Foreword and acknowledgements

Coming from a clinical oriented bachelor education little awareness was raised about the relatively new and booming research field within biomedical sciences namely bioelectronics and nanotechnology. Since I was ready for something different and the conviction that new ideas arise from making multidisciplinary connections made me choose a chemistry oriented bachelor thesis in 2012 within the research group of prof. Thomas Junkers. From there on the interest for the importance of polymers in the biomedical field of nanotechnology and bioelectronics started to grow. As a result the choice of the subject of my thesis was easily made.

That being said I especially would like to thank my promotor prof. dr. Thomas Junkers a lot for welcoming me to his research group (for the second time) and the tremendous guidance throughout the entire project. It is really motivating to work with a passionated and friendly professor who listens and has faith in what I'm doing.

I would also like to thank dr. Anitha Ethirajan as my co-promotor for facilitating the relations with the IMO-physics department and for providing an extra dimension to the synthesis of polymers with the production of polymer particles and I would like to thank Prof Wanda Guedens for the midterm evaluation of my project.

Special thanks go to my mentor dr. Joke Vandenbergh. Everything I learned concerning labwork, research strategies, chemical insights and result analysis is thanks to Joke. In other words the results and built-up of the project is mainly made possible by her. Off course this was facilitated by a pleasant cooperation.

Furthermore I would like to thank the entire PRD research group for helping me out when needed. They showed me how to properly work as a team and how to handle more difficult situations as a group. Special thanks go to mr. Benjamin Wenn for being a helpful and nice colleague (and for the BiDoPAT). In the office and when Joke was not around he was my first aid. Also thanks to mrs. Kayte Ranieri for the preliminary study on the Baylis-Hillman polymers, for always being available to help me out and for the cake, mrs. Mandy Berden for introducing me to the synthesis of polymer particles, mrs Neomy Zaquen. and dr. Matthias Conradi for the maintenance of the GPC (which is an important and difficult job) and for introducing me to the machine and its software, mr. Joachim Laun and mr. Felipe Bordini do Amaral for heated discussions about chemistry and for being good friends.

Last but not least special thanks go to my family. Apart from the financial support they were always there to motivate me in good and bad times and they taught me the right principles of life, to put everything in perspective and to always stay humble. Also thanks to my girlfriend for supporting me throughout my five year education and for choosing a scientific education with lots of biochemistry. That way I am also able to talk to her about what the biggest part of what my life is about. Lastly I would still like to thank the people I forgot to mention.

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Abstract

Biodegradable polymers can be used in variable applications within the field of biomedical sciences such as drug delivery systems, scaffolding, sutures and other. An example of such a polymer is polycaprolactone. To produce these structures a novel and efficient method is explored to synthesize new materials with superior characteristics that may replace existing polymers in the future. In this project polymer networks are synthesized using thiol-ene click step growth polymerization of thiols with bi- or multifunctional acrylates. The non-radical thiol-ene Michael addition is a fast reaction with high yields, which proceeds at room temperature with small amounts of catalyst, a tertiary phosphine.

For the purpose of this thiol-ene step growth polymerization a dithiol homotelechelic polystyrene polymer is synthesized via RAFT polymerization using a bifunctional RAFT-agent (BiDoPAT) and subsequent aminolysis of the RAFT end groups. The resulting polymer is successfully conjugated with linear and branched acrylic linkers. The final product has a number average molecular weight (M_n) of 7000 and tailing towards 100000 $g \cdot mol^{-1}$. By using acrylates to link the dithiol blocks together, biodegradability is introduced via its ester moieties, which are spread throughout the entire molecule.

Step growth thiol-ene click polymerization reactions were not equally successful for homotelechelic BiDoPAT RAFT polyacrylate polymers since they are more difficult to purify. Furthermore, the use of a trithiocarbonate group containing BiDOPAT agent resulted in the formation of undesired dodecanethiol side products after aminolysis. These side products are able to end cap and therefore inhibit step growth polymerization. As an alternative, a BiDTB RAFT-agent is tested, which has a dithiocarbonate moiety instead. Aminolysis of polymers containing these dithiobenzoate end groups results in the formation of the desired telechelic dithiol polymers while any other thiol side products are avoided. MMA was polymerized using BiDTB and subsequently aminolyzed. End group analysis revealed that dithiol PMMA suffers from backbiting reactions, forming thiolactone end groups, which impedes any subsequent thiol-ene step growth process. These reactions have to be further optimized.

A Baylis-Hillman step growth polymerization is a different method to obtain degradable polymers. By reacting terefthalaldehyde to an activated diacrylate a polymer containing ester moieties and accessible vinyl functionalities is obtained. The latter can be exploited to crosslink the residual Baylis-Hillman polymer via thiol-ene click reactions by using multifunctional thiol monomers and the above mentioned dithiol polystyrene.

In another project a preliminary study was carried out using a suspension technique for the synthesis of degradable particles. These particles were made from simple di- or tetra-acrylates like HDDA and PETA and di-or tetrathiols like butanedithiol and PETMP. Different particle sizes (30 µm < Ø < 200 µm) were obtained by varying the relative amount of stabilizer. Porosity was introduced when using a higher ratio of linear linkers over branched linkers. Different polymer to crosslinker ratios are tested and optimized according to the above mentioned crosslinking strategies.

All the above mentioned polymer products can be subjected to phosphate buffers to study degradation and exposed to cells to study biocompatibility in the future. These characteristics can then be further tuned depending on the product's fate.

Samenvatting

Binnen het biomedisch onderzoek kunnen biodegradeerbare polymeren gebruikt worden voor allerhande toepassingen zoals hechtingen en doelgerichte medicatie. Een voorbeeld van zo een polymeer is polycaprolacton. Een nieuwe synthese methode werd onderzocht om materialen te produceren met verbeterde superieure eigenschappen die in de toekomst bestaande polymeren zouden kunnen vervangen. Voor dit project werden polymere netwerken gesynthetiseerd door middel van een thiol-een klik "stapgroei" polymerisatie van dithiolen met bi- of multifunctionele acrylaten. De gebruikte base/nucleofiel gekatalyseerde thiol-een Michaël additie is een snelle reactie met hoge opbrengsten. De reactie kan uitgevoerd worden op kamertemperatuur met kleine hoeveelheden katalysator, nl. een tertiair fosfine.

Om deze stapgroei polymerisatie uit te kunnen voeren werd een homotelechelisch polystyreen gesynthetiseerd via RAFT polymerisatie. Hiervoor werd het bi-functioneel RAFT agens BiDoPAT gebruikt. Vervolgens werden de RAFT-eindgroepen geaminolyseerd. Het uiteindelijke product heeft een gemiddelde massa (M_n) van 7000 g·mol^{-1} en loopt uit tot 100000 g·mol^{-1}. Omdat er acrylaten als linker werden ingebouwd is er verspreid over de gehele lengte van het polymer via de estergroepen biodegradeerbaarheid geïntroduceerd.

Voor de geteste homotelechelische BiDoPAT RAFT polyacrylaten werden minder goede resultaten bekomen dan voor polystyreen. Het gebruik van BiDoPAT dat een trithiocarbonaat groep bevatte, resulteerde in de vorming van ongewenste dodecaanthiol bijproducten na aminolyse. Deze bijproducten kunnen de stapgroei polymerisatie bemoeilijken door de eindgroepen te bezetten. BiDTB dat een dithiocarbonaat groep bevat is getest als alternatief. Aminolyse van deze polymeren resulteerde in de vorming van de gewenste telechelische dithiol polymeren zonder de vorming van de thiol bijproducten. MMA werd gepolymeriseerd door middel van BiDTB en vervolgens geaminolyseerd. Na analyse werd de vorming van thiolacton eindgroepen via backbiting vastgesteld. Aangezien hierdoor een thiol eindgroep wordt verbruikt hindert dit het stapgroei proces.

