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Syntéza kopolymerů reagujících na vnější podněty pomocí radikálové polymerizace s vratným adičně-fragmentačním přenosem řetězce

Synthesis of stimuli-responsive copolymers via reversible addition-fragmentation chain transfer polymerization

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, 22. 5. 2024

Kristýna Dušková

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ABSTRAKT

Předkládaná bakalářská práce se zaměřuje na syntézu (ko)polymerů reagujících na vnější podměty pomocí RAFT polymerace. Žádané termoresponzivní chování připravených (ko)polymerů bylo zajištěno použitím vhodných monomerů di(ethylen glykol) methyl ether akrylátu (DEGMA) a tri(ethylene glykol) methyl ether akrylátu (TEGMA), které byli vybrány na základě literární rešerše. Inkorporace pinakol esteru 2-/3-akrylamidofenylboronové kyseliny (2-/3-APBAE) do struktury připravených kopolymerů je zodpovědná za citlivost vůči pH a 1,2- nebo 1,3-diolům, což vyžaduje odchránění fenylboronové kyseliny (odstranění pinakol esteru). (Ko)polymerizační proces byl optimalizován s využitím tří přenosových činidel (CTA) a tří rozpouštědel s odlišnou polaritou, přičemž všechny připravené (ko)polymery byly charakterizovány pomocí nukleární magnetické rezonance (^{1}H NMR) a gelové permeační chromatografie (GPC). Kopolymery obsahující 3-APBAE byly důkladně přečištěny pomocí dialýzy. Termoresponzivní chování připravených (ko)polymerů bylo potvrzeno měřením hodnot teploty zákalu (T_{cp}) pomocí rozptylu světla v závislosti na změně teploty. Jeden z kopolymerů tvořený monomery DEGMA a 3-APBAE byl odchráněn v mírně kyselém prostředí a rovněž charakterizován změřením T_{cp} hodnoty.

ABSTRACT

Submitted bachelor thesis focuses on the synthesis of stimuli-responsive (co)polymers via RAFT polymerization. Desired thermoresponsive behavior of prepared (co)polymers was ensured by using suitable monomers di(ethylene glycol) methyl ether acrylate (DEGMA) and tri(ethylene glycol) methyl ether acrylate (TEGMA), which were chosen based on literature research. The incorporation of 2-/3-acrylamidophenylboronic acid pinacol ester (2-/3-APBAE) into the structure of prepared (co)polymers is responsible for pH and 1,2- or 1,3-diols responsivity, which requires the deprotection of phenylboronic acid (removal of pinacol ester). (Co)polymerization process was optimized by utilizing three chain transfer agents (CTA) and three solvents with different polarity. All prepared (co)polymers were characterized by nuclear magnetic resonance (^{1}H NMR) and gel permeation chromatography (GPC). Copolymers containing 3-APBAE were thoroughly purified with dialysis. Thermoresponsive behavior of prepared (co)polymers was confirmed by measuring cloud point (T_{cp}) values using light scattering in dependency on temperature change. One of the copolymers containing DEGMA and 3-APBAE was deprotected in mild acidic environment and characterized by measuring T_{cp} value as well.

ABBREVIATIONS

2-APBAE – 2-acrylamidophenylboronic acid pinacol ester
3-APBAE – 3-acrylamidophenylboronic acid pinacol ester
APBA – acrylamidophenylboronic acid
AIBN − azobisisobutyronitrile
CRP – controlled radical polymerization
CSIRO − Commonwealth Scientific and Industrial Research Organisation
CTA – chain transfer agent
CTA 1 − cyanomethyl dodecyl trithiocarbonate
CTA 2 − 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid
CTA 3 − 3-(benzylthiocarbonylthioylthio)propanoic acid
DEGMA − di(ethylene glycol) methyl ether acrylate
DCM – dichloromethane
DMA – *N*,*N*-dimethylacetamide
DMSO – dimethyl sulfoxide
EGMA − ethylene glycol methyl ether acrylate
FRP – free radical polymerization
GPC – gel permeation chromatography
LAMs – less activated monomers
LCST − lower critical solution temperature
MAMs – more activated monomers
MTT – (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetra-zolium bromide)
MWCO – molecular weight cut-off
NIPAM − *N*-isopropylacrylamide
NMR – nuclear magnetic resonance
OEG – oligo ethylene glycol
PBA – phenylboronic acid
P(DEGMA) – poly(di(ethylene glycol) methyl ether acrylate)
P(NIPAM) − poly(*N*-isopropylacrylamide)
P(TEGMA) – poly(tri(ethylene glycol) methyl ether acrylate)
RAFT − reversible addition-fragmentation chain transfer
RDRP − reversible deactivation radial polymerization
RI – refractive index
T_{cp} – cloud point
TEA – triethylamine
TEGMA − tri(ethylene glycol) methyl ether acrylate
THF − tetrahydrofuran
UCST – upper critical solution temperature

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1 INTRODUCTION

Stimuli-responsive (co)polymers alter their properties (chemical and physical) upon the exposure to an external stimulus (temperature, pH, etc.). Thermoresponsive (co)polymers are the most studied examples, possessing the ability to change their solubility upon temperature evolution (heating or cooling). Polyacrylates with short oligo ethylene glycol (OEG) side chain are an arising group of thermoresponsive polymer thanks to their tunable lower critical solution temperature (LCST) behavior and biocompatibility. Polymers containing phenylboronic acid (PBA) are well known for binding with 1,2- or 1,3- diols resulting in the formation of boronate ester. PBA exists in charged tetragonal or neutral trigonal form depending on pH with charged form being preferred in pH above pK_a. The copolymerization between OEG acrylates and PBA containing monomers therefore results in a triply responsive copolymer. The use of controlled radical polymerization (CRP) techniques allows the synthesis of copolymers with predictable molecular weight and low dispersities.

In this thesis, stimuli responsive (co)polymers were synthesized via RAFT polymerization. The thermoresponsive behavior was achieved by using two OEG acrylate monomers (di(ethylene glycol) methyl ether acrylate (DEGMA) and tri(ethylene glycol) methyl ether acrylate (TEGMA)). Moreover, incorporation of PBA based monomers (2-/3-acrylamidophenylboronic acid pinacol ester (2-/3-APBAE)) resulted in responsivity towards pH and the presence of 1,2- or 1,3-diols after pinacol ester deprotection. The resulting (co)polymers were characterized using NMR and GPC. Their thermoresponsive behavior was investigated by measuring their cloud points (T_{cp}) values.

2 OVERVIEW OF THE LITERATURE

2. 1 RAFT polymerization

Reversible addition-fragmentation chain transfer (RAFT) polymerization was first introduced in 1998[1] by CSIRO researchers from Australia. Since then, RAFT polymerization has become one of the most versatile polymerization techniques. RAFT is a reversible deactivation radical polymerization (RDRP)[2] also referred to as living/controlled radical polymerization (CRP). RDRP copies features of living polymerization while combining them with the benefits of radical process's flexibility (wide range of reactions components can be used).

RAFT polymerization allows the synthesis of polymers with predictable molecular weight, low molar mass distribution and capacity for additional chain growth (blocks or polymers with higher molecular weight can be produced by further monomer addition).[1] RAFT process can be used in both homogenous and heterogenous[3] systems. Versatility of the RAFT process can be demonstrated by its compatibility with a wide range of monomers, solvents, and initiators. The usage of RAFT process to provide a variety of materials with different architectures[4] has also been reported.

2. 1. 1 Mechanism of RAFT process

The most important RDRP characteristic is the equilibrium between active and dormant chains. In RAFT process this equilibrium is carried out by a degenerative transfer. In degenerative transfer system the overall number of radicals throughout the activation-deactivation process remains the same. Source of radicals is therefore needed, in most cases a radical initiator.

After radical initiation (I), new radical reacts with monomer unit and forms a propagating radical (II). Addition of propagating radical to the thiocarbonylthio compound called chain transfer agent (CTA) is followed by fragmentation of the intermediate radical which results in polymeric CTA and another radical (III). The new radical reacts with another monomer unit to form a new propagating radical (IV). Rapid equilibrium between dormant polymeric CTAs and active propagating radicals results in the same likelihood of growth for all chains (V) thus ensuring narrow dispersity. Radicals can also react with each other which

leads to termination (VI).[5] The mechanism is depicted in Figure 1. The process is considered to be effective when the rate of addition-fragmentation equilibrium is higher than the propagation rate meaning less than one monomer unit is added within activation cycle.[6]

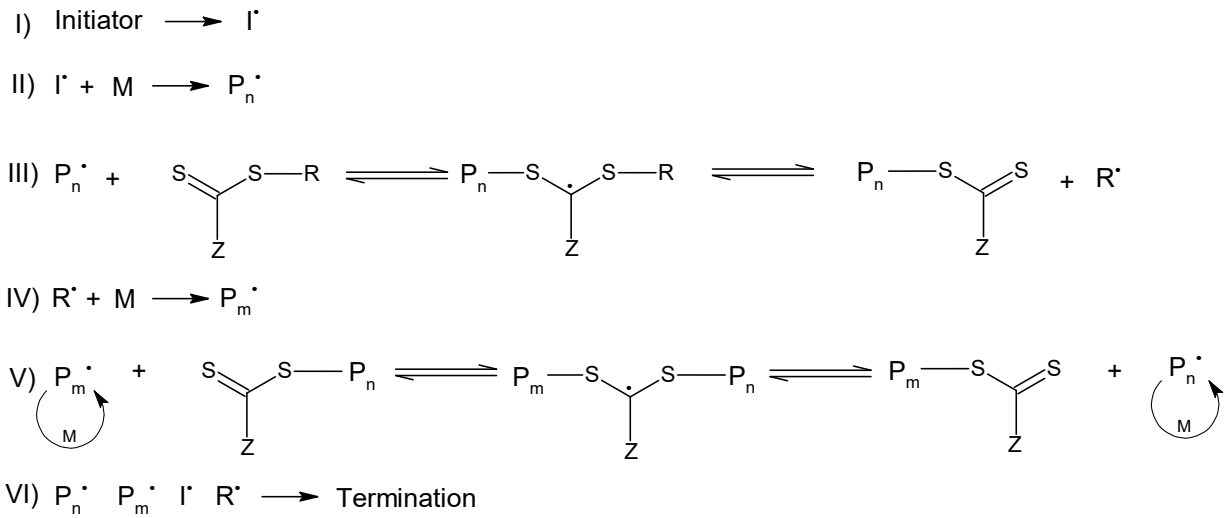


Figure 1: The mechanism of RAFT process.[6]

Throughout the whole process, monomer units are inserted between the R and Z−C(=S)−S group of the CTA. These group therefore form the alpha and omega end group of most of the resulting polymeric chains. There are four types of polymeric chains. Those that contain omega end group (living chains) and those without the group (dead chains). In regard to the alpha end group, chains may be initiated by the CTA's R group or by radicals from the initiator. Based on the number of generated radicals from the initiator, the number of chains in each class can be predicted.[6]

2. 1. 2 Role of the CTA

There are two groups of vinyl monomers based on their reactivity. First group consists of more activated monomers (MAMs) with vinyl group conjugated to carbonyl group double bond, aromatic ring, or nitrile (e.g., (meth)acrylates, styrene, isoprene). Second one is formed by less activated monomers (LAMs) with double bond next to lone pairs of oxygen, nitrogen, sulfur, or halogen (e.g., vinyl acetate, vinyl chloride). This wide monomer compatibility is result of CTA's reactivity.

The double bond between carbon and sulfur in the CTA must be more reactive towards radical addition than the carbon-carbon double bond of the monomer for successful

RAFT polymerization. This can be ensured by choosing the appropriate Z and R group of the CTA towards the targeted monomer class. The Z group is responsible for the stability of an intermediate radical. It alters rate of addition of propagating radicals and the fragmentation rate of intermediate radical. MAMs form more stable propagating radicals thanks to an electronic stabilization from their substituent. Thus, they need a Z group that will stabilize the intermediate radical, ensuring that addition on carbon-sulfur double bond is favored. Most used CTAs are therefore trithiocarbonates with *S*-alkyl as the Z group or dithiobenzoates with phenyl acting as the Z group. LAMs, on the other hand, require less stable intermediate radicals, thus xanthates and dithiocarbamates with *O*-alkyl and *N*-alkyl in the place of the Z group respectively are used. The R group must be a good leaving group to ensure the fragmentation of intermediate radical, but it also must be able to efficiently reinitiate propagation.[7] The R group radical therefore must be stable enough for its formation while simultaneously reactive enough for the addition to the monomer unit.

