144	8 100	1.45	8 100	1.43
RM_145	3 800	1.33	4 400	1.25

[1] determined by GPC analysis

The subsequent T_{cp} measurements were performed with the dialyzed samples.

5.2.5 Deprotection of copolymers

Sample EM_20 was deprotected via the method described above to remove the pinacol ester and reveal the more reactive -OH groups (Figure 24). This was confirmed via ^{1}H NMR analysis (Figure 24) where the pinacol ester peak at 1.32 ppm does not appear on the deprotected copolymer spectrum. GPC analysis of the deprotected copolymer was not performed due to polymeric boronic acids being highly hygroscopic and therefore would be challenging to characterize via GPC. [29] The number average molar mass was assumed to not have changed as shown in previously published literature. [28]

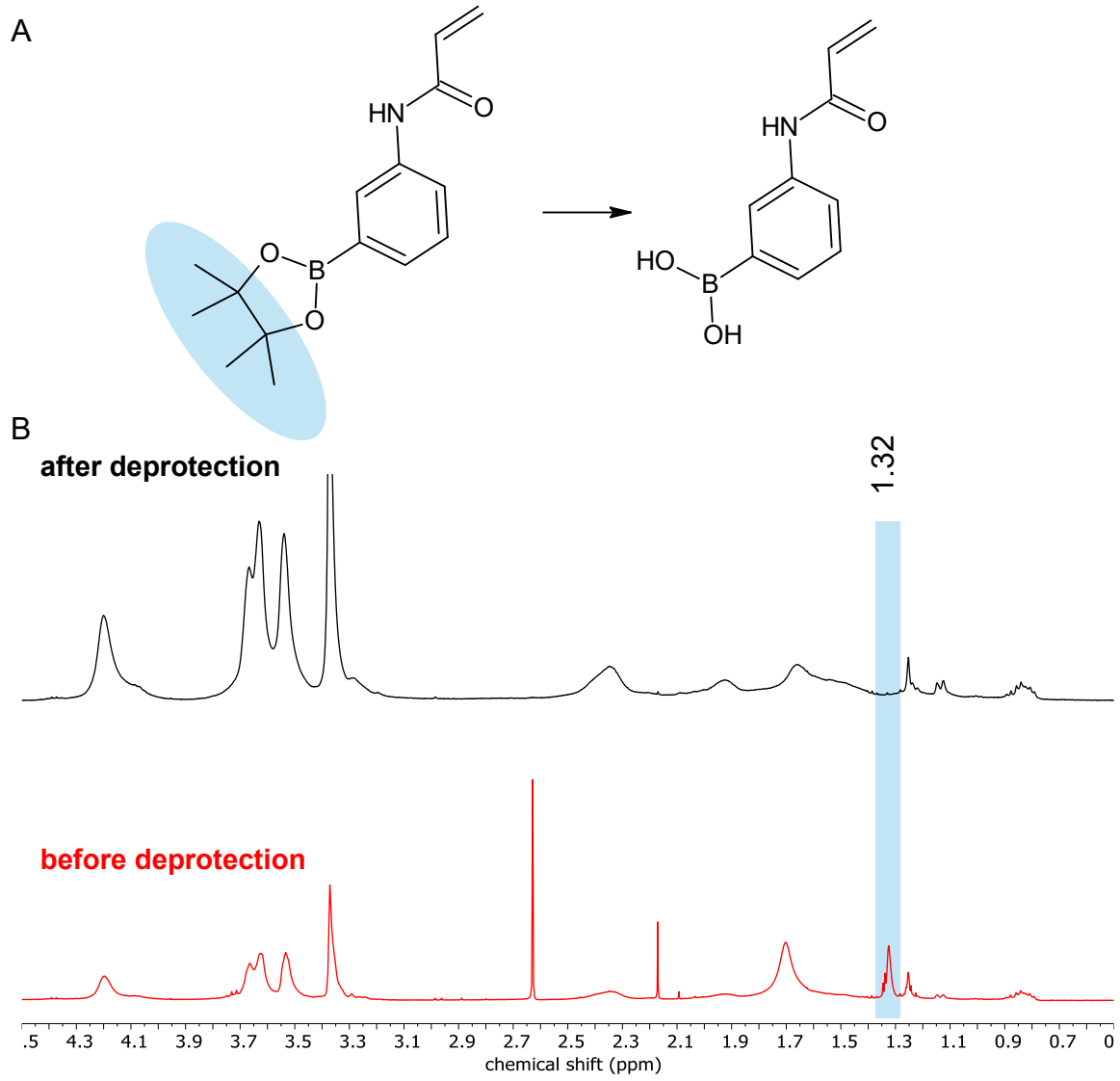

Figure 24: (A) Schematic diagram of deprotection, marked blue is the pinacol ester group; (B) Comparison of the ^{1}H NMR spectra of EM_20 copolymer after deprotection (top) and before (bottom), marked blue is the pinacol ester peak.

5.3 Measurements of T_{cp} values

5.3.1 Measurements of T_{cp} values for homopolymers

Both investigated homopolymers (Table 8) were synthesized via the ATRP method using reaction conditions with the best results after screening experiments. Homopolymer p(mDEGA) - EM_7 was prepared using CuBr as a catalyst, PMDTA as a ligand, and EBP as an initiator in DMSO at 90°C for 4 h. The p(mTEGA) – RM_142 was synthesized using the same conditions except for the reaction being run at 70°C for 2 h.

Table 8: Summary of properties of homopolymers used for T_{cp} analysis and the results at given concentrations

experiment	*monomer*	M_n [1] [g·mol^{-1}]	M_w/M_n [1]	*c [mg/ml]*	$T_{cp,\ heating}$ [°C]	$T_{cp,\ cooling}$ [°C]