Een Baylis-Hillman stapgroei polymerisatie is een alternatieve methode om degradeerbare polymeren te bekomen. Door een terafthalaldehyde te reageren met een geactiveerd diacrylaat wordt er een polymeer bekomen die naast estergroepen ook vinyl functionaliteiten bevat. Die laatste kunnen gebruikt worden om Baylis-Hillman polymeren te crosslinken door middel van multifunctionele thiolen (eenvoudige di-of tetra-thiolen maar ook het dithiol polystyreen) via thiol-een reacties.

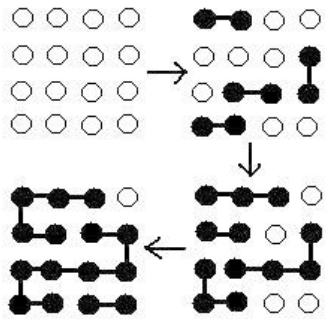
Voor een ander project is er preliminair onderzoek gedaan naar het gebruik van een suspensietechniek voor de synthese van partikels. De partikels werden opgebouwd uit eenvoudige di/tetra-thiolen zoals PETMP en butaandithiol en di/tetra-acrylaten zoals HDDA en PETA. Er werden partikels bekomen met variërende diameter (30µm < Ø < 200µm) afhankelijk van de hoeveelheid stabilisator. Een zekere porositeit werd geïntroduceerd door gebruik te maken van een hogere verhouding aan lineaire moleculen. Er werden verschillende linkers getest en ratio's geoptimaliseerd naar gelang de crosslink methode.

Alle eerder genoemde materialen kunnen blootgesteld worden aan fosfaatbuffers om de degradatie te bestuderen. Ook kunnen cellen blootgesteld worden aan de netwerken om de biocompatibiliteit te onderzoeken. Deze eigenschappen kunnen vervolgens worden aangepast naargelang de bestemming van het product.

1 Introduction

In the field of biomedical research biodegradable polymers play an important role in various applications like scaffolding, sutures, drug delivery and others. For these examples polymer residues are automatically removed by degradation when the polymers are no longer needed. Early in the 20th century biodegradable polymers were rejected by industry due to their high instability. However, they were picked up again in the 1960's to investigate their huge potential for medical devices, surgery and sutures[1]. Natural biodegradable polymers like cellulose and starch[2] mostly excel in biocompatibility but suffer from batch to batch variations, which make them difficult to investigate. Synthetic polymers, however, have significant advantages over natural polymers concerning versatility, which makes them usable for a wide spectrum of applications. Their properties can be easily tuned towards desirable characteristics by modification depending on the polymers purpose. Furthermore the variety of available monomers and polymerisation techniques provides the possibility to choose a suitable monomer for each respective application. However, to make synthetic polymers more interesting for medical purposes also biodegradability and biocompatibility have to be thought of.

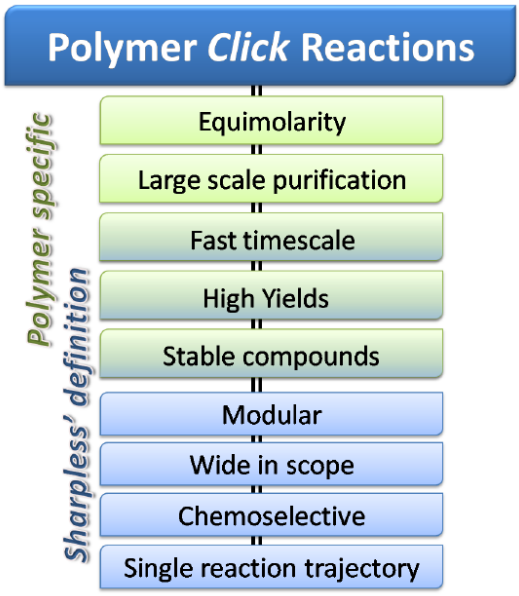
In this project the main aim is to explore the synthesis of biodegradable polymer networks using thiols and acrylates via a thiol-ene Michael addition step growth polymerization. Polymers formed in this way show favorable characteristics like the formation of uniform and dense networks, narrow glass transition temperatures (T_g) and low shrinkage stress[3]. In step growth polymerization multifunctional polymer blocks are conjugated via a stepwise intermolecular reaction. Unlike chain growth polymerizations there is no need for an initiator. Step growth polymerization is completed by only one orthogonal reaction type between two opposite functionalities producing few side reactions and by products. The reactivity of these functionalities is assumed to be independent from the chain length. Monomer units react rapidly with each other into dimers, dimers react into trimers and tetramers and so on so forth (Scheme 1). Statistically this implies that no monomer can exist next to larger macromolecules and a very high conversion is needed to obtain polymers of reasonable size. Due to the nature of step growth polymerizations end groups and average molecular weights can be controlled via stoichiometry[4].



$$\bar{X}_n = \frac{1}{1-p} \qquad \text{(Carothers equation)}$$

Scheme 1: Schematic representation of a step growth polymerization[5]. The given Carothers equation describes the formula to calculate the degree of polymerization ($\bar{X}_n$) in a step growth polymerization.

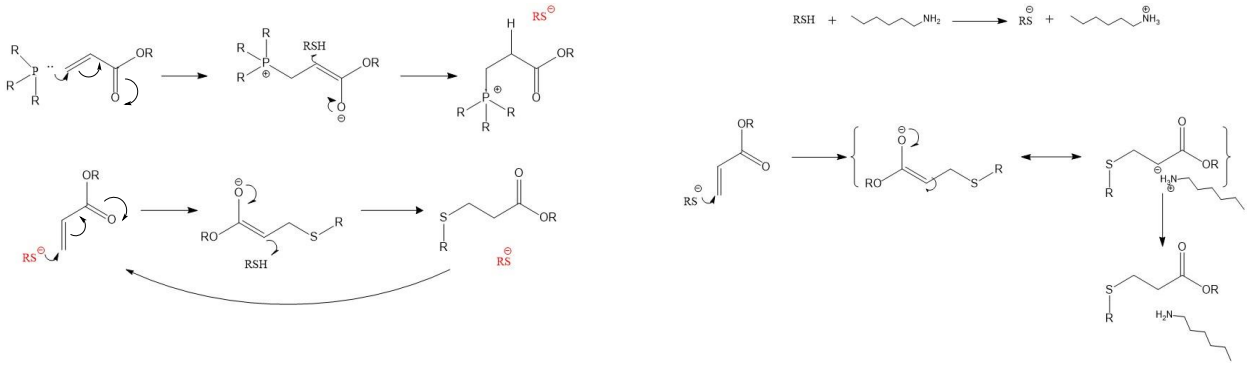
A Michael addition takes place between an electron deficient moiety, the so called Michael acceptor and a nucleophilic electron rich Michael donor. The vinyl functionality in an acrylate is connected with the electron withdrawing ester group, which makes it a good Michael acceptor. Thiols on their part serve as nucleophiles. Michael addition thiol-ene reactions are assumed to be click reactions. Chemical reactions have to fulfill several conditions to be acknowledged as click reaction[6,7](Scheme 2). Among others, main criteria are the relative good performance under equimolar conditions, feasible purification (non-chromatographic) and fast reactions with high yields following a single reaction trajectory. For the non-radical thiol-ene Michael addition this is the case. The radical thiol-ene route (initiated by UV-light or heat) is extensively investigated[8] but should not be considered a true click reaction based on the definition. Although it can be executed between a thiol and any ene-compound (even not activated alkenes) it does not follow a single reaction trajectory and thus fails to reach more than 99 % reaction efficiency, which is of major importance in step growth polymerizations.