Thiocarbonylthio group present in initial CTA is retained in the resulting polymer. Presence of the thiocarbonylthio group is responsible for the living character of RAFT process. However, it also means that the polymers are usually colored, in some cases might even be odorous. This can be a problem for some applications. Therefore, the thiocarbonylthio group could be removed via several strategies, such as thermolysis[8] or radical-induced reduction by hydrogen atom donor[9] resulting in a double bond and hydrogen atom at the end of polymer chain respectively (Figure 2).

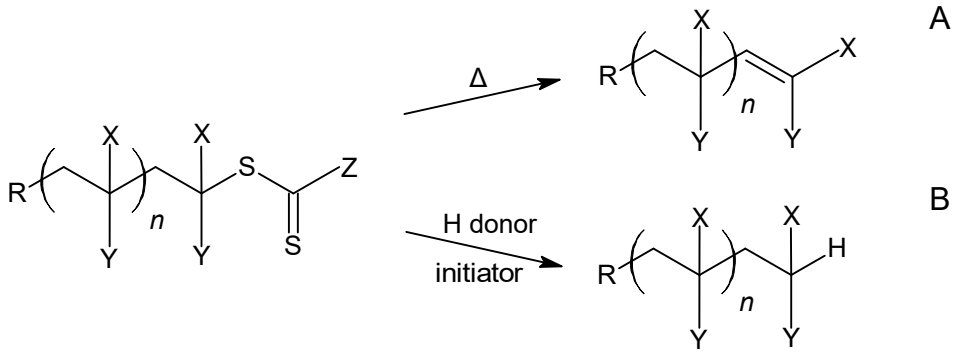


Figure 2: Process of thiocarbonylthio end group removal by thermolysis (A) and radical-induced reduction by hydrogen atom donor (B).

2. 1. 3 Role of the initiator and solvent

Given that the overall number of radicals stays the same during the whole RAFT process, source of radicals (initiator) is therefore required for the introduction of radicals into the system. Initiator allows control of polymerization rate, which increases with its higher concentration. Number of chains that end in a bimolecular termination is in a direct correlation with the number of radicals initially introduced via initiator decomposition. This permits for the prediction and control over the number of dead chains. Bimolecular termination isn't responsible for the loss of living chain end. The number of chains with thiocarbonylthio group at the omega chain end doesn't change. This presents the advantage of RAFT compared to other RDRP systems.

Lowering initiator concentration leads to optimal livingness of the system. Higher initiator to CTA concentration allows to achieve higher rate of polymerization.[10] However, polymerization rate follows that of standard radical polymerization and can also be influenced by other parameters such as the decomposition rate coefficient of the initiator. A higher polymerization rate allows for shorter polymerization time or lesser amount of initiator needed to gain full conversion. To reach an optimal polymerization rate a high rate of radical generation or solvent induced acceleration is needed. Polar solvents are able to stabilize the transition state of propagating radicals, which leads to their lower activation energy[11]. Typical reaction setups aim to balance the living character of resulting polymeric chains and the reaction speed by controlling the ratio between CTA and the initiator, typical range being 5−10. [6]

Most popular form of initiation is thermal initiation using diazo or peroxide compounds. The radical formed form the initiator should be a good leaving group regarding the propagating radical. This is important to avoid retardation. Azobisisobutyronitrile (AIBN) is seen as a good initiator choice for acrylates polymerizations since the 2-cyano-2-propyl radical is a good leaving group with reference to most propagating radicals.[12] Another mechanisms of initiation are redox initiation[13] and photoactivation[14] (Figure 3).

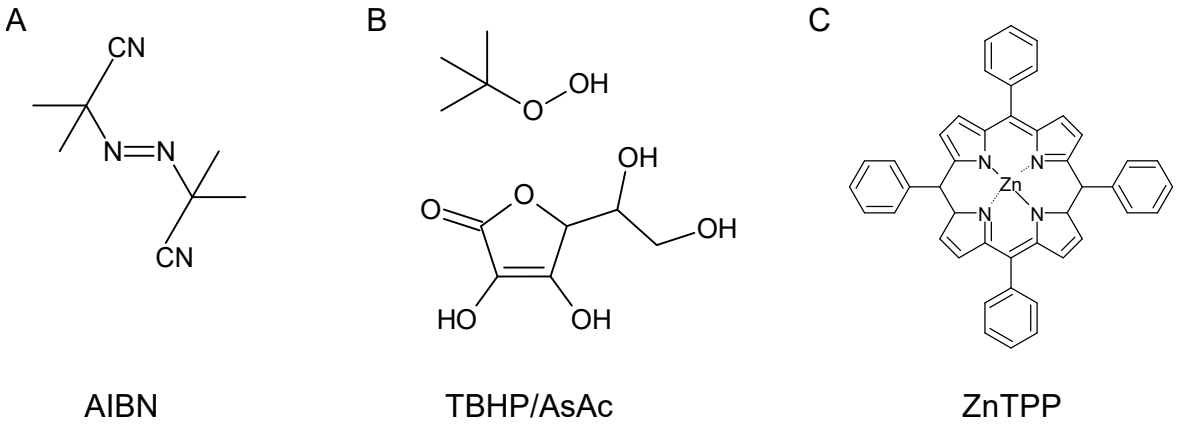


Figure 3: The examples of structures for thermal initiator (azobisisobutyronitrile) (A), redox initiation (tert-butyl hydroperoxide/ascorbic acid) (B) and photoredox catalyst (zinc tetraphenylporphyrin) (C).

2. 2 Stimuli-responsive (co)polymers

Stimuli-responsive (co)polymers are capable of changing their physical and/or chemical properties in a response to the exposure to an external stimulus. These stimuli include temperature, pH, or small molecules (e.g. glucose or lactate). Stimuli response can result in numerous responses including phase separation, optical, color or shape change.[15] Stimuli responsive (co)polymers have a wide range of applications such as pH sensitive membranes[16], drug delivery carriers[17], smart coatings[18] and CO_2 sensors.[19]

2. 2. 1 Thermoresponsive (co)polymers

Thermoresponsive (co)polymers represent one the most investigated group of stimuli-responsive (co)polymers. That is because of their easily controlled stimulus and promising application in biomedicine.[20] Various mechanisms can be used to obtain a temperature response, although solution phase transition around a certain temperature point is the most used. This temperature is known as a lower critical solution temperature (LCST) in case of polymers losing solubility upon heating. If polymer gains solubility with rising temperature, the temperature is referred to as an upper critical solution temperature (UCST).

The term LCST should be used only in the case when the phase diagram has been determined, where LCST corresponds to the minimum of the diagram. If the phase diagram has not been determined, it's better to refer to the transition between soluble and insoluble

state as transition temperature or cloud point temperature (T_{cp}).[15] During heating of a polymer with a LCST behavior (LCST polymer), phase separation happens at the T_{cp} (Figure 4). This coincides with the forming of droplets with high polymer concentrations leading to clouding of the solution. Phase separation is caused by the change in a hydration state. This change is a result of competing hydrogen bonding properties. Upon a heating of a LCST polymer, intra- and inter- molecular hydrogen bonding between polymer chains are preferred to a hydrogen bonding between polymer and water leading to a phase separation.[15]

From a thermodynamic point of view, entropic loss can no longer be compensated by enthalpic gain from hydrogen bonding of water molecules to the polymer at T_{cp}. This leads to Gibbs free energy being equal to zero, suggesting that water acts as a theta solvent. Increasing hydrophobicity of a polymer leads to the decrease of enthalpic gain and therefore to lower T_{cp}. Balance of hydrophilic/hydrophobic properties thus allows wide and tunable thermoresponsive behavior. Cloud point can therefore be manipulated by monomer identity and its side chain length, type of backbone, and end group.[21] T_{cp} can also be influenced by other polymer characteristics such as molecular weight, dispersity and alpha and omega end groups.[22] Furthermore, T_{cp} can also differ based on the concentration of polymeric solution or the presence of salt ions in the solution.[23]

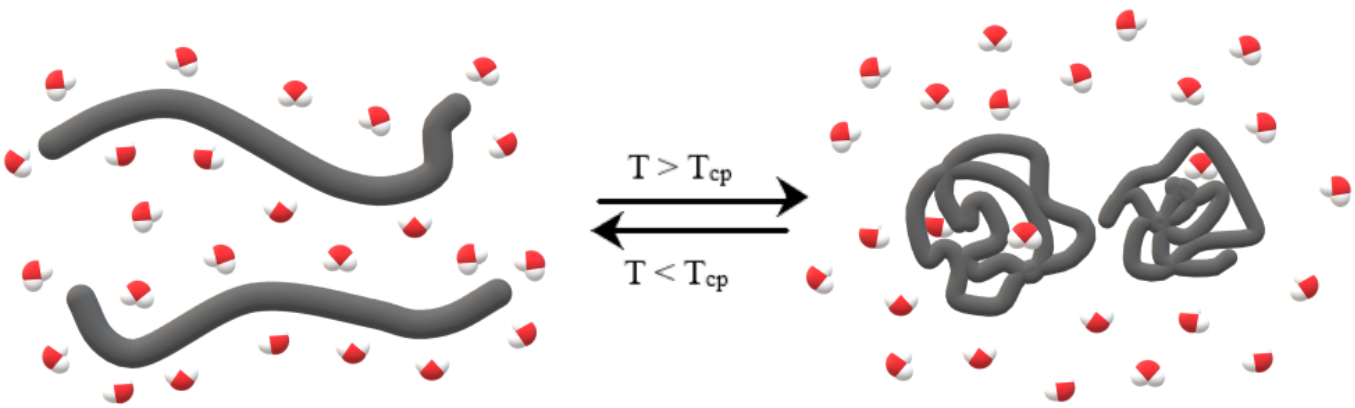


Figure 4: Depiction of a polymer phase transition in aqueous solution from a completely dissolved homogenous state (left) to a high polymer concentration droplets and a low polymer concentration aqueous phase (right).

Poly(*N*-isopropylacrylamide) (P(NIPAM)) is viewed as the benchmark of thermoresponsive polymers. This comes from the value of its T_{cp} in water (32 °C)[24] which is

comparable to a body temperature making it a good candidate for in vivo biomedical applications[25]. Another advantage of P(NIPAM) comes from its relative insensitivity to environmental conditions. The T_{cp} varies only by a few degrees when exposed to small variations in concentration or pH.[25] Because of this almost independent phase transition, the T_{cp} is very similar to its LCST at any concentration.[15]

Polymers with short oligo ethylene glycol (OEG) side chains are an arising group of thermoresponsive polymers. OEG monomers typically comprise of polymerizable group of (meth)acrylate carrying an OEG chain terminated with a methyl/ethyl ether group. Polymers with short OEG chains have both the biocompatibility of poly(ethylene glycol) and flexible, easy to control LCST behavior.[26]

The characteristics of P(NIPAM) and a copolymer comprising of two OEG methacrylates with the same T_{cp} as P(NIPAM) have been compared. When heated, P(NIPAM) exhibits a very sharp transition, however a broad hysteresis can be seen during cooling. OEG methacrylate copolymer exhibits more uniform temperature profile. Both polymers show a typical salting-out effect in the presence of NaCl and a similar behavior in physiological medium. The degree of polymerization has been shown to have a bigger influence on the T_{cp} of P(NIPAM) than methacrylate copolymer.[27]

2. 2. 2 Thermoresponsive polyacrylates bearing short OEG chain

While the most studied types of OEG monomers are methacrylates, acrylate monomers have some more advantageous characteristics. Because of the missing hydrophobic methyl group, the acrylate backbone is more hydrophilic than the one of methacrylates which makes possible to synthesize monomers with shorter side chains, comprising of less ethylene glycol units. This also results in their higher T_{cp} compared to the methacrylate analogs.[28] The end groups of polyacrylates can be easily functionalized.[29] On the other hand, acrylates tend to be more prone to hydrolysis.[30] OEG acrylates can be polymerized by all CRP techniques.[26] The chosen technique however has an effect on the toxicity of the polymer. Evident differences in the cytotoxicity determined by a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetra-zolium bromide) assay have been observed for the same polymer depending on used CTA.[31] Copolymers based on OEG acrylates exhibit a tunable T_{cp} based on a monomer composition.[32]

The T_{cp} of poly(di(ethylene glycol) methyl ether acrylate) (P(DEGMA)) has been shown to have the widest range of all OEG acrylate monomers with average T_{cp} of 40 °C[26]. In the first report of its thermoresponsive properties, T_{cp} has been determined to be 38 °C for 0.5 wt% aqueous solution.[33] The T_{cp} of P(DEGMA) seems to be strongly dependent on the concentration of the polymer solution. The T_{cp} decreases with an increase in concentration. Higher concentration makes the aggregation of dehydrated polymer chains easier, resulting in a lowered T_{cp}. The 1.0 wt% concentration seems to be a turning point. In lower concentrations, the T_{cp} increases dramatically (> 8 °C between 1.0 and 0.05 wt%). In comparison, above 1.0 wt%, the T_{cp} variation is less than 2 °C.[33] The effect of molar mass and end groups on T_{cp} has also been investigated. P(DEGMA) exhibits an increase in T_{cp} with increasing molar mass. Modification of end group from more hydrophobic *tert*-butyl benzoate to a more hydrophilic benzoic acid group lead to an increase in T_{cp}.[28] The slightly more hydrophilic poly(tri(ethylene glycol) methyl ether acrylate) (P(TEGMA)) has been shown to have a T_{cp} of 58 °C for 0.5 wt% solution. It displays the same dependence on concentration as described above for P(DEGMA)[33] with average T_{cp} of 70 °C[26] (Figure 5).