EM_7	mDEGA	7 800	1.35	1	56.9	53.5
RM_142	mTEGA	3300	1.15	10	82.5	80.7

[1] determined by GPC analysis

For the p(mDEGA) homopolymer it was possible to measure the T_{cp} values at a concentration of 1 mg/ml, however for the p(mTEGA) homopolymer, the concentration had to be increased to 10 mg/ml to be able to detect T_{cp} in an aqueous solution – the cloud point temperature had to be lowered below the boiling point of water. Because critical aggregation concentration is temperature dependent, by increasing the concentration of polymer in solution, it is possible to reach critical aggregation concentration at a lower temperature. [31] The samples for analysis were prepared via the method described above. The analysis showed the cloud point temperature for p(mDEGA) at T_{cp} = 56.9°C for heating and T_{cp} = 53.5°C for cooling (Figure 25, left). According to published literature [6], this is higher than T_{cp} measured for p(mDEGA) prepared via RAFT and NMP methods with similar molar mass.

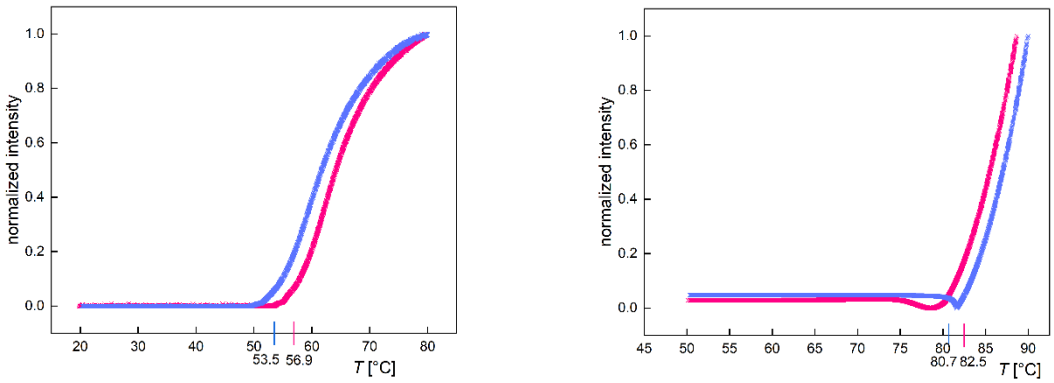

Figure 25: Recorded spectra for 1 mg/ml p(mDEGA) (left) and 10 mg/ml p(mTEGA) (right), Spectra were recorded for both heating (pink) and cooling (blue) with temperature rate 1 °C/min. Markers on the x-axis indicate calculated T_{cp} values for heating (pink) and cooling (blue)

For p(mTEGA), the cloud point temperature was found at T_{cp} = 82.5°C for heating and T_{cp} = 80.7°C for cooling (Figure 25, right). For p(mTEGA) the cloud point temperatures were also higher than previously synthesized homopolymers [6]. The cloud point temperature is higher for the p(mTEGA) homopolymer because of the higher hydrophilicity derived from additional ethylene glycol unit on the monomer (Figure 20).