Scheme 2: Overview of characteristics a reaction must have to be acknowledge as a click reaction[6]

For the thiol-ene Michael addition reaction there are two considered catalysis routes (Scheme 3). First, there is a base catalyzed route. This route is widely explored for the purpose of small molecule synthesis, polymer modifications and other[8]. To obtain a reaction, which can be qualified as a click reaction, reaction conditions need to be optimized. Frequently, these reactions suffer from relatively long reaction times, solvent dependency (high polarity, low volatile), elevated temperatures and high catalyst concentrations. In literature[9] primary amines like hexylamine are often the catalyst of choice since they are known for the efficient catalysis of Michael addition reactions. Primary amines also have a nucleophilic character, which is, however, less pronounced compared to thiols. In high excess, also amines can attack the electron deficient acrylates, giving rise to side products. The second route follows a nucleophilic catalytic step. This catalysis route is only mildly explored. These reactions allow to perform a fast and orthogonal thiol-ene addition and can be performed under non demanding conditions like ambient temperature and minimal amounts of catalyst. To make use of the nucleophilic route phosphines are often used. These (often

tertiary) phosphines are known as relatively weak bases but can react very fast using their nucleophilic property. Unlike one would expect the relative effect of steric hindrance (compared to amines) is small due to the bigger atom size of phosphor (> nitrogen). To induce the reaction the nucleophilic phosphor attacks the electron deficient vinyl moiety of the acrylate forming a zwitterion ion, which subsequently reacts with a thiol yielding a thiolate anion. This zwitterion is a highly reactive species, which causes higher reaction rates but also the formation of side products when formed in excess (when too much catalyst is present). Another reason for the high reaction rates compared to the base catalyzed reaction is the absence of a second proton source[3]. The most important parameter concerning the reaction rate is the structure of the ene-functionality rather than the catalyst and the thiol structure. In general, solvents can have an influence on the reaction rate as well since solvents with high polarity stabilize the thiolate anion and favors its formation. Furthermore protic solvents should be avoided. They can interact with enolate ion and slow down the ionic cycle.

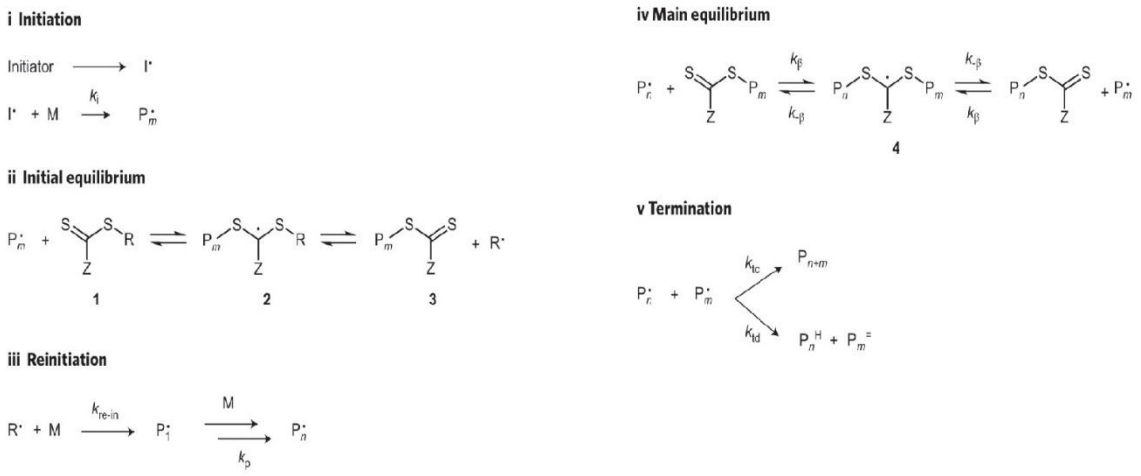


Scheme 3: Schematic representation of nucleophilic catalysis route (left) and base catalyzed route (right)

The thiol-ene Michael addition is mostly used to modify surfaces and grafting assays or to conjugate up to three small polymer blocks in bulk to block copolymers . Vandenbergh et al.[10] showed promising results for thiol-ene step growth polymerizations for small multifunctional thiols and acrylates. Also degradable thiol-ene networks could be formed using multifunctional thiols and acrylates[11]. During this project thiol-ene step growth polymerization with larger polymers was investigated. Polystyrene was the polymer of choice since it is relatively easy to manipulate and to purify. Furthermore other monomers like butyl acrylate (BuA), isobornyl acrylate (*i*BoA), N-isopropyl amide (NIPAM) and methyl methacrylate (MMA) were tested.

Like mentioned earlier multifunctional narrowly dispersed building blocks are desired for step growth polymerizations. To this end the polymers are synthesized via reversible addition fragmentation transfer (RAFT) polymerization (Scheme 4)[12]. RAFT is a controlled radical polymerization (CRP) technique. CRP techniques provide control over molecular weight distributions, dispersity and display high end-group fidelity[13]. Also complications, which are part of living anionic polymerizations like extreme reaction conditions and complex reaction procedures are avoided[14]. For RAFT a chain transfer agent (CTA) is needed. A typical RAFT-agent contains two groups, namely the R and Z-group, which are of strong influence to the characteristics and an electron deficient center mostly provided by a thiocarbonyl thio moiety where radicals can attack. When the radical has attacked, the R-group functions as the (radical) leaving group (RAFT pre-equilibrium), which can initiate new growing chains (re-initiation). The R-group leaves when released a new active center on the RAFT-agent, which is vulnerable for radical attacks. When this happens an intermediate is formed with two chains attached to the RAFT-agent. The lifetime of such

an intermediate is determined by the Z-group. Afterwards one of the two chains is released (RAFT main-equilibrium). This way the Z-group containing thiocarbonyl thio compound is always found at the ω-end of the polymer chain, while the R group is found at the α-chain end. Via this RAFT main equilibrium the CTA controls the radical reaction by transferring the active center from one growing polymer chain to another.



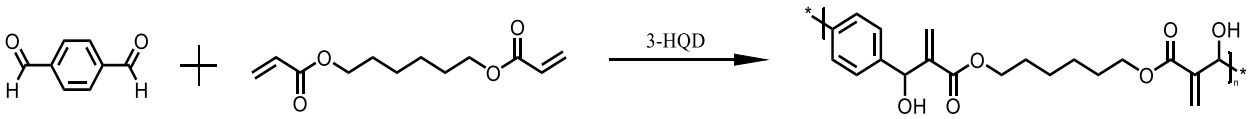
Scheme 4: Schematic representation of the RAFT-polymerization mechanism[15]

However, to ensure these end-groups are maintained a maximum conversion of 70% is suggested since higher conversion can lead to side reactions eliminating the RAFT end groups[13,16]. Depending on the RAFT-agent a specific dithio or trithio end group functionality is incorporated at the end of the polymer chain, which is susceptible to various modification reactions[17]. This makes RAFT an interesting method to produce building blocks for further conjugation reactions. RAFT agents contain thiocarbonyl thio moieties[13,16,17], which can be transformed via aminolysis into telechelic thiol functionalities that are suitable for further conjugations. To obtain bifunctional polymers for step growth polymerization, RAFT polymerizations were carried out using custom-made bifunctional RAFT agents namely bis 2-(Dodecylthiocarbonothioylthio)propionic acid (BiDoPAT)[18,19] and ethane-1,2-diyl bis(2-phenyl-2-((phenylcarbonothioyl)thio)acetate), which is a Bis dithiobenzoate (BiDTB)[14]. The thiocarbonyl thio ends of the resulting bifunctional RAFT polymers were converted into thiols using aminolysis. These telechelic dithiol polymers could then be used for thiol-ene step growth conjugations.