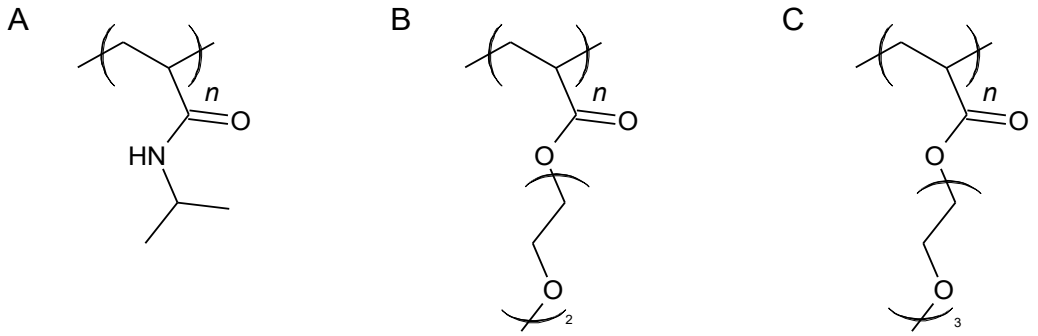


Figure 5: The structures of poly(N-isopropylacrylamide) (A), poly(di(ethylene glycol) methyl ether acrylate) (B) and poly(tri(ethylene glycol) methyl ether acrylate) (C) with their respective average value[26] of T_{cp}.

Their T_{cp} can be lowered by incorporating a hydrophobic comonomer into the copolymer. On the other hand, a hydrophilic comonomer leads to an increase in T_{cp}.[34] In comparison, block copolymers often exhibit two different T_{cp}. This can be used for the formation of micelles as shown for the block copolymer between ethylene glycol methyl

ether acrylate (EGMA) and DEGMA. The block copolymer was soluble in water at low temperature but underwent self-assembly into micelle upon heating.[35]

2. 3 Phenylboronic acid

Phenylboronic acid (PBA) is derived from boric acid by substituting one of its hydroxyl groups with phenyl. PBA is electron-deficient compound due to the vacant p-orbital on the sp^2 hybridized atom of boron. PBA containing moieties have the properties of mild Lewis acid and the ability to form complexes with electron donors such as amines, citrate, phosphate or imidazole.[36] They're well known for forming boronate esters with 1,2-diols and 1,3-diols. (Co)polymers containing PBA have therefore found many applications, for example saccharide sensors,[37] holographic sensors,[38,39] self-healing hydrogels[40,41] and drug delivery.[42,43]

The effectiveness of saccharide sensing is largely defined by the pK_a value of PBA containing monomer. pK_a is the measurement of Lewis acidity and it is determined as the ratio of trigonal and tetragonal PBA at a specific pH. Neutral or charged esters are formed after the addition of 1,2- or 1,3-diols. Because of the ring strain on the sp^2 hybridized boron atom, the neutral ester is very unstable and transforms into neutral PBA or charger ester[44] (Figure 6).

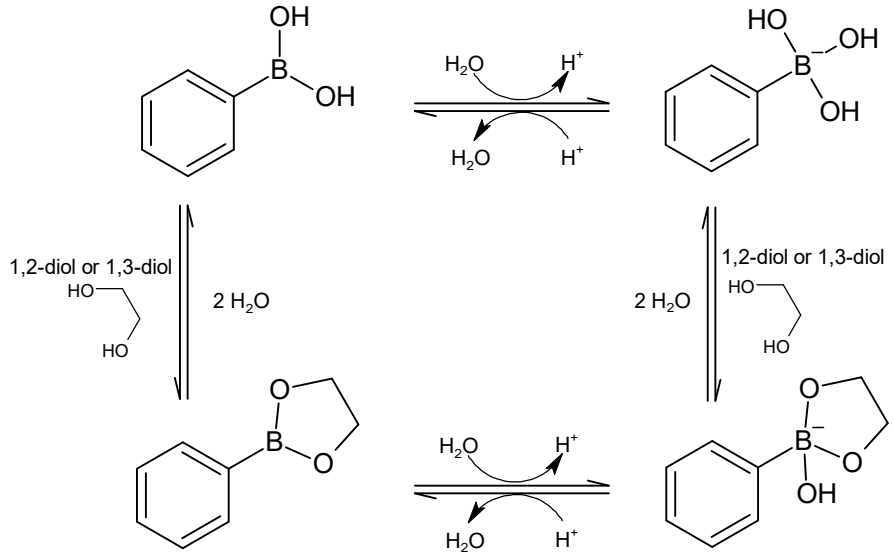


Figure 6: Equilibrium of PBA in the presence of 1,2-diols or 1,3-diols.

Polymers made of PBA in a neutral form tend to be hydrophobic. On the other hand, polymers containing anionic form of PBA are soluble in water. Therefore, upon the addition of diols, increase of charged PBA species can be observed. This represents the main mechanism of sugar-responsive behavior. Solubility of PBA containing polymers is thus influenced by both the pH and the diol concentration.[45] The presence of charged PBA is necessary for the formation of stable charged esters. This form is favored at pH higher than pK_a of PBA (pK_a = 8.9).[46] The pK_a of formed boronate ester is noticeably lower than that of a free PBA. This is most likely caused by the change in the electronic structure after substituting the hydrogen atoms in the PBA by a cyclic ester. A reduced structural flexibility of the boronic ester also plays a small part.[47]

The pK_a of PBA can be tuned by substituents on the aromatic ring. The main aim is to lower the pK_a (increase acidity) to get closer to physiological pH (7.4), making PBA decorated (co)polymers available for applications like drug delivery.[48] This can be accomplished by incorporating electron withdrawing groups (e.g. nitro, halogen) on the aromatic ring (pK_a = 7.1 for 3-nitrophenylboronic acid, pK_a = 6.7 for 2,4,5-trifluorophenylboronic acid). [49]

The properties of 2-substituated PBA vary when compared to other isomers. They are affected by steric effects and bonds formed between substituent and boronic group.[50] The equilibrium between planar trigonal and tetragonal form of PBA results in a large proximity change for substituents at the 2- position on the aromatic ring of the PBA.[44] It has been shown that the functionalization of the aromatic ring with just methyl group in 2- position, increases the pK_a to the value of 9.7.[51] This is caused by the steric hindrance during the formation of tetragonal form of the PBA.

2. 3. 1 Acrylamidophenylboronic acid

Acrylamidophenylboronic acid (APBA) is the most widely used monomer containing PBA being available in 2-, 3- and 4- form.[44] The pK_a values determined in solution with ^{11}B NMR spectroscopy were shown to be 10.48, 8.87 and 8.93 for 2-APBA, 3-APBA and 4-APBA respectively (Figure 7).[38]

Intramolecular coordination between the electron deficient boron and an electron rich carbonyl oxygen in 2- APBA is responsible for the PBA to be in its tetragonal form. This is

believed to be true throughout a wide pH range, including physiological pH.[39] The bond between boron and carbonyl oxygen stabilizes the formed ester which can therefore be formed at a lower pH. The internal coordination has been shown to help stabilize cross-links in hydrogels formed at neutral and acidic pH.[40]

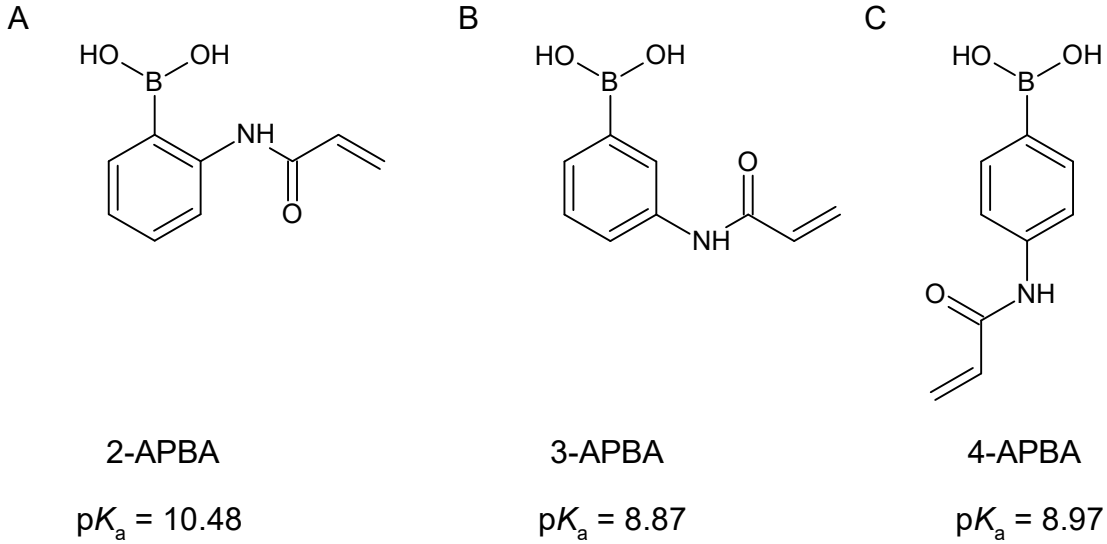


Figure 7: The structures of 2-acrylamidophenylboronic acid (A), 3-acrylamidophenylboronic acid (B) and 4-acrylamidophenylboronic acid (C) with their respective pK_a *values.*[38]

The thermoresponsive and glucose sensitive copolymer of NIPAM and 3-APBA has been reported to exhibit an increase in T_{cp} upon the addition of glucose accompanied by an increase of number of charged PBA species.[52] However, the copolymer of NIPAM and 2-APBA displayed the opposite trend and the addition of glucose resulted in a lower T_{cp}.[53] Given that the PBA group in 2-APBA is dominantly in its negatively charged tetragonal form, significant change in the ionization degree is not observed upon the glucose addition. The observed decrease in T_{cp} can be explained by glucose acting as an additive. Additives change the quality of solvent (typically water) and therefore affect the interactions between polymer chains and water [15] resulting in shifted T_{cp} as shown for salt, surfactants[54] and saccharides.[55]

2. 3. 2 (Co)polymerization of PBA containing monomers

Formerly, nearly all PBA containing (co)polymers discussed in the literature were prepared via free radical (co)polymerization (FRP).[38,40,52,56,57] Using this mechanism,

polymeric gels with glucose responsivity were easily prepared. However, FRP doesn't provide much control over polymerization resulting in broad dispersities and polymer crosslinking. This has been changed with the utilization of controlled polymerization techniques.