5.3.2 Measurements of T_{cp} values for copolymers

5.3.2.1 Measurements of T_{cp} values for mDEGA/3-APBAE copolymer

For the mDEGA/3-APBAE copolymer analysis, sample EM_20 (Table 3, entry 3), was chosen. This sample was prepared via the ATRP method using CuBr as a catalyst, PMDTA as a ligand, and EBiB as an initiator in DMSO at 70°C for 2 h, as the most suitable conditions based on screening experiments. The sample was then purified through dialysis before the analysis, which improved its M_w/M_n slightly (Table 7). The results showed the cloud point temperature, measured at 1 mg/ml concentration of copolymer, at T_{cp} = 37.9°C for heating and T_{cp} = 36.0°C for cooling (Figure 26).

5.3.2.2 Measurements of T_{cp} values for mTEGA/3-APBAE copolymer

For the mTEGA/3-APBAE copolymer analysis, sample RM_145 (Table 6, entry 4), was chosen. This sample was prepared via the ATRP method under conditions with the best results according to screening experiments, which were similar to EM_20, except for using EBP as an initiator. The results showed the cloud point temperature, measured at 1 mg/ml concentration of copolymer, at T_{cp} = 48.4°C for heating and T_{cp} = 42.1°C for cooling (Figure 26).

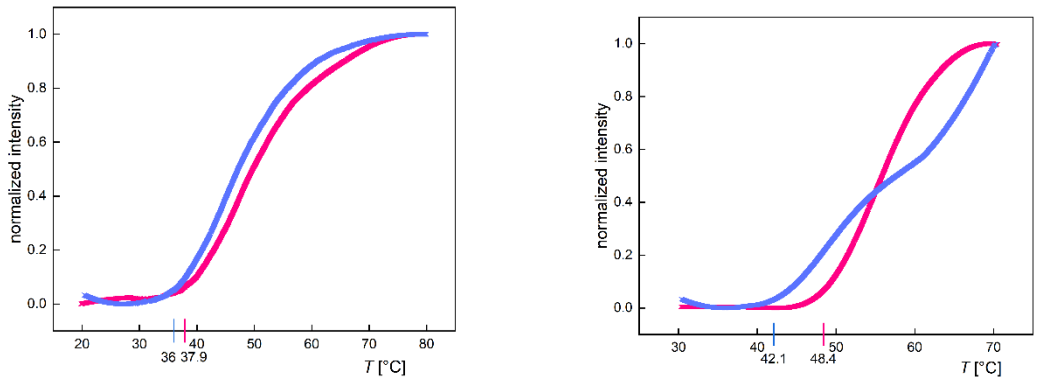

Figure 26: Recorded spectra for 1 mg/ml mDEGA/3-APBAE copolymer (left) and mTEGA/3-APBAE (right); spectra were recorded for both heating (pink) and cooling (blue) with temperature rate 1 °C/min. Markers on the x-axis indicate calculated T_{cp} values for heating (pink) and cooling (blue)

The copolymer T_{cp} was recorded to be lower than its corresponding homopolymer in both cases (Table 9). This is due to the introduction of a more hydrophobic 3-APBAE monomer into the copolymer structure.