Biacrylic linkers are used to conjugate the bifunctional thiol polymers together. Since these linkers contain ester moieties they are assumedly vulnerable for hydrolysis. This means a certain level of biodegradability is incorporated in the polymer chain. Apart from diacrylic linkers also multi branched acrylates can be used. This way crosslinking is achieved and dense and insoluble polymer networks can be produced, which are nonetheless degradable.

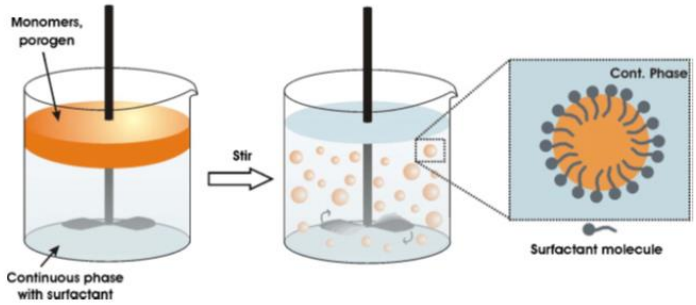
Next to thiol-ene click step growth polymerization another non-radical step growth method is explored namely Baylis-Hillman polymerization. A Baylis-Hillman reaction is performed between an aldehyde and an electron deficient vinyl functionality (like in acrylates) and is catalyzed by a tertiary amine. After addition a typical linkage is formed consisting of a ketone, a vinyl group and an alcohol (Scheme 5)[20]. From a Baylis-Hillman reaction no by products are generated[21]. However, it most often shows slow reaction

rates. The newly formed hydroxyl and especially the vinyl functionality are useful for functionalizing the Baylis-Hillman polymers. When using linear and multifuntional thiols these polymers can be crosslinked into dense networks via thiol-ene click reactions.



Scheme 5: general mechanism for a Baylis-Hillman reaction

As a last project, synthesis of particles using thiol-ene step growth suspension polymerization was targeted. Suspension polymerization is a technique in which an organic phase, containing the monomers of choice, is dispersed in a continuous water phase by intense mechanical stirring (Scheme 6)[22]. The formed droplets are stabilized using polyvinylalcohol as stabilizing agent. Stabilizers lower the surface tension between the two distinct phases keeping the droplets for a longer time in dispersion. In this first preliminary study, multifunctional acrylates and thiol monomers were polymerized in suspension to produce particles between 50 and 500 µm. In a follow up study, the biocompatibility of these particles with cell cultures will be investigated.



Scheme 6: General working principle of the suspension technique[22]

2 Materials and experimental

2.1 Materials

For the following experiments the products used are:, 2,2'-azobis(2-methylpropionitrile) (AIBN, Sigma-Aldrich, 98%), 2-bromopropionic acid (Acros 99%), butanedioldiacrylate (BDDA, Sigma-Aldrich, 90%), butanedithiol (Sigma-Aldrich, ≥ 97%), butyl acetate (Acros, 99%), butyl acrylate (BuA, Acros, 99%), carbon disulphide (Acros, 99.9%), chloro-2-phenyl acetyl chloride (Sigma Aldrich, 90%), 1,4-Diazabicyclo[2.2.2]octane (DABCO, Acros, 97%), , N,N'-Dicyclohexylcarbodiimide (DCC, Acros, 99%), di(ethylene glycol) diacrylate (DEGDA, Sigma-Aldrich, 75%), 4-(dimethylamino)pyridine (DMAP, Acros, 99%), dimethylformamide (VWR, pa.), dodecanethiol (Acros, 98 hexanediol diacrylate (Sigma-Aldrich, 80%), hexylamine (Acros, 99%), 3-hydroxyquinuclidine (3-HQD, Sigma-Aldrich, 99%), iodine (Acros, 99.2%), isobornyl acrylate (*i*BoA, Sigma-Aldrich, technical grade), N-isopropyl acrylamide (NIPAM, Acros, 99%), , methyl methacrylate (MMA, Acros, 99%), pentaerythritol tetraacrylate (PETA, Sigma-Aldrich, 10-40% triester), pentaerythritol tetrakis(3-mercaptopropionate) (PETMP, Sigma-Aldrich, > 95%), phenylmagnesium bromide (Sigma-Aldrich, in 1M THF), polyvinyl alcohol (PVA, Acros, 88% hydrolyzed), sodium hydroxide (NaOH, VWR), Styrene (St, Acros, 99.5%), terefthalaldehyde (TA, Acros, 98%), tetra(ethylene glycol) diacrylate (TEGDA, Sigma-Aldrich), tetrapropylammonium bromide (Acros, 98%), tributylphosphine (TBP, Acros, 95%), triethylamine (TEA, Acros, 99%), triethylphosphite (Acros 97%), tris[2-(acryloyloxy)ethyl] isocyanurate (TAEIC, Sigma-Aldrich). All solvents used were obtained from commercial sources (Acros, VWR, Sigma-Aldrich) and used without further purification.

2.2 Characterization

^{1}H-NMR spectra were recorded in $CDCl_3$ on a Varian Inova 300 spectrometer at 300 MHz using a 5 mm probe.

Analytical SEC (Size Exclusion Chromatography) was performed on a Tosoh EcoSEC HLC-8320GPC, comprising an autosampler, a PSS guard column SDV (50 x 7.5 mm), followed by three PSS SDV analytical linear XL (5 µm, 300 x 7.5 mm) columns thermostated at 40 °C (column molecular weight range: 1 x 10^2 – 1 x 10^6 g·mol^{-1}), and a differential refractive index detector (Tosoh EcoSEC RI) using THF as the eluent at with a flow rate of 1 mL·min^{-1}. Toluene was used as a flow marker.

Analytical SEC (DMF GPC) was performed using a Spectra Series P100 (Spectra Physics) pump equipped with two mixed-B columns (10 µm, 2 cm × 30 cm, Polymer Labs) and a refractive index detector (Shodex) at 70 °C. DMF was used as the eluent at a flow rate of 1.0 mL·min-1. Molecular weight distributions were determined relative to polystyrene standards because Mark-Houwink parameters are not available for the polymers under investigation.

Analysis of the MWDs of the polymers with UV detection at λmax of the polymer (Chlorobenzene GPC) was performed using a Spectra Series P100 (Spectra Physics) pump equipped with two mixed-B columns (10 µm, 2 cm x 30 cm, Polymer Laboratories) and an Agilent 1100 DAD UV detector at 60 °C. Chlorobenzene (CB) was used as the eluent at a flow rate of 1.0 mL·min-1. Molecular weights were determined relative to polystyrene standards.

Electrospray ionization - mass spectroscopy (ESI-MS) was performed on an LTQ Orbitrap Velos Pro mass spectrometer (ThermoFischer Scientific) equipped with an atmospheric pressure ionization source

operating in the nebulizer-assisted electrospray mode. The instrument was calibrated in the m/z range 220-2000 using a standard solution containing caffeine; MRFA, and Ultramark 1621. A constant spray voltage of 5 kV was used, and nitrogen at a dimensionless auxiliary gas flow rate of 5 and a dimensionless sheath gas flow rate of 10 were applied. The S-lens RF level, the gate lens voltage, the front lens voltage and the capillary temperature were set to 50 %, -90 V, -8.5 V, and 275°C respectively. A 250 µL aliquot of polymer solution with a concentration of 10 $\mu g \cdot ml^{-1}$ was injected. A mixture of THF and methanol (THF:MeOH = 3:2), all HPLC grade, were used as solvent.