RAFT is the most used technique for polymerization of protected monomers. It has been reported that unprotected monomers containing PBA can be directly polymerized using RAFT polymerization.[58] Unfortunately, polymeric PBAs are hygroscopic, leading to difficulties in their handling and characterization. Thus, a polymerization of protected monomers (in the form of boronate ester) with their subsequent deprotection is usually a preferred approach.[45] The usage of protected monomers limits the influence of the boronic acid group on the mechanism of polymerization. Moreover, it also stops the influence of the boronic acid on kinetics and increases the solubility of both monomer and polymer in organic solvents.[44]

Pinacol ester is the most used form of protection. The ester can be formed in anhydrous organic solvents by removing water from mixture with activated molecular sieves.[45] The deprotection can be carried out by hydrolysis in basic water with pH higher than pK_a of used PBA containing monomer. Dialysis can be used in the next step to remove the resulting diol.[45] Another way of deprotection is a transesterification process using a resin functionalized with a boronic acid.[59]

3 AIMS OF THESIS

The primary aim of this thesis is to synthesize stimuli-responsive copolymers via RAFT polymerization. This was planned to achieve by following steps:

1. Synthesis of appropriated monomers and chain transfer agents (CTAs)
2. Synthesis of homopolymers and copolymers
3. Characterization of the synthesized (co)polymers through NMR and GPC
4. Evaluation of thermoresponsive properties (T_{cp} values) of the synthesized (co)polymers

4 MATERIALS AND METHODS

4. 1 Experimental methods

4. 1. 1 Nuclear magnetic resonance (NMR)

^{1}H NMR spectra were recorded on a Bruker 400 MHz or Bruker Avance Neo 400 MHz spectrometer at room temperature in $CDCl_3$. For solid samples, approximately 5 mg of sample was dissolved in 0.7 ml of $CDCl_3$ directly into the NMR tube. For liquid samples, few drops were put into NMR tube and dissolved in 0.7 ml of $CDCl_3$. The chemical shifts were calibrated by using the residual resonance of $CDCl_3$.

4. 1. 2 Gel permeation chromatography (GPC)

Gel permeation chromatography was used for the determination of number-averaged molecular weight (M_n) and dispersity ($Đ$) of the prepared (co)polymers. GPC was performed at 55 °C in *N*,*N*-dimethylacetamide (DMA) containing 50 mM of LiCl at an elution rate of 0.5 ml/min. GPC was carried out using OMNISEC RESOLVE system (degasser, pump, autosampler and column oven) in combination 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. Molar masses and dispersities were calculated against poly(methyl methacrylate) standards using data obtained from refractive index detector (RI). The samples were prepared by dissolving 5 mg of (co)polymers in 1 ml of GPC solvent and (co)polymer solutions were pushed through a syringe filter (0.45 μm).

GPC analysis of copolymers containing 2-APBAE was performed in chloroform. An assembly consisting of an RI detector, S 1125G Quaternary Gradient HPLC Pump (Sykam) and two columns, PLgel 5 mm 100 Å and DeltaGel Mixed-B, connected in series was used for SEC analysis. A mixed mobile phase ($CHCl_3$:TEA:IPA 94:4:2) with a flow rate of 1 ml/min was used. The calibration required for the calculations was made using polymethyl methacrylate standards.

4. 1. 3 T_{cp} values measurements

T_{cp} values of prepared (co)polymers were measured by using a Fluorolog-QM (Horiba, Canada). All measurements were conducted in 1 cm quartz glass cuvettes equipped with a magnetic stirrer. The spectra were measured at excitation and emission wavelength 600 nm. Both excitation and emission slit were set to 0.5 nm. The samples were prepared by dissolving between 2.5 mg and 3.0 mg of (co)polymer in a corresponding amount of distilled water to get a 1 mg/ml solution. 0.1 mg/ml samples were prepared by diluting 1 mg/ml samples. T_{cp} values were calculated from measured spectra. Measured intensity values were normalized, steep part was fitted with linear function. T_{cp} was determined as intersection between linear fit and initial intensity (equal to zero).

4. 2 Materials

Following chemicals were purchased from commercial suppliers and used as received unless stated otherwise:

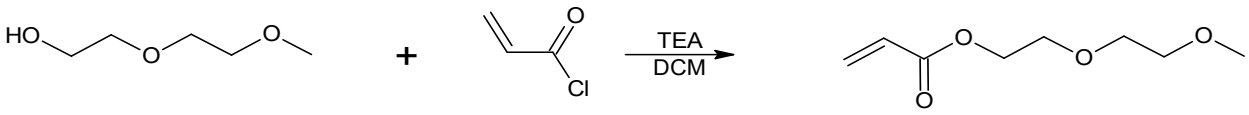
1,4-dioxane (Lach-Ner, ≥99%), 2-aminophenylboronic acid pinacol ester (Sigma-Aldrich, ≥95°%), 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (CTA 2) (Sigma-Aldrich, 98%), 3-mercapro propionic acid (Sigma-Aldrich, ≥99%), acetone (Lach-Ner, ≥99%), acryloyl chloride (Sigma-Aldrich, ≥97%), AIBN (Sigma-Aldrich, 98%, recrystallized from ethanol prior to use), aluminum oxide (activated, basic, Brockmann I) (Sigma-Aldrich), benzyl bromide (Sigma-Aldrich, reagent grade, 98%), carbon disulfide (Sigma-Aldrich, ≥99%, anhydrous), chloroform-d (Sigma-Aldrich, 99.8 atom% D), cyanomethyl dodecyl trithiocarbonates (CTA 1) (Sigma-Aldrich, 98%), DCM (Lach-Ner, ≥99%), di(ethylene)glycol monomethyl ether (Sigma-Aldrich, for synthesis), DMSO (Penta, ≥99%), DMSO anhydrous (Sigma-Aldrich, ≥99.9%), hexane pure (Lach-Ner), hydrochloric acid (35%, Lach-Ner), hydroquinone (Sigma-Aldrich, ReagentPlus®, ≥99%), magnesium sulfate anhydrous (Lach-Ner), potassium phosphate tribasic (Sigma-Aldrich, reagent grade, ≥98°%), sodium chloride (Lach-Ner), sodium hydrogen carbonate (Lach-Ner), sodium hydroxide (Penta, ≥98%), sodium sulfate anhydrous (Lach-Ner), TEA (Sigma-Aldrich, ≥99%), THF (Lach-Ner, ≥99%), toluene (Lach-Ner, ≥99%), tri(ethylene)glycol monomethyl ether (Sigma-Aldrich, for synthesis).

3-acrylamidophenylbornic acid pinacol ester (3-APBAE) was kindly provided by our colleague, Mgr. Martin Orságh.

The rest of used monomers and CTAs was synthesized following procedures found in the literature. Synthesis of these species is described in the following chapters.

4. 2. 1 Synthesis of DEGMA

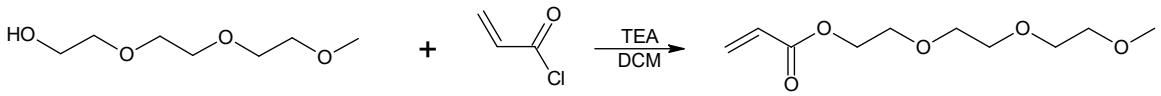
DEGMA was prepared by procedure similar to one previously described.[33] Di(ethylene glycol) monomethyl ether (17.96 g, 0.149 mol), TEA (17.96 g, 0.177 mol) and DCM (140 ml) were added to Schlenk flask placed in ice bath. Acryloyl chloride (16.07 g, 0.178 mol) was mixed with DCM (30 ml) in another flask. Both flasks were stirred. Acryloyl chloride solution was added to Schlenk flask dropwise via cannula. The ice bath was removed. Mixture was left to stir overnight at room temperature. The precipitate was filtered into separation funnel. The solution was treated with saturated $NaHCO_3$ (100 ml). Two layers were formed. Organic layer was washed with water (200 ml), saturated NaCl (100 ml) and water (100 ml) in this sequence. The aqueous layer was washed with DCM (2 × 50 ml). Organic extracts were combined with organic layer and dried with anhydrous Na_2SO_4 (for approximately 90 minutes). Na_2SO_4 was removed by filtration via filter paper. Solvents were removed with rotavapor. A small amount of hydroquinone was added for stabilization. Product was purified via vacuum distillation (55 °C / 26 mbar) affording a colorless liquid (17.46 g, 67 % yield). The synthesis of DEGMA is depicted in Scheme 1.



Scheme 1: Synthesis of DEGMA.

4. 2. 2 Synthesis of TEGMA

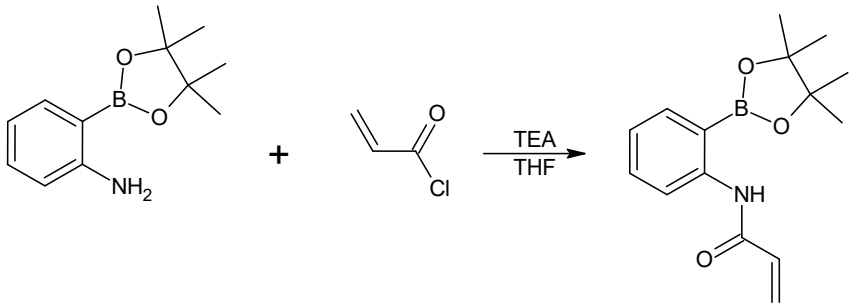
TEGMA was synthesized by similar procedure to synthesis of DEGMA. Tri(ethylene glycol) monomethyl ether (24.4 g, 0.149 mol), DCM (140 + 30 ml), TEA (17.87 g, 0.177 mol) and acryloyl chloride (15.93 g, 0.176 mol) were used for the synthesis. The product was purified with vacuum distillation (124 °C / 33 mbar) affording a colorless liquid (19.74 g, 61 % yield). The synthesis is of TEGMA is depicted in Scheme 2.



Scheme 2: Synthesis of TEGMA.

4. 2. 3 Synthesis of 2-acrylamidophenylboronic acid pinacol ester (2-APBAE)

The procedure was modified from one previously described in literature.[40] 2-aminophenylboronic acid pinacol ester (2.00 g, 9.13 mmol) was dissolved in round bottom flask with THF (60 ml) and TEA (1.39 ml). The solution was chilled in ice bath. Slight excess of acryloyl chloride (0.8 ml) was mixed with THF (20 ml). Solution was added dropwise via cannula using small nitrogen overpressure to the mixture in round bottom flask. Ice bath was removed and the mixture was left to stir for 24 hours. The mixture was filtered, solvent was removed with rotavapor. Product was twice recrystallized from hot toluene to yield a light purple solid (1.16 g, 47 % yield). The synthesis of 2-APBAE is depicted in Scheme 3.

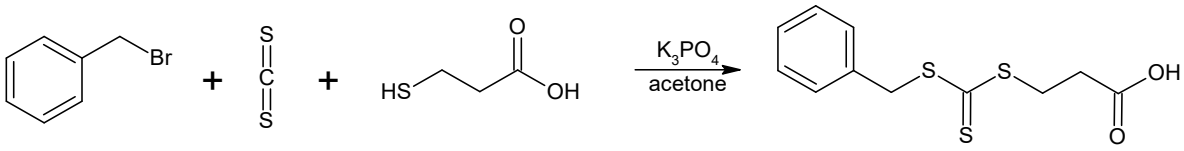


Scheme 3: Synthesis of 2-APBAE.

4. 2. 4 Synthesis of 3-(benzylthiocarbonylthioylthio)propanoic acid (CTA 3)

The procedure was modified from one previously described in literature.[60] Acetone (20 ml), K_3PO_4 (2.00 g, 9.42 mmol) and 3-mercapto propionic acid (0.82 ml, 9.41 mmol) were added into a Schlenk flask and stirred for ten minutes. Next, CS_2 (1.7 ml, 28.27 mmol) was added and resulting solution turned into bright yellow. After stirring for another ten minutes, benzyl bromide (1.1 ml, 9.25 mmol) was added. The solution was thickened under vacuum after another ten minutes of stirring. Residue was mixed with saturated NaCl (100 ml), moved into a separation funnel and extracted with DCM (2 × 100 ml). The DCM layer was then washed with saturated NaCl (3 × 100 ml). Organic extracts were dried over anhydrous $MgSO_4$ (for approximately 2 hours). $MgSO_4$ was removed by filtration via filter paper. Solvent was removed with rotavapor. Product was further dried under vacuum

affording a canary yellow crystalline solid (1.75 g, 68 % yield). The synthesis of CTA 3 is depicted in Scheme 4.