Table 9: Results of cloud point temperature measurements

acrylate	*comonomer*	$x_{3\text{-}APBAE}$ [1] [%]	$T_{cp,\ heating}$ [°C]	$T_{cp,\ cooling}$ [°C]
mDEGA	-	-	59.6	53.5
mTEGA	-	-	82.5	80.7
mDEGA	3-APBAE	14	37.9	36.0
mTEGA	3-APBAE	3	48.4	42.1

[1] determined by ^{1}H NMR using monomer conversion

5.3.2.3 Measurements of T_{cp} values for deprotected copolymer

After the deprotection of the copolymer mDEGA/3-APBAE, T_{cp} measurements were also performed at 1 mg/ml concentration of the comonomer. The T_{cp} for heating was found to be 40.4 °C and for cooling at 37.4 °C (Figure 27). These results do show a slight increase in the cloud point temperature (Figure 27) as compared to protected (Figure 26) copolymer but the change is very small (+2.5 °C for heating and only +1.4 °C for cooling). A larger increase in the T_{cp} was expected due to the removal of the pinacol ester and the subsequent increase in the hydrophilic of 3-APBAE. However, the increase might be smaller than expected because of the formation of hydrogen bonds with other 3-APBA units instead of water. [30]

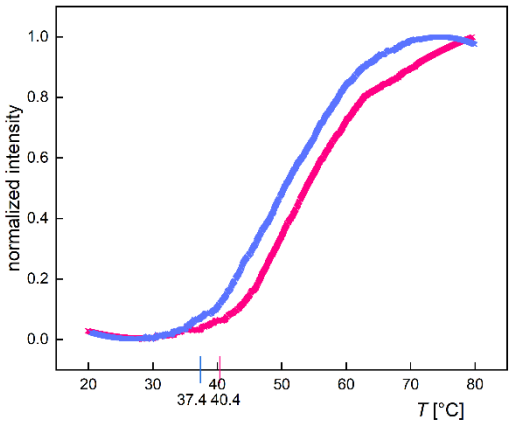

Figure 27: Recorded spectra for 1 mg/ml mDEGA/3-APBAE copolymer after deprotection); spectra were recorded for both heating (pink) and cooling (blue) with a temperature rate of 1 °C/min. Numbers in the upper right corner indicate calculated T_{cp} values for heating (pink) and cooling (blue)

6 Conclusion

Stimuli-responsive copolymers were prepared by the ATRP method, and all were characterized with ^{1}H NMR and GPC. To optimize the (co)polymerization procedure, several components of the catalytic system and different reaction conditions were tested. Firstly, homopolymers of two acrylates (mDEGA and mTEGA) were synthesized. During this step, DMSO was quickly determined as the most promising solvent and after performing a kinetic analysis, 70°C showed to be the best temperature for the reaction. These conditions were then used as a benchmark for following experiments. The p(mDEGA) homopolymer showing the best results, yielding the lowest polymer dispersity and simultaneously highest monomer conversion, was obtained using EBP as an initiator, CuBr as a catalyst and PMDTA as a ligand run for 1 h at 70°C. For the p(mTEGA) homopolymer, similar conditions provided results, with the exceptions being EBiB as an initiator and the reaction time being two hours. These results were then taken into consideration while preparing the copolymers.

The same requirements regarding dispersity and monomer conversion were applied to the copolymer synthesis. Here, 3-APBAE was copolymerized with mDEGA and mTEGA while continuing to test several conditions for the reaction. The best combination of conditions for the mDEGA/3-APBAE copolymer was determined to be EBiB as an initiator, CuBr as a catalyst and PMDTA as a ligand for 2 hours. Using a low molar ratio (10 %) of the comonomer and a higher molar ratio of catalyst to initiator (2/1) while maintaining the ratio of ligand to initiator at 1/1 proved beneficial. The mTEGA/3-APBAE copolymer was again prepared under the same conditions except for the initiator being EBP.

T_{cp} values were determined for samples of both homopolymers and both combinations with the comonomer (after dialysis of the sample). It was found that the copolymers exhibited lower T_{cp} values than their corresponding homopolymers, which can be attributed to the more hydrophobic comonomer being introduced into the polymer structure. An increase in the concentration of copolymer in the tested sample shows a decrease in T_{cp}, which can be attributed to a lower critical aggregation concentration. Lastly, the T_{cp} for a deprotected copolymer was measured and only a slight increase in the value was found. This can be attributed to the fact that although the deprotected 3-APBAE unit becomes more hydrophilic, it also forms hydrogen bonds with other units therefore reducing its capacity to form hydrogen bonds with water.

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