Optical microscopy images were made using a Olympus BX 41 optical microscope. 10x and 20x magnification was used.

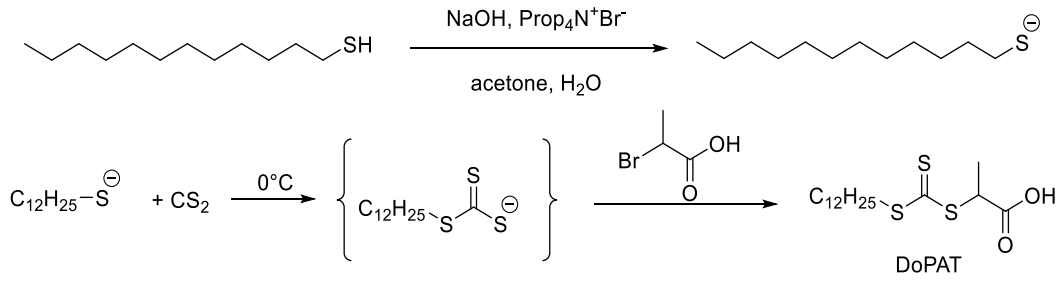
The column used for product separation was made with S-X1 beads from biobeads® (BIO-RAD laboratories) with a particle size between 200-400 mesh. DCM is the used eluent.
Purification of polymers was performed on a recycling preparative HPLC LC-9210 NEXT system in the manual injection mode (3 mL) comprising a JAIGEL-1H and JAIGEL-2H column and a NEXT series UV detector using $CHCl_3$ as the eluent with a flow rate of 3.5 $mL \cdot min^{-1}$. Fractions were collected manually.

2.3 Synthesis

2.3.1 Reversible addition fragmentation transfer (RAFT) polymerization

Synthesis of 2-([(Dodecylsulfanyl)carbonothioyl]sulfanyl) propanoic acid (DoPAT) (RAFT-agent) [18]

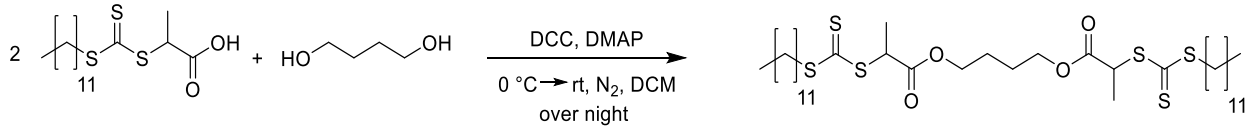
Monofunctional RAFT agent was synthesized using following procedure (Scheme 7). NaOH (1eq.) was dissolved in a mixture of dodecanethiol (1eq.), acetone (80mL/ 1g NaOH), water (10 mL/ 1 g NaOH) and tetrapropylammonium bromide (0.08 eq.) while stirred and heated with a heatgun. When everything was dissolved carbon disulfide (1 eq.) was added to the solution while the solution was cooled in an ice bath. After 20 min, 2-bromopropanoic acid (1 eq.) was added and the mixture was stirred at room temperature overnight. The solution was slowly acidified with 37% HCL/ H2O 1:4 until precipitation was obtained. The product was filtered and rinsed with water repeatedly. The precipitate was placed on a vacuum pump overnight and afterwards collected and recrystallized from diethylether/petroleumether 1:1, giving 34.25g (yield=85%) of pure DoPAT. $_1$H NMR: (ppm) 10.2 (br, 1H, CO2H), 4.88 (q,J) 7.4 Hz, 1H, SCH), 3.36 (t,J) 7.4 Hz, 2H, CH2S), 1.70 (quint,J) 7.5 Hz, 2H, CH2CH2S),1.63 (d,J) 7.4 Hz, 3H, SCHCH3), 1.40 (br quint,J) 7.5 Hz,2H, CH2CH2CH2S),1.22-1.35 (br, 16H, CH3(CH2)8), 0.88 (t,J) 7.4 Hz, 3H, CH3CH2).



Scheme 7: Reaction scheme for the synthesis of DoPAT

Synthesis of BiDoPAT (bifunctional RAFT-agent)(Scheme 8)[19]

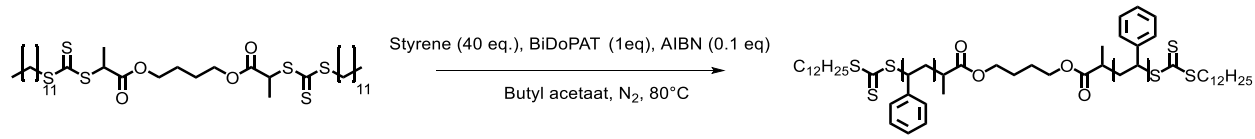
DoPAT (2 eq.) and 1,4-Butanediol (1 eq.) are dissolved in DCM (15 ml / 1 g DoPAT) in a three neck flask. The solution is stirred in an ice bath and continuously purged with nitrogen. In another beaker DCC (2 eq.) and DMAP (0.1 eq.) are added together in DCM (about 1/3 of the volume used for the first solution). This mixture is added drop wise to the solution with DoPAT. After everything is added the ice bath is removed. The reaction mixture was stirred overnight at room temperature and then washed with 100 mL H_2O. The water phase was washed three times with DCM. The recombined organic phases were dried over magnesium sulfate, filtered off and the solvent removed under lowered pressure. The yellow solid product is purified by column chromatography [SiO_2, *n*-Hexane : Ethyl acetat (1:1)] to yield the product as a yellow solid (76 %). ^{1}H NMR ($CDCl_3$): δ = 4.79 (q, 2H), 4.21 – 4.06 (m, 4H), 3.33 (t, *J* = 7.20 Hz, 4H), 1.75 – 1.60 (m, 8H), 1.58 (d, *J* = 7.4 Hz, 6H), 1.45 – 1.18 (m, 36H), 0.93 – 0.78 (m, 6H)



Scheme 8: Reaction scheme for the synthesis of Bifunctional DoPAT

RAFT-polymerization with BiDoPAT

Polystyrene (Scheme 9). In order AIBN (0.1 eq), BiDoPAT (1 eq) and styrene monomer (40 eq.) are added in a vial. Approximately 1 g of butyl acetate per gram of styrene is used as the solvent. The solution is stirred and flushed with N_2 for 5 minutes. Afterwards the reaction vial is transferred to a glovebox and placed in a pre-heated copper block of 80°C. The polymerization is carried out for 6 hours with samples taken after 1, 2 and 4 hours. The reaction was quenched by removing the sample from the glovebox and placing it in an icebath. The product was analyzed using GPC. Conversion^{NMR} = 44 %.