Scheme 4: Synthesis of CTA 3.

4. 3 (Co)polymerization processes

All (co)polymers were synthesized via RAFT polymerization. Three different CTAs were tested (Figure 8) and AIBN was used as a thermal initiator (Figure 3).

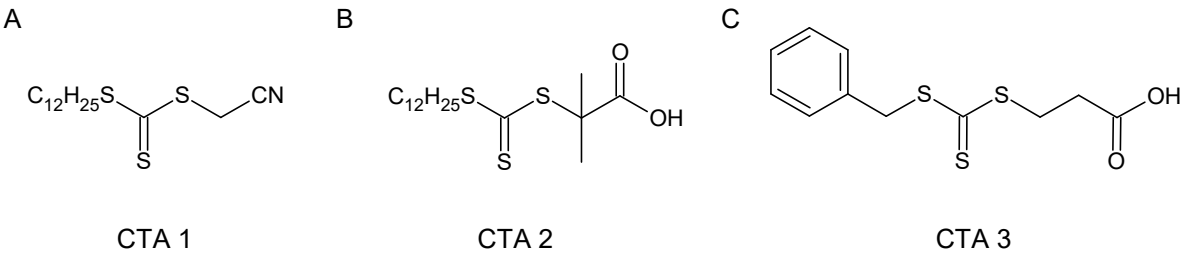


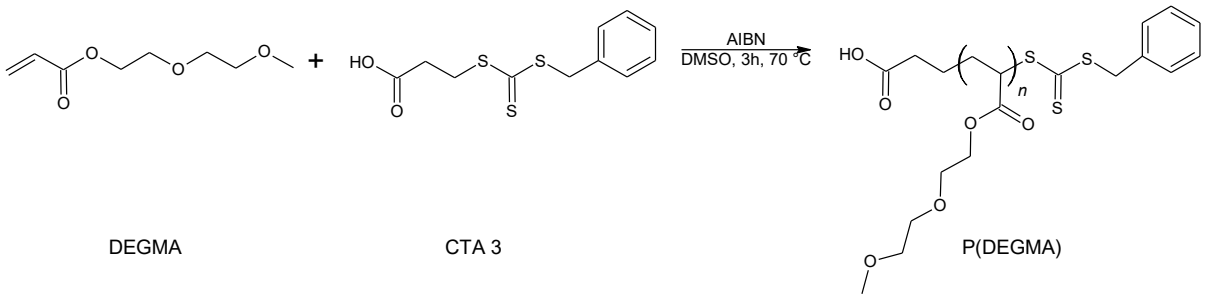
Figure 8: The structures of cyanomethyl dodecyl trithiocarbonate (A), 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (B) and 3-(benzylthiocarbonylthioylthio)propanoic acid (C).

4. 3. 1 Homopolymerization process

The example of typical homopolymerization process is as follows (Scheme 5):

DEGMA (216.7 mg, 1.50 mmol) was pushed through a small column of basic activated Al_2O_3 to remove hydroquinone and directly collected into a reaction vial designed for microwave synthesis (Biotage). Column was rinsed with DMSO (1.3 ml) and pushed through with nitrogen. CTA 3 (5.0 mg, 0.018 mmol) and AIBN solution in DMSO (0.27 ml, c = 1 mg/ml) were added to the vial. The concentration of DEGMA in the reaction mixture was 11 % wt. The vial was sealed with septum cap and purged with nitrogen for 15 minutes and then placed into heating block preheated at 70 °C. After 3 hours, the polymerization was terminated by placing the flask into an ice bath and opening it to the air. The resulting yellow viscous liquid was diluted with acetone and twice precipitated into hexane. The resulting polymer was dissolved in acetone and transferred into a vial. The polymer was dried *in vacuo*

overnight to yield yellow viscous oil (215.3 mg, 82 % yield). The homopolymerization of TEGMA followed the analogous procedure as described above for DEGMA, using the same TEGMA concentration in the reaction mixture.



Scheme 5: Synthesis of P(DEGMA) in DMSO using CTA 3.

4. 3. 2 Copolymerization process

The example of typical copolymerization process is as follows:

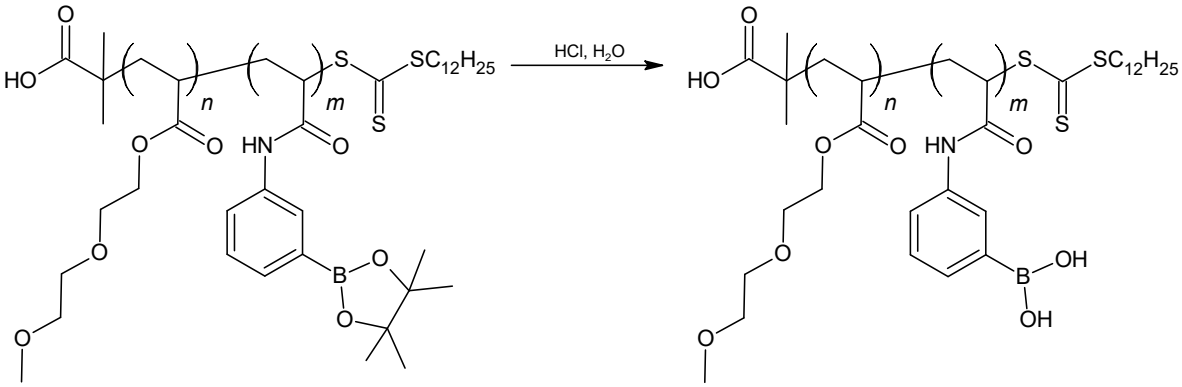
DEGMA (222.0 mg, 1.27 mmol) was pushed through a small column of basic activated Al_2O_3 to remove hydroquinone and directly collected into a reaction vial designed for microwave synthesis (Biotage). Column was rinsed with DMSO (1.1 ml) and pushed through with nitrogen. 3-APBAE (50.7 mg, 0.19 mmol), CTA 2 (5.6 mg, 0.015 mmol) and AIBN solution in DMSO (0.23 ml, c = 1 mg/ml) were added to the vial. The vial was sealed with septum cap and purged with nitrogen for 15 minutes and then placed into heating block preheated at 70 °C. After 3 hours, the polymerization was terminated by placing the flask into an ice bath and opening it to the air. The resulting yellow viscous liquid was diluted with acetone and twice precipitated into hexane. The resulting polymer was dissolved in acetone and transferred into a vial. The polymer was dried *in vacuo* overnight to yield yellow viscous oil (264.7 mg, 97 % yield).

4. 3. 3 Dialysis of copolymers

The copolymer was dissolved in 5 ml of acetone. The solution was dialyzed (SpectraPor® dialysis membrane, MWCO = 3.5 kDa) against acetone. The dialysis was left to run for 24 hours. The acetone bath was changed four times. The copolymer solution was moved back into a vial, acetone was left to evaporate, and the purified copolymer was further dried *in vacuo*.

4. 3. 4 Deprotection of copolymer

Copolymer (24.8 mg) was dissolved in 5 ml of acetone. Distilled water (5 ml) was added under constant stirring. The solution was dialyzed (SpectraPor® dialysis membrane, MWCO = 3.5 kDa) against 0.3% hydrochloric acid solution in distilled water. The dialysis was left to run for 31 hours, changing the acid bath twice. The solution was then dialyzed against distilled water for 46 hours, changing the bath three times. The pH of the water was checked with indicator paper until neutral. The solution was transferred into a vial and its volume was measured. The solution was diluted to get 1 mg/ml concentration for T_{cp} values measurements. The scheme of deprotection is depicted in Scheme 6.



Scheme 6: Deprotection of copolymer composed of DEGMA and 3-APBAE.

5 RESULTS AND DISCUSSION

5. 1 Targeted monomers

DEGMA and TEGMA are a thermoresponsive OEG acrylates. Average T_{cp} values of their corresponding homopolymers have been reported as 40 °C and 70 °C for P(DEGMA) and P(TEGMA) respectively (Figure 5).[26] The T_{cp} value of P(DEGMA) is in the range of physiologically interesting temperature.[28] Both P(DEGMA) and P(TEGMA) combine the biocompatibility of poly(ethylene glycol) and flexible and easily controlled LCST behavior.[26] DEGMA and TEGMA also ensure the solubility of the resulting copolymer in water when hydrophobic 2-/3-APBAE is integrated as comonomer.

APBA is the most used PBA containing monomer.[44] 2-/3-APBA are responsive towards pH and the presence of 1,2- or 1,3-diols. Because of the intramolecular interaction between boron and carbonyl oxygen, 2-APBA is present in its tetragonal charged form in wide pH range.[39] In contrast, 3-APBA is mostly in trigonal uncharged form around neutral pH.[44] Given the fact that polymeric PBAs are hygroscopic and therefore difficult to characterize, monomers protected with pinacol ester (2-/3-APBAE) were used in the (co)polymerizations.[45]

5. 2 Synthesis of (co)polymers

5. 2. 1 Synthesis of P(DEGMA)

The synthesis of homopolymer P(DEGMA) was studied using three different CTAs (Figure 8) in combination with AIBN as thermal initiator. All used CTAs are trithiocarbonates which are considered to be a good choice for the polymerization of MAMs monomers[6] and have been previously used for acrylate (co)polymerizations.[61–63] Three solvents of different polarity (toluene, 1,4-dioxane and DMSO) were tested as the polarity of reaction medium is known to lower the activation energy.[11] All carried out experiments along with the properties of resulting polymers are summarized in Table 1.

Table 1: Synthesis of homopolymers P(DEGMA).

entry	solvent	CTA	[M]/[CTA]/[I][a]	time [h]/ T [°C]	conv.[b] [%]	M_n GPC[c] [kg·mol^{-1}]	$Đ$[c]	M_n theo[d] [kg·mol^{-1}]
1	toluene	**1**	31/1/0.2	3/70	87	2.8	1.58	5.0
2	dioxane	**1**	50/1/0.1	3/70	81	4.2	1.49	7.4
3		**2**	90/1/0.1	3/70	79	9.1	1.27	12.8
4	DMSO	**2**	90/1/0.1	3/70	89	10.5	1.23	14.3
5		**2**	90/1/0.1	4/60	61	5.7	1.31	9.9
6		**3**	90/1/0.1	3/70	72	7.9	1.27	11.6
7	DMSO anhydrous	**2**	90/1/0.1	3/70	82	10.4	1.19	13.2

[a]molar ratio of monomer (M)/chain transfer agent (CTA)/initiator (I), [b]determined by ^{1}H NMR; [c]determined by GPC with RI detector, [d]theoretical molecular weight calculated using stoichiometry and conversion ($M_n^{theo} = \frac{[M]\cdot M_M \cdot conversion_M}{[CTA]} + M_{CTA}$)

The polymerization process was adapted from the procedure previously described in the literature.[28] However, toluene was used instead of benzene as the less toxic solvent. The polymerization was left to run for 3 hours at 70 °C with the ratio of monomer/CTA/initiator 31/1/0.2 (entry 1, Table 1). A high monomer conversion (87 %) was achieved, however the resulting polymer displayed a relatively broad and trimodal molar mass distribution ($Đ$ = 1.58). For the following experiment (entry 2), the monomer/CTA 1 ratio was adjusted to 50/1 and CTA 1/initiator ratio to 1/0.1 to obtain better polymerization control (lower dispersity). More polar solvent, 1,4-dioxane, was tried, which resulted in a bimodal molar mass distribution ($Đ$ = 1.49).

For the following experiments, the monomer/CTA/ ratio was adjusted to 90/1 (target molecular weight 15.0 kg·mol^{-1}) and CTA/initiator ratio was kept at 1/0.1. CTA 2 bearing carboxyl group (Figure 8) was therefore employed. In combination with 1,4-dioxane, 79 % monomer conversion was achieved, however the resulting polymer displayed a small low molar mass shoulder ($Đ$ = 1.27, entry 3) (green curve, Figure 9).