Scheme 9: Reaction scheme for the synthesis of polystyrene via RAFT using BiDoPAT as CTA

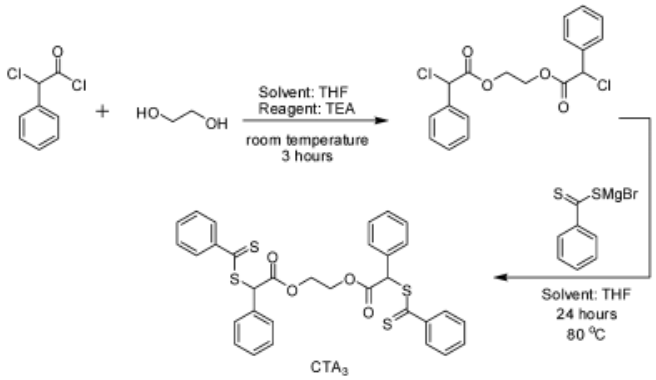
Poly (butyl acrylate) (BuA). Polymerization of BuA was performed with AIBN (0.01 eq), BiDoPAT (1 eq.) and BuA monomer (10 eq.) in butyl acetate (1 g for each gram of monomer). The same procedure was followed as above mentioned for styrene. The reaction was stirred at 80 ˚C in inert atmosphere for 90 minutes. The sample was analyzed using GPC and ESI-MS. Conversion^{NMR}= 77 %

Poly (isobornyl acrylate) (*i*BoA). For *i*BoA 0.01 eq. AIBN, 1 eq of BiDoPAT and 10 eq. of *i*BoA monomer was dissolved in butyl acetate (1g per g of monomer). The above mentioned (see styrene) procedures were followed. The reaction was stirred at 80 °C for 90 minutes in inert atmosphere. Samples were analyzed using GPC and ESI-MS. The polymer was separated over a DCM wetted biobeads-SX1® column to purify the polymer sample from monomer leftovers. Conversion^{NMR} = 78 %.

Poly (N-isopropyl acrylamide) (NIPAM). NIPAM is polymerized using AIBN (0.01 eq.), BiDoPAT (1 eq.) and NIPAM monomer (20 eq.). Dioxane was used as solvent (3 g for 1 g of monomer). The reaction was heated at 80 °C and stirred for 6 hours. The rest of the procedure is similar to the procedure for styrene. Samples are analyzed using GPC and ESI-MS. The polymer is run over a DCM wetted biobeads-SX1® column to purify the polymer sample from monomer leftovers. Conversion^{NMR} = 98 %.

Synthesis of bidithiobenzoate (biDTB) RAFT agent [(14)]

To synthesize a dithiobenzoate BiRAFT agent two dithiobenzoate compounds are connected via a linker. First this linker needs to be synthesized. Therefore ethylene glycol (1 eq.) and triethylamine (TEA, 2 eq.) are added together in dry THF (150 ml/ g ethylene glycol). The solution is cooled down to 0 °C. Thereafter 2-chloro-2-phenyl acetyl chloride (2 eq.) is added drop wise to the solution. Then the mixture is stirred for 3 hours while allowing it to reach room temperature. Diethylether is added to the product after which the organic phase is washed with $NaHCO_3$ and water and subsequently dried with $MgSO_4$. The product is concentrated by evaporation of the solvent, yielding a slight yellow oil (94%). ^{1}H NMR (CDCl3, 300 MHz): 4.36 (2H, s, HC-O), 5.59 (2H, s, HC-Cl), 7.28-7.33 (10H, m, Ar-H).



Scheme 10: Reaction scheme for the synthesis of BiDTB[(14)]

Secondly the dithiobenzoate component is synthesized. 1 eq. of a 1M phenylmagnesium bromide (in THF) was further dissolved in THF (2 ml per ml phenylmagnesium bromide). Then the mixture was cooled down to 0 °C. Carbon disulfide (2 eq.) was added dropwise and the solution was stirred for 30 minutes allowing it to reach room temperature. Finally a solution of the dichloride linker (0.5 eq.) in dry THF (15ml / 10 g)

was added and stirred for 24 hours on 80 °C. After that the reaction was placed in an ice bath and extracted with ethyl acetate/ H_2O. Afterwards the product is washed with $NaHCO_3$ and dried with $MgSO_4$. The crude product was purified by column chromatography (SiO_2, hex/EtOAc 4/1) and subsequent recrystallization in hex/EtOAC 4/1, yielding dark pink crystals (26%). ^{1}H NMR for a racemic mixture of both isomers. For the first isomer: (CDCl3, 300 MHz): δ (ppm from TMS) 4.4-4.3 (4H, m, O-CH2CH2), 5.67 (2H, s, C-H), 7.20-7.48 (16H,m, Ar-H), 7.91 (4H, dd, J) 7.26, 1.31 Hz, SC(Ar-H)S). ^{1}H NMR for the second isomer: (CDCl3, 298K, 400 MHz): δ (ppm from TMS);4.4-4.3 (4H, m, O-CH2CH2), 5.69 (2H, s, C-H), 7.20-7.48 (16H,m, Ar-H), 7.91 (4H, dd, J) 7.26, 1.31 Hz, SC(Ar-H)S).

RAFT polymerization with BiDTB

Polystyrene. PS was synthesized using AIBN (0.1 eq.), BiDTB (1 eq.) and monomer (40 eq.). For 1 g styrene 1 g of butyl acetate was used as solvent. After degassing (5 min) the vial was transferred to a glove box and stirred for 6 hours at 80 °C. Reaction was quenched by cooling in an ice bath. The polymer was purified from monomer leftovers by precipitation in cold MeOH and subsequent filtration. Samples were analyzed by GPC. Conversion^{NMR} = 16 %.

Poly (methyl methacrylate) (PMMA). In order AIBN (0.1 eq.), BiDTB (1 eq.) and MMA monomer (40 eq.) were weighted in a glass vial. The solvent of choice was butyl acetate 1 g/ 0.5 g of monomer. The mixture was degassed (N_2) for 5 minutes and transferred into a glovebox. The vial was placed on a preheated hotplate of 80 °C and stirred for 4 hours. Thereafter the reaction was quenched in an ice bath. The polymer was purified from monomer leftovers by precipitation in cold MeOH and subsequent filtration. The polymer was analyzed using GPC (after drying overnight to get rid of monomer excess). Conversion^{NMR} = 74 %. Also a second batch of PMMA with a lower molar mass was synthesized in order to study the end group fidelity with ESI-MS. Therefore AIBN (0.1 eq.), BiDTB (1 eq.) and 20 eq. of MMA monomer were used in 1 g butyl acetate per 0.5 g monomer. After purging with N_2 the mixture was stirred for 4 hours at 80 °C in a glovebox. Samples were analyzed in GPC and ESI-MS. Conversion^{NMR} = 25%.

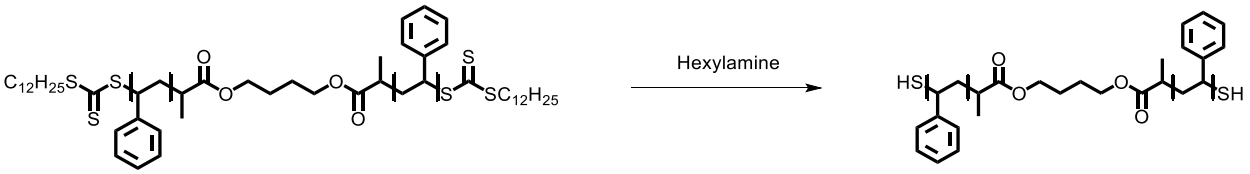
MMA polymer styrene chain extension. For the chain extension 20 eq of styrene monomer were mixed with 1 eq. of diDTB-PMMA (M_n=4300 g/mol) and 0.1 eq. of AIBN. The reaction was purged and stirred in a glovebox for 4 hours at 80 °C. NMR and GPC (after overnight drying) were taken for analysis. Conversion^{NMR} = 37%.

2.3.2 Aminolysis and (in-situ) Step growth click polymerization of BiDoPAT polymers

Polystyrene

Aminolysis and in-situ thiol-ene click (one-pot) reaction (Scheme 11). For the one-step reactions multiple parameters were varied and tested. These click reactions were tested with different ratio's between hexanediol diacrylate (HDDA) and BiDoPAT-PS going from 0.7 equivalent (eq.) until 2.2 eq. of acrylates compared to 1 eq. of PS. These tests were performed in THF at room temperature (rt.) and stirred overnight. Hexylamine was the catalyst of choice (initially 3 eq., later on 5 eq). To investigate the influence of the oxygen, a reaction with equimolar acrylate/thiol ratios was also tested under N_2 atmosphere. Furthermore, an experiment with different reducing agents was performed. Therefore 5 eq. of the

reducing agent, tributylphosphine (TBP) or triethylphoshite (TEP) was used and compared with a blank. All three reactions were accompanied by 5 eq. of hexylamine.

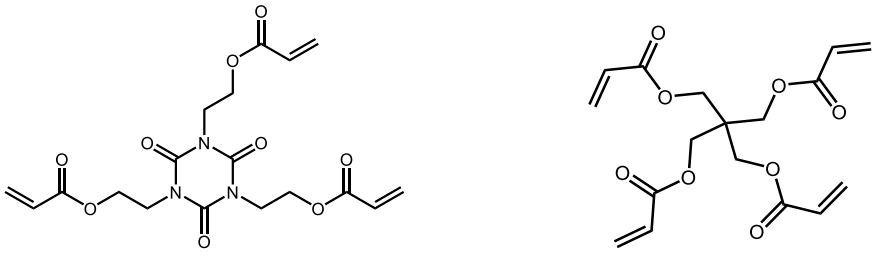


Scheme 11: Graphic description of the result of aminolysis of polystyrene

Aminolysis. The aminolysis of BiDoPAT-PS was carried out at room temperature in ambient atmosphere with 5eq. and 30 eq. of hexylamine and stirred ON and for 0.5 h respectively. Furthermore different solvents like DCM, DMF and THF were tested. TBP (1 eq) was added as the reducing agent. The product was afterwards purified by precipitation in cold methanol. The sample was collected by decantation of the methanol and measured in GPC.

Thiol-ene click step growth. Purified dithiol-PS was used (1 eq.) together with an equimolar amount (1.25 eq.) of HDDA (80 % purity). Reactions were performed at room temperature in ambient atmosphere in THF (0.3g/ 0.1 g dithiol PS). The catalysts were hexylamine (0.5 eq.) and TBP (0.2 eq.). Reaction times varied between two minutes and overnight reactions. Reactions were stopped and purified by precipitation over ice cold methanol. Samples were isolated by decantation and measured in THF-GPC.

Branched thiol-ene step growth polymerization. The synthesis of branched polymers through step growth polymerization was performed using tris[2-(acryloyloxy)ethyl] isocyanurate (TAEIC) and Pentaerythritol tetraacrylate (PETA) as the branched acrylates (Scheme 12). These compounds were mixed with the linear HDDA in different ratio's going from 0.2/1 to 100% branched acrylate. Equimolarity towards dithiol PS was respected. Reactions were performed at room temperature in ambient atmosphere. THF was used as solvent and the reactions were stirred for 2-3 hours. One drop (0.2 eq.) of TBP was added to initiate the reaction. The resulting networks were precipitated in cold methanol.



Scheme 12: The skeleton formula of TAEIC (left) and PETA (right)

Poly (butyl acrylate) and poly (isobornyl acrylate)

Aminolysis and in-situ thiol-ene click (one-pot) reaction. One equivalent of BiDoPAT-PBuA was dissolved in acetone. Reactions were carried in ambient atmosphere and at room temperature. 2.5 eq of HDDA was

added (80 % purity). Hexylamine (4-5 eq) was added together with TBP (0.5-1.5 eq.). The reactions were stirred overnight. The product was isolated using a rotavap.

Aminolysis. Aminolysis was performed with one equivalent of PBuA or P*i*BoA with hexylamine (30 eq.) and TBP (1 eq.). This reaction was carried out in ambient atmosphere at room temperature. The reactions were stirred for 2 hours. For PBuA the reaction was carried out in acetone and the sample was isolated afterwards using the rotavap. Aminolysis on P*iBoA* was performed in acetone or DCM. The product was purified in three different ways. 1) The sample was precipitated in ice cold methanol and placed in the fridge overnight. The sample could be isolated via decantation afterwards. 2) The sample was purified via separation over a DCM wetted biobeads® SX-1 column. The collected fractions were isolated using a rotavap. 3) The product was purified using a recycling GPC. Again the desired fraction was isolated with a rotavap. All products were analyzed using a THF-GPC.

Thiol-ene click step growth. Dithiol PBuA (1 eq.) was mixed with 1.25 eq. of HDDA and 1 drop of TBP (0.1-0.2 eq.). The reaction was performed in THF at room temperature and ambient atmosphere. The reaction times were varied between 2-60 minutes. The product was isolated using a rotavap. P*i*BoA click reactions were performed with 1.25 eq. of HDDA and 0.1-0.2 eq. of TBP in THF. The samples were precipitated in ice-cold methanol and placed in the fridge overnight when required. The methanol is decanted afterwards.

Poly (N-isopropyl acrylamide)

Aminolysis. To one eq. of BiDoPAT-PNIPAM 30 eq. of hexylamine was added together with one eq. of TBP. Reactions were carried out in acetone or DCM in ambient atmosphere and at room temperature. After aminolysis dithiol-PNIPAM is precipitated in cold hexane.

Thiol-ene click step growth. The conditions for the step growth polymerization of dithiol-PNIPAM were the same as for the polyacrylates. 1 eq. of BiDoPAT-PNIPAM was mixed with 1.25 eq. of HDDA and 0.1-0.2 eq. of TBP. The resulting product was precipitated in cold hexane afterwards and measured via THF-GPC.

2.3.3 Aminolysis and (in-situ) step growth click polymerization of BiDTB polymers

BiDTB RAFT agent

Aminolysis and in-situ thiol-ene click (one-pot) reaction. 1 eq. of the BiDTB RAFT agent was add together with 1 eq. of butanedioldiacrylate (BDDA). Hexylamine (2.1 eq.) was used together with TBP (0.2 eq.). Everything was dissolved in acetone. The reaction was performed at room temperature in ambient atmosphere and stirred for 2 hours. The product was dried overnight and a GPC sample was taken.

Poly(methyl methacrylate)

Aminolysis and in-situ thiol-ene click (one-pot) reaction. For the step growth polymerization with in situ aminolysis 1 eq. of PMMA is mixed with 1 eq. of BDDA and hexylamine (2.1 eq.) is added. The reaction is stirred for three hours at room temperature in ambient atmosphere. The polymer is precipitated in cold methanol afterwards. The sample is dried and analyzed using GPC.

Aminolysis. Aminolysis is performed on one equivalent of PMMA. For that hexylamine (10 eq.) was used and TBP (0.5 and 1 eq.) as reducing agent. The reaction with 0.5 eq. of TBP was carried out in ambient atmosphere while the reaction with 1 eq. of TBP was carried out while being purged with N_2 continuously. Both reactions were stirred for two hours in acetone. The aminolysis product was precipitated in cold methanol and analyzed in GPC after drying.

Thiol-ene click step growth. The dithiol PMMA (1 eq.) was dissolved in THF(1 mL/g) with TBP (1 eq). After 30 minutes BDDA (1 eq.) was added. The reaction was stirred for one hour at room temperature in ambient atmosphere. The product was precipitated in cold methanol and dried before a GPC sample was taken.