Finally, DMSO was used as a solvent with the highest polarity. The combination of CTA 2 and DMSO showed promising results thanks to high conversion (89 %) and acceptable dispersity with no shoulder ($Đ$ = 1.23, entry 4) (red curve, Figure 9). The effect of time and temperature has also been investigated. The homopolymerization was performed at 60 °C and left to run for 4 hours (entry 5). This resulted in a lower conversion (61 % vs.

89 %) and broader dispersity (1.31 vs. 1.23) comparing to our standard conditions (3 hours, 70 °C). For CTA 2, the homopolymerization was also carried out in anhydrous DMSO (entry 7). The obtained polymer showed a slightly lower conversion (82 % vs. 89%) and dispersity (1.19 vs. 1.23) compared to the use of standard DMSO. Given these comparable results and easier manipulation with standard DMSO, all other (co)polymerizations were carried out in standard DMSO. The use of CTA 3 (Figure 8) (entry 6) showed a lower conversion (72 % vs. 89 %) and slightly higher dispersity ($Đ$ = 1.27 vs. 1.23) in comparison with the homopolymer synthesized with CTA 2. This is probably caused by the structure of leaving/initiating group R which is in form of more stable tertiary radical in CTA 2 (Figure 8, Figure 1).

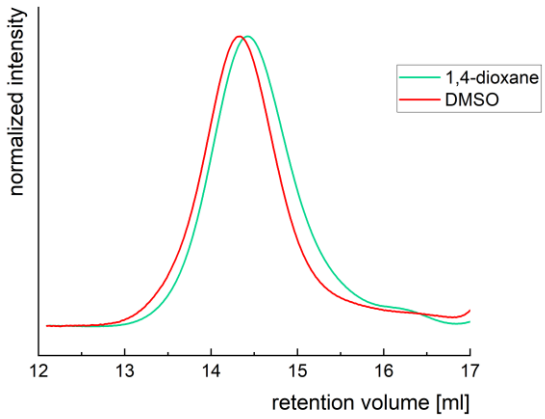


Figure 9: GPC chromatograms of P(DEGMA) homopolymers prepared with CTA 2 in 1,4-dioxane (entry 3, green) and in DMSO (entry 4, red).

The utilization of CTA 2 in DMSO showed the most promising results in terms of molar mass and its distribution, the corresponding homopolymer (entry 4, Table 1) was used for T_{cp} values measurement (5. 3. 1).

5. 2. 2 Synthesis of P(TEGMA)

Based on our previous results of DEGMA polymerization, P(TEGMA) was synthesized using CTA 2 and 3 in DMSO and the ratio of monomer/CTA/initiator was fixed at 90/1/0.1. All carried out experiments along with the resulting polymer properties are summarized in Table 2.

Table 2: Synthesis of homopolymers P(TEGMA).

entry	solvent	CTA	[M]/[CTA]/[I][a]	time [h]/ T [°C]	conv.[b] [%]	M_n [GPC c] [kg·mol^{-1}]	$Đ$ [c]	M_n [theo d] [kg·mol^{-1}]
8		**2**	90/1/0.1	3/70	69	5.2	1.18	13.9
9	DMSO	**2**	90/1/0.1	4/60	38	3.4	1.27	7.8
10		**3**	90/1/0.1	3/70	35	3.4	1.64	7.1

[a]molar ratio of monomer (M)/chain transfer agent (CTA)/initiator (I), [b]determined by ^{1}H NMR; [c]determined by GPC with RI detector, [d]theoretical molecular weight calculated using stoichiometry and conversion ($M_n^{theo} = \frac{[M]\cdot M_M \cdot conversion_M}{[CTA]} + M_{CTA}$)

The use of CTA 2 in DMSO at our standard conditions (3 hours, 70 °C) resulted in a reasonable conversion (69 %) and low dispersity ($Đ$ = 1.18, entry 8). Lowering the temperature from 70 °C to 60 °c while simultaneously prolonging polymerization time from 3 to 4 fours (entry 9), resulted in a lower conversion (38 % vs. 69 %) and broader dispersity ($Đ$ = 1.27 vs. 1.18). Finally, the use of CTA 3 (entry 10, Table 2) resulted in significantly lower conversion (35 % vs. 69 %) and higher dispersity ($Đ$ = 1.64 vs. 1.18) in comparison to polymer synthesized with CTA 2.

The conversions of TEGMA are consistently lower compared to analogous P(DEGMA) experiments (Table 1). This could be explained by the fact that TEGMA has an extra OEG unit in the side chain, thus making the polymerization of bulkier TEGMA monomer slower. This was more pronounced in the case of CTA 3 (entry 10, Table 2) where is the simultaneous effect of less reactive monomer in combination with less active CTA 3, as discussed previously (5. 2. 1).

Analogously to P(DEGMA), the combination of CTA 2 and DMSO showed the most promising results and the homopolymer (entry 8,Table 2) was therefore used for T_{cp} values measurement (5. 3. 1).

5. 2. 3 Synthesis of copolymers

Following the homopolymerization results, copolymerizations of 2-/3-APBAE with DEGMA or TEGMA were carried out in the same manner, using CTA 2 and 3 in DMSO as a reaction medium. All copolymerizations were prepared in a way that the molar fraction of 2-/3-APBAE in the reaction feed was approximately 15 %. Copolymerization experiments

were left to run for 3 hours at 70 °C with the molar ratio of acrylate monomer/APBAE monomer/CTA/initiator equal to 90/13.5/1/0.1.

Copolymers containing 2-APBAE were hardly soluble in common solvents, except of chloroform, and displayed broad distribution of molar masses ($Đ \approx 2.50$). Thus, we decided to focus on copolymerizations with 3-APBAE comonomer (Table 3), which has been previously used in controlled radical polymerizations. [45,48]

Table 3: Synthesis of copolymers between 3-APBAE (13 mol%) and OEG acrylates DEGMA or TEGMA in DMSO within 3 hours at 70 °C using acrylate monomer/ 3-APBAE/CTA/initiator ratio of 90/13.5/1/0.1.

entry	copolymer	CTA	acrylate conv. [a] [%]	APBAE conv. [a] [%]	x_{APBAE} [a] [%]	M_n GPC [c] [kg·mol^{-1}]	$Đ$ [c]	M_n theo [d] [kg·mol^{-1}]
11	3/D	**2**	87	87	11 (13[b])	17.6	1.48	17.2
12		**3**	83	79	17 (14[b])	18.9	1.28	16.2
13	3/T	**2**	63	75	23 (15[b])	8.6	1.23	15.5
14		**3**	31	55	24 (21[b])	8.3	1.39	8.4

[a]determined by ^{1}H NMR, [b]determined by ^{1}H NMR using monomer conversion, [c]determined by GPC with RI detector for dialyzed copolymers, [d]calculated using stoichiometry and conversion($M_n^{theo} = 90 \cdot M_{acrylate} \cdot conversion_{acrylate} + 13.5 \cdot M_{APBAE} \cdot conversion_{APBAE} + M_{CTA}$)

The copolymer of 3-APBAE and DEGMA (3/D) prepared by CTA 2 showed higher conversions of both monomers but broader dispersity ($Đ$ = 1.48 vs. 1.28) compared to the same copolymer obtained with CTA 3 (entry 11 and 12). In the case of 3-APBAE/TEGMA copolymers (3/T), the same trend, regarding conversions and dispersities, was observed when comparing CTA 2 and CTA 3 (entry 13 and 14).

When comparing the conversions of both acrylate monomers within copolymerization, lower conversions corresponded to TEGMA monomer, as observed for acrylates homopolymerization (Table 1 and Table 2). Moreover, the lower reactivity of bulkier TEGMA led to the lower conversions of 3-APBAE comonomer in comparison with DEGMA.

The molar fraction of 3-APBAE incorporated to the resulting copolymer was determined by ^{1}H NMR using two different methods of calculation: the first one used integrals of characteristic signals in purified copolymers and the second one was based on conversions determined from the quenched polymerization mixture. Both values were in

a good agreement suggesting the accurate determination of 3-APBAE. Furthermore, these data revealed that CTA 3 is slightly better incorporator of acrylamide than CTA 2. Last but not least, the 3-APBAE containing copolymers displayed a different structure from 2-APBAE based (co)polymers. Incorporation of 2-APBAE resulted in rubbery like material which is in contrast with viscous and oily 3-APBAE containing copolymers and both homopolymers.

^{1}H NMR spectrum of 3/D (CTA 2) can be seen in Figure 10 compared to spectra of monomers and P(DEGMA) (CTA 2).

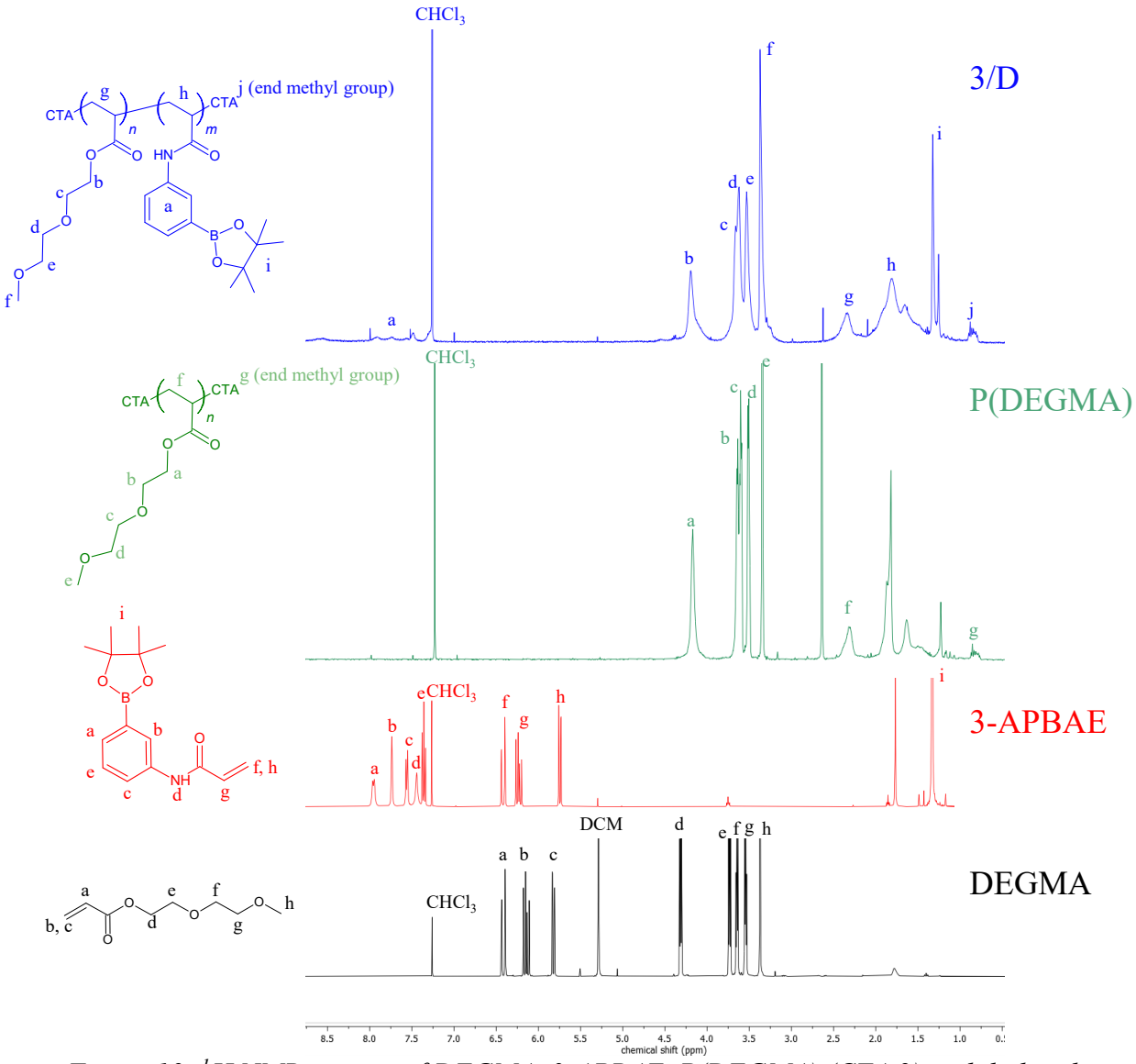


Figure 10: ^{1}H NMR spectra of DEGMA, 3-APBAE, P(DEGMA) (CTA 2) and dialyzed copolymer 3/D (CTA 2).

5. 2. 4 Dialysis of copolymers

All prepared copolymers (Table 3) displayed low molar mass peak (red curves in Figure 11) in their corresponding GPC chromatograms. Obtained copolymers were therefore purified via dialysis against acetone using membrane with MWCO = 3.5 kDa (4. 3. 3). The successful dialysis was confirmed by subsequent GPC analysis (blue curves in Figure 11) demonstrating the disappearance of low molar mass fractions. The disappearance of low molar mass peak (M_n = 2.6 kg·mol^{-1}, $Đ$ = 1.13), from the copolymer 3/D synthesized with CTA 2, can be observed (Figure 11A), however a small increase of refractive index is still visible at the end of the chromatogram of dialyzed copolymer. This was most likely caused by the solvent residue in the copolymer, as the maximum of blue low molar mass peak represents much smaller molar mass value than minor red peak. The loss of a low molar mass shoulder can be clearly observed for 3/D copolymer prepared with CTA 3 (Figure 11B). The same behavior was observed for 3/T copolymers. Measurements of T_{cp} values were performed with dialyzed copolymers summarized in Table 3 (5. 3. 2).

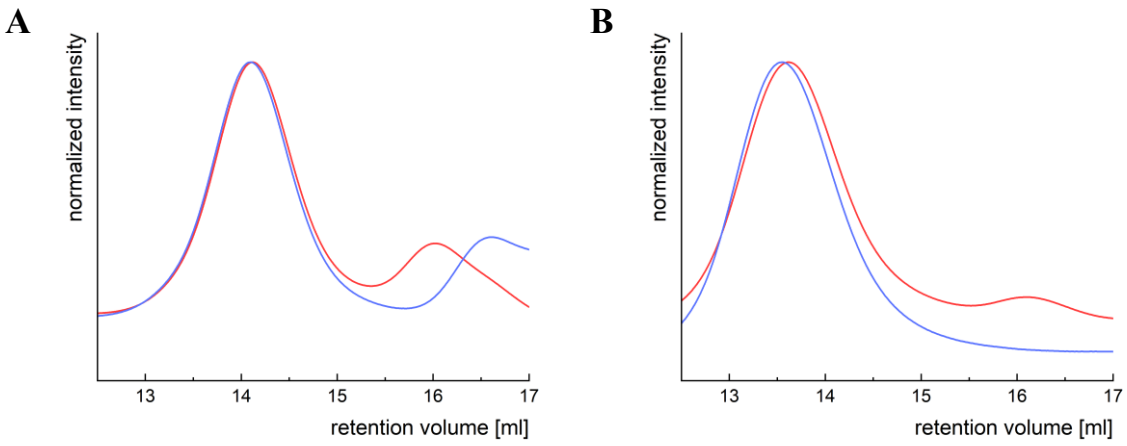


Figure 11: Representative GPC chromatograms of 3/D copolymers synthesized with CTA 2 (A) (entry 11, Table 3) and CTA 3 (B) (entry 12, Table 3). Red curves represent copolymers before dialysis and blue curves after dialysis.

5. 2. 5 Deprotection of copolymer

Successful deprotection with diluted HCl (Scheme 6, 4. 3. 4) of 3/D copolymer synthesized with CTA 2 was confirmed with ^{1}H NMR. The disappearance of pinacol ester peak at 1.32 ppm is nicely displayed in Figure 12. Multiplet at 0.84 ppm that belongs to the methyl end group of CTA 2 (Figure 8) confirmed the presence of CTA 2 the deprotected copolymer.

GPC of the deprotected copolymer was not measured because polymeric boronic acids tend to be very hygroscopic and are therefore challenging to be characterize by GPC.[59] Even copolymers synthesized with unprotected boronic acid monomer require protection by pinacol esterification before GPC measurement.[58] Molar mass of the copolymer is considered to be unchanged as previously assumed for deprotection process conducted in a similar acidic environment.[45]

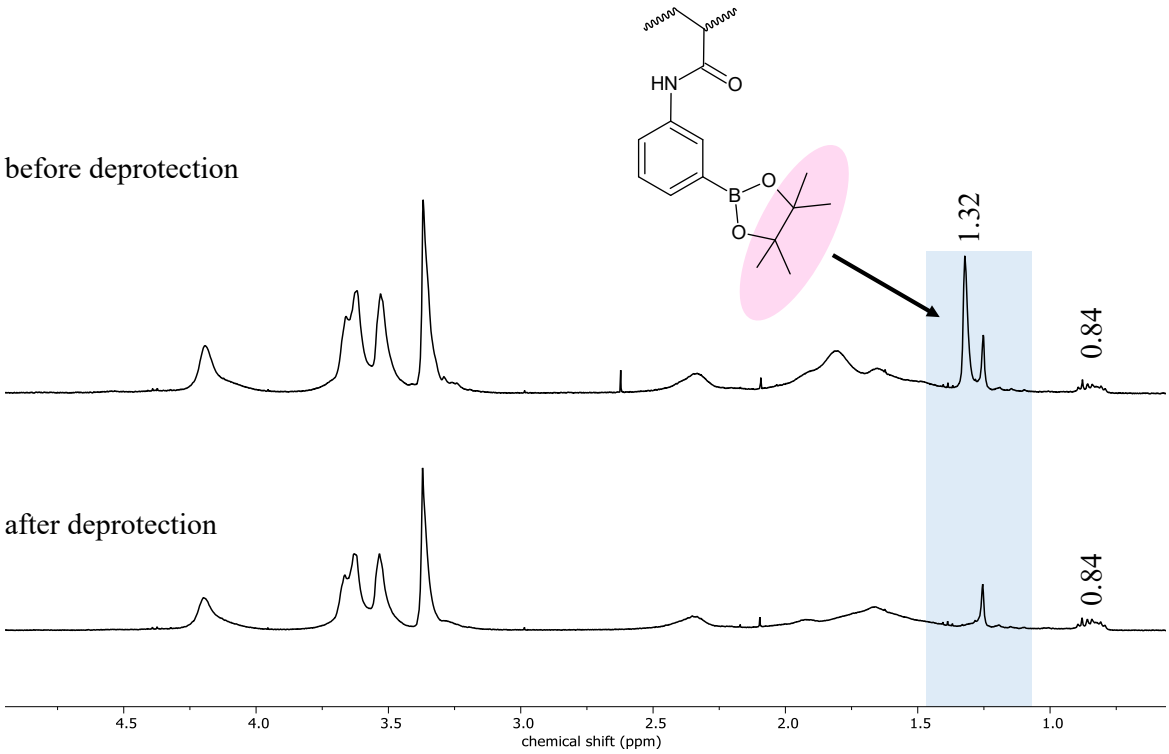


Figure 12: ^{1}H NMR spectra of copolymer before and after deprotection.

5. 3 T_{cp} values measurements

5. 3. 1 T_{cp} values measurements of homopolymers

Both investigated homopolymers were synthesized by typical RAFT polymerization procedure (CTA 2, AIBN as an initiator and DMSO was used as a solvent). Firstly, T_{cp} values were determined for 1 mg/ml aqueous solutions at heating/cooling rate of 1 °C/min. The T_{cp} od P(DEGMA) was calculated to be 49 °C for heating and 45 °C for cooling (Figure 13A). For P(TEGMA) calculated T_{cp} values were 75 °C for heating and 72 °C cooling (Figure 13B). These values are in agreement with T_{cp} ranges previously obtained for these polymers.[26] P(TEGMA) displays higher T_{cp} values compared to P(DEGMA) because of its more hydrophilic character caused by an additional ethylene glycol unit in the P(TEGMA) acrylate side chain.

Later, the polymer solutions were diluted to a 0.1 mg/ml concentration. For P(DEGMA) T_{cp} values exhibited a rise in T_{cp} compared to 1 mg/ml solution. The T_{cp} values were determined as 57 °C for heating and 54 °C for cooling (Figure 13C). A rise, although a smaller one, was also observed for P(TEGMA) with T_{cp} values of 77 °C for heating and 78 °C for cooling (Figure 13D). Based on the literature data, higher T_{cp} values corresponding to lower concentration of polymer solutions were expected.[33] This is explained by the aggregation of the polymer chains, which is facilitated at higher concentrations, resulting in lower T_{cp} values.

Calculated T_{cp} values are summarized in Table 4.

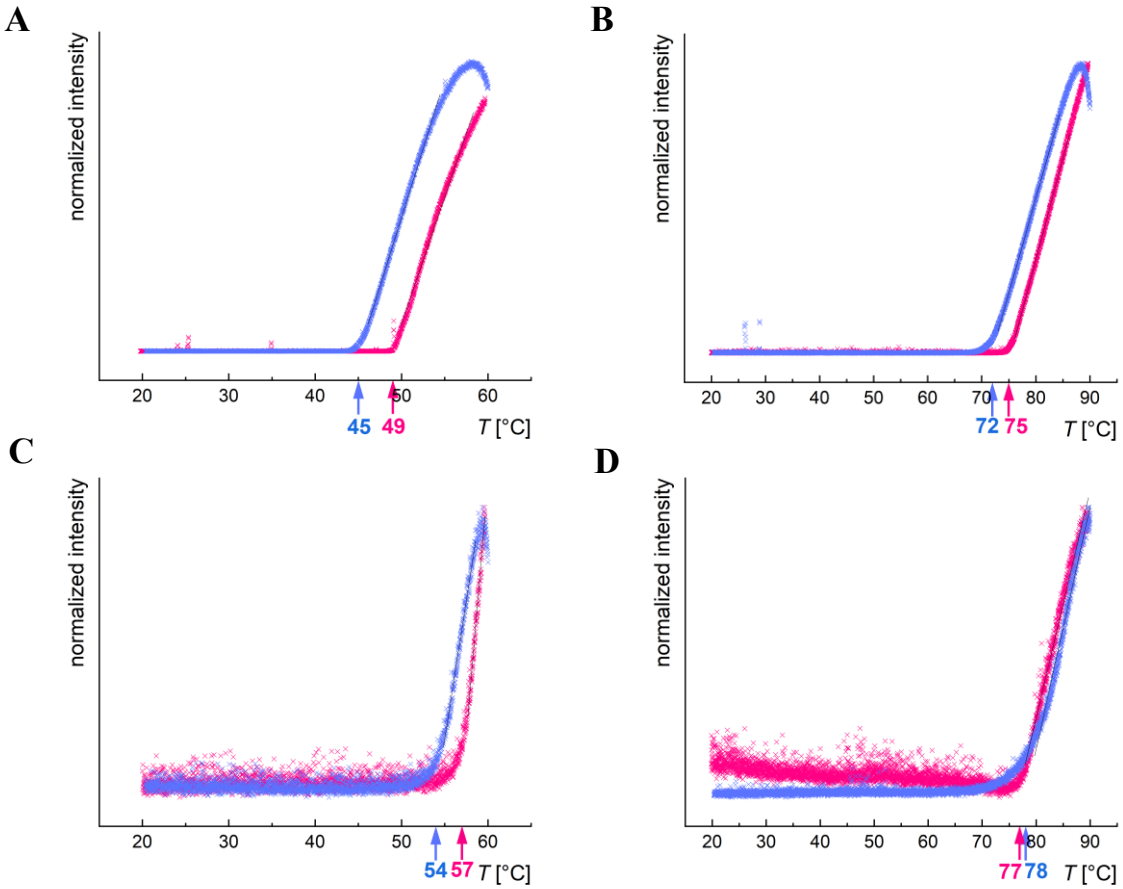


Figure 13: Recorded spectra for 1 mg/ml P(DEGMA) (A), 1 mg/ml P(TEGMA) (B), 0.1 mg/ml P(DEGMA) (C) and 0.1 mg/ml P(TEGMA) (D). Spectra were recorded for both heating (×) and cooling (×) with temperature rate 1 °C/min. Arrows on temperature axis mark calculated Tcp for heating (pink) and cooling (blue).

Table 4: Calculated T_{cp} values for P(DEGMA) and P(TEGMA). All T_{cp} values are in °C.

polymer	M_n [a] [kg·mol^{-1}]	$Đ$ [a]	c [mg/ml]	T_{cp} (heating)	T_{cp} (cooling)
P(DEGMA)	10.5	1.23	1	49	45
			0.1	57	54
P(TEGMA)	5.2	1.18	1	75	72
			0.1	77	78

[a]determined by GPC with RI detector

5. 3. 2 T_{cp} values measurements of copolymers

All measured copolymers were synthesized via RAFT polymerization in DMSO with AIBN as initiator and using CTA 2 and 3 (Figure 8). All measurement were performed with

1 mg/ml polymer solution concentration and the heating/cooling rate of 1 °C/min. Recorded spectra can be seen in Figure 14, all calculated values are summarized in Table 5.

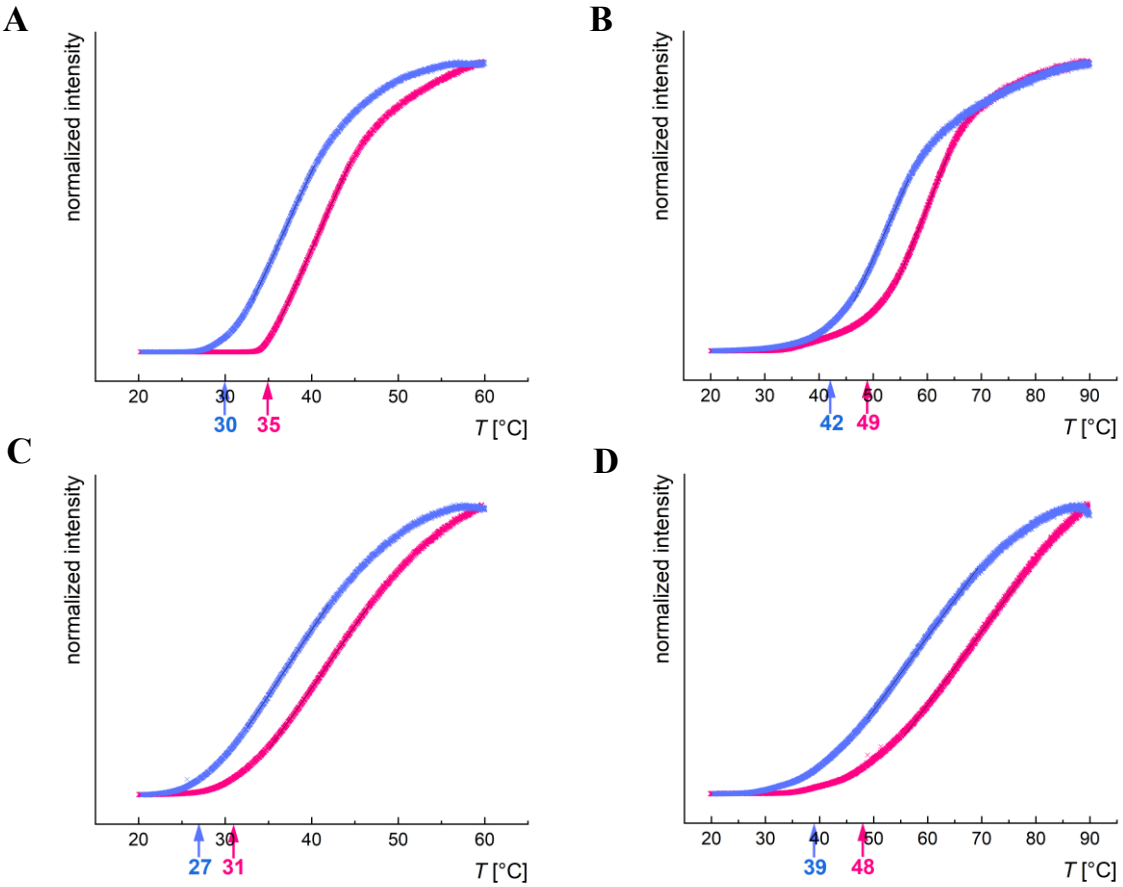


Figure 14: Recorded spectra for 3/D (CTA 2) (A), 3/T (CTA 2) (B), 3/D (CTA 3) (C) and 3/T (CTA 3) (D). Spectra were recorded with 1 mg/ml solutions for both heating (×) and cooling (×) with temperature rate 1 °C/min. Arrows on temperature axis mark calculated T_{cp} for heating (pink) and cooling (blue).

Table 5: Calculated T_{cp} values of copolymers along with their molar masses and 3-APBAE content. All T_{cp} values are in °C.

copolymer	CTA	M_n [a] [kg·mol^{-1}]	$Đ$ [a]	x$_{3\text{-APBAE}}$ [b] [%]	T_{cp} (heating)	T_{cp} (cooling)
3/D	2	17.6	1.48	11	35	30
	3	18.9	1.28	17	31	27
3/T	2	8.6	1.23	23	49	42
	3	8.3	1.39	24	48	39

[a]determined by GPC with RI detector, [b]determined by ^{1}H NMR

All copolymers exhibit lower T_{cp} values in comparison with corresponding P(DEGMA) and P(TEGMA) homopolymers (Table 4). This is caused by the incorporation

of hydrophobic 3-APBAE comonomer into the polymeric chain. When comparing the values of same copolymer synthesized using different CTA, small difference can be observed. This could be the result of different CTA being incorporated to the polymer chain or rather by the different content of 3-APBAE in the copolymer. Both CTAs (Figure 8) are relatively equal regarding polarity, containing carboxyl group at one chain end as well as a hydrophobic group (dodecyl in the case of CTA 2 and benzyl for CTA 3) at the other chain end. Moreover, both types of copolymers reached similar molar mass, regardless the used CTA (Table 5). Thus, higher T_{cp} values clearly correspond to a lower 3-APBAE content.

This is also supported by the fact that higher 3-APBAE content corresponds to more significant drop in T_{cp} values when comparing copolymer to its corresponding OEG acrylate homopolymer. For 3/D (CTA 2) T_{cp} value for heating is 35 °C, for P(DEGMA) (CTA 2) 49 °C, thus the difference is 14 °C. For 3/T (CTA 2) and P(TEGMA) (CTA 2), the difference is 26 °C.

5. 3. 3 T_{cp} values measurements of deprotected copolymer

For the copolymer 3/D synthesized with CTA 2, T_{cp} measurements were also performed after its deprotection (section 4. 3. 4, Scheme 6). T_{cp} values measured for the sample of concentration 1 mg/ml showed no change for heating and only a negligible change (~1.0 °C) for cooling compared to the protected copolymer (Figure 14A, Table 5). The T_{cp} value was found to be 35 °C for heating and 31 °C for cooling (Figure 15A). However, higher T_{cp} value was expected due to the increase of hydrophilic character of 3-APBA after deprotection. This behavior could be explained by preferential formation of hydrogen bonds only between hydroxyl groups of 3-APBA units rather than with water. Another factor could be that deprotected 3-APBA favor its trigonal more hydrophobic form at neutral pH.[44] In contrast, 2-APBA has been shown to prefer its tetragonal charged form due to the intramolecular coordination between the boron and the carbonyl oxygen.[39] This results in higher T_{cp} value of NIPAM and 2-APBA copolymer when compared to neat P(NIPAM).[64]

Few drops of 1 M NaOH aqueous solution were added into the sample to ensure that the PBA in 3-APBA is almost exclusively in its tetragonal charged form. The addition of NaOH solution changes the pH to values higher than pK_a of PBA, ensuring that tetragonal form is largely favored. No significant rises in intensity were observed during the subsequent

measurement (Figure 15B). This could be caused by the polyelectrolyte character of polymer chains, that are highly charged and therefore unable to form high concentration droplets. Thus, phase separation was not observed.

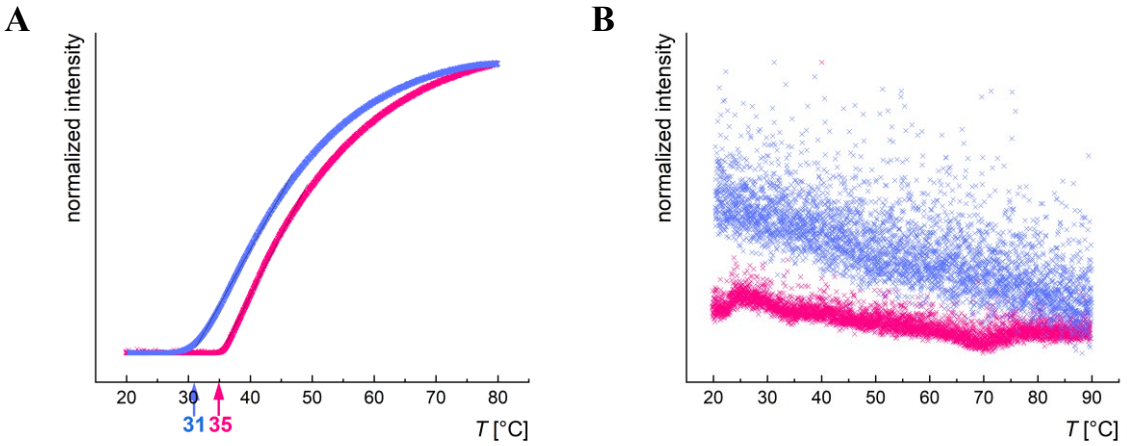


Figure 15:Recorded spectra for 3/D (CTA 2) (A) after deprotection and 3/D (CTA 2) after the addition of 1 M NaOH solution(B). Spectra were recorded with 1 mg/ml solutions for both heating (×) and cooling (×) with temperature rate 1 °C/min. Arrows on temperature axis mark calculated T_{cp} for heating (pink) and cooling (blue).

6 CONCLUSION

Stimuli-responsive (co)polymers have been prepared via RAFT polymerization. Three CTAs and three solvents with different polarity have been investigated for the co(polymerization) processes, using AIBN as a thermal initiator. The (co)polymerization was conducted at 70 °C for 3 hours and obtained (co)polymers were characterized with ^{1}H NMR and GPC. The combination of CTA 2 (2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid) and DMSO showed the best results in terms of monomer conversion and dispersity for both homopolymers P(DEGMA) and P(TEGMA). Based on the homopolymerization results, CTA 2 and DMSO were thus utilized for copolymerization experiments.

2-APBAE containing copolymers displayed broad molar mass distributions and were hardly soluble in common solvents. Thus, we focused on the synthesis of copolymers comprising of 3-APBAE and DEGMA/TEGMA. Copolymers containing 3-APBAE displayed a low molar mass peak in their GPC chromatograms and were therefore purified via dialysis against acetone. Successful dialysis was confirmed by another GPC analysis showing no low molar mass fractions. Last but not least, copolymer 3/D synthesized with CTA 2) was successfully deprotected via dialysis against 0.3% HCl, which was confirmed via ^{1}H NMR, showing the disappearance of peak corresponding to the methyl protons of pinacol ester. Moreover, ^{1}H NMR analysis also confirmed that CTA 2 is still present in the deprotected copolymer, suggesting no changes of molar mass within deprotection, as GPC could not be performed due the high hygroscopicity of polymeric boronic acids.

T_{cp} values measurement were performed for homopolymers P(DEGMA) and P(TEGMA) with two concentrations (0.1 and 1 mg/ml). Both homopolymers displayed higher T_{cp} value in lower concentration, which can be attributed to the higher critical aggregation concentration of polymer chains under those condition. T_{cp} values were also measured for dialyzed copolymers, showing lower T_{cp} values compared to their corresponding homopolymers due the incorporation of hydrophobic 3-APBAE comonomer. The influence of hydrophobic comonomer was further supported by the fact that more significant drop in T_{cp} values corresponded to higher 3-APBAE content in copolymer. Finally, the T_{cp} measurement of deprotected copolymer showed virtually no change, compared to the protected analogue. This was most likely caused by the fact, that deprotected

copolymer prefers to form hydrogen bonds within its own polymer chains rather than with water. Extensive crosslinking cam prevent polymer chains from undergoing the conformational changes necessary to maintain LCST behavior.

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