2.3.4 Baylis-Hillman polymerization

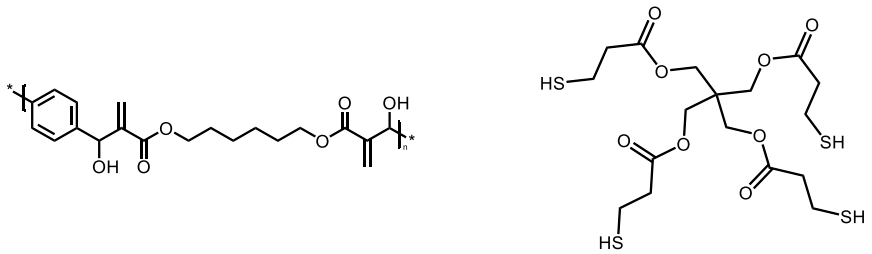
Baylis-Hillman polymerization

Terefthalaldehyde (TA, 1eq.) was add together with 1 eq. of HDDA and 1.63 eq. of methanol. For 0.1 g of TA 0.2 g of DMF was used as the solvent. The catalyst was added in 1 eq. and varied between DABCO, 3-HQD and DMAP. The mixture was stirred overnight in ambient atmosphere at room temperature.

For the next experiment the catalyst of choice was 3-HQD (1eq.). This was again mixed with TA (1 eq.) and methanol (1.63 eq.). 0.2 g of DMF was used for 0.1 g of TA. This time the acrylates are varied between BDDA, diethyleneglycoldiacrylate (DEGDA) and tetraethylene glycol diacrylate (TEGDA).

Crosslinking of Baylis-Hillman polymers

Multifunctional thiols. The equivalents of Baylis-Hillman polymer used for these reactions are based on the amount of repeating units (361 $g \cdot mol^{-1}$) (Scheme 13). This because the functionalities reside in each repeating unit and not only at the end of the polymer. This means for 1 eq. of pentaerythritol tetrakis(3-mercaptopropionate) (PETMP) (Scheme 13) 2 eq. of repeating units are needed. For 0.1 g of PETMP about 0.5 g of THF is used. First the Baylis-Hillman polymer is dissolved in THF before adding the PETMP. The mixture is stirred for a couple minutes. Lastly TBP (0.2 eq.) is added. The reaction was placed on a stirring plate at room temperature until the reaction was finished (< 2h)



Scheme 13: The repeating unit in a Baylis-Hillman polymer (left) and the skeleton formula of PETMP (right)

This experiment is repeated with butanedithiol instead of PETMP. Therefore for 1 eq. is mixed with 1 eq. of Baylis-Hillman polymer repeating units. Again TBP was added to start the reaction and stirred until the end product was formed (< 2h). The product was isolated by decantation.

Dithiol Polystyrene. Baylis-Hillman polymers were crosslinked with linear homotelechelic dithiol PS. For that experiment 1 eq. of Baylis-Hillman repeating units was added together with 1 eq. of PS in THF. The catalyst was varied among different experiments. TBP was used in 1 eq. Also hexylamine (0.5 eq) was tested and accompanied by 0.2 eq. of TBP. This reaction was left on a stirring plate for approximately 2 hours at room temperature. The product was isolated by precipitation in ice-cold methanol (when needed) and subsequent decantation.

2.3.5 Suspension polymerization

First the continuous phase is prepared in a separate vial. Polyvinyl alcohol (PVA) was added to H_2O (3 ml and 6 ml) with a concentration of 5 and 10 mg/ml respectively and extensively stirred or shaken. In a second vial the organic phase is prepared existing from HDDA (0.2 g, 1 eq.), (PETMP) (0.5 eq.) and 0.4 g of DCM and stirred for a couple minutes. Then the organic phase is added to the continuous phase and placed on a stirring plate (1200 rpm) for 30 minutes at room temperature. 1 drop hexylamine is then added to the stirring solution to initiate the reaction.

A second composition is tested. The continuous phase is prepared first as mentioned above. 5 mg/ml PVA was added to 3 ml H_2O. To prepare the organic phase, HDDA (0.2 g, 1 eq.), PETA (0.1 eq.) and butanedithiol (1.2 eq.) were dissolved in 0.4 g DCM. Subsequently, the organic phase was added to the water phase and placed on a stirring plate (1200 rpm) for 30 minutes. Then one drop of hexylamine was added to start the polymerization.

The formed particles are analyzed using an optical microscope to determine the size distribution.

3 Results and discussion

3.1 Reversible addition fragmentation transfer (RAFT) polymerization

RAFT-polymerization with BiDoPAT

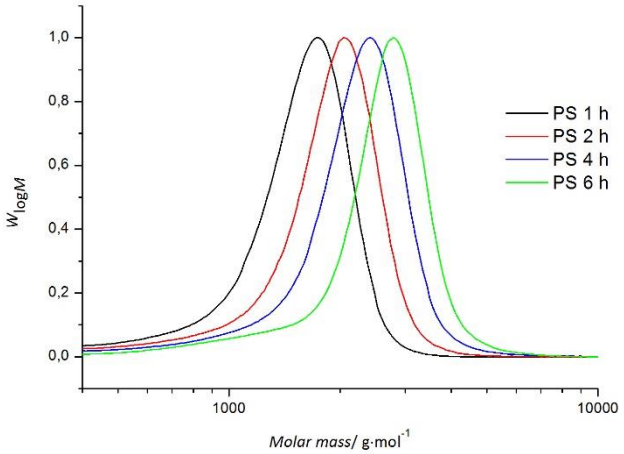


Figure 1: Overlay of GPC elugram of polystyrene made with 40 eq. of monomer via RAFT polymerization with varying reaction times going from 1 to 6 hours

Polystyrene. When comparing the obtained GPC results for the different reaction times (Figure 1) a direct proportional growth of PS was observed with increasing reaction times. The largest polymer (6 hours) had an M_n of approximately 2300 g·mol^{-1} and $Đ$ of 1.17[(11)]. PS is not easily visible in ESI-MS, which makes the investigation of end groups very cumbersome. Therefore, also other polymers were targeted.

Poly (butyl acrylate) (BuA). BuA polymerization was first tested with 0.1 eq. and 0.05 eq. of AIBN with 40 eq. and 20 eq. of monomer respectively for 30 minutes. The spectra obtained via GPC showed an M_n around 5000 g·mol^{-1}, which is too large for ESI-MS measurements since polymer sizes are preferably limited to 2000-3000 g·mol^{-1}. ESI-MS measurements are desired to analyse end-group fidelity. The preferred polymer size is obtained after using a protocol of 10 eq. of BuA and 0.01 eq. of AIBN (Figure 2). These polymers had a M_n of 1600 and $Đ$ of 1.18 [23]according to GPC measurements. When analysing the polymers with ESI-MS the synthesis of a bifunctional RAFT polymer is confirmed (Figure 3). The observed peaks represent PBuA with different chain lengths and both BiDoPAT end groups. Few peaks with low relative abundance caused by unknown side products are observed. This means a rather pure polymer is obtained.

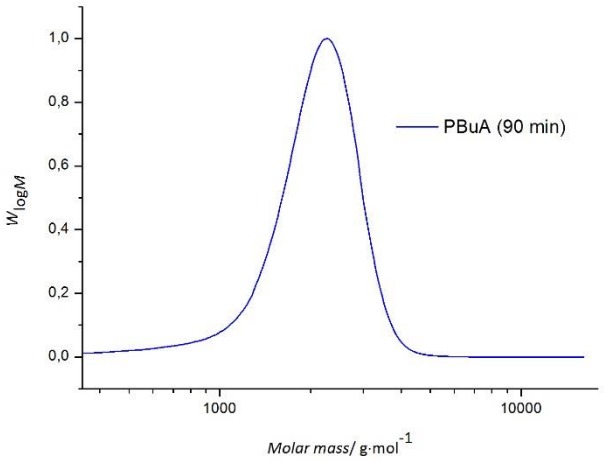


Figure 2: GPC trace of PBuA made with 10 eq. of BuA monomer after a reaction time of 90 minutes. The obtained M_n is 1600 g·mol^{-1} with a Đ of 1.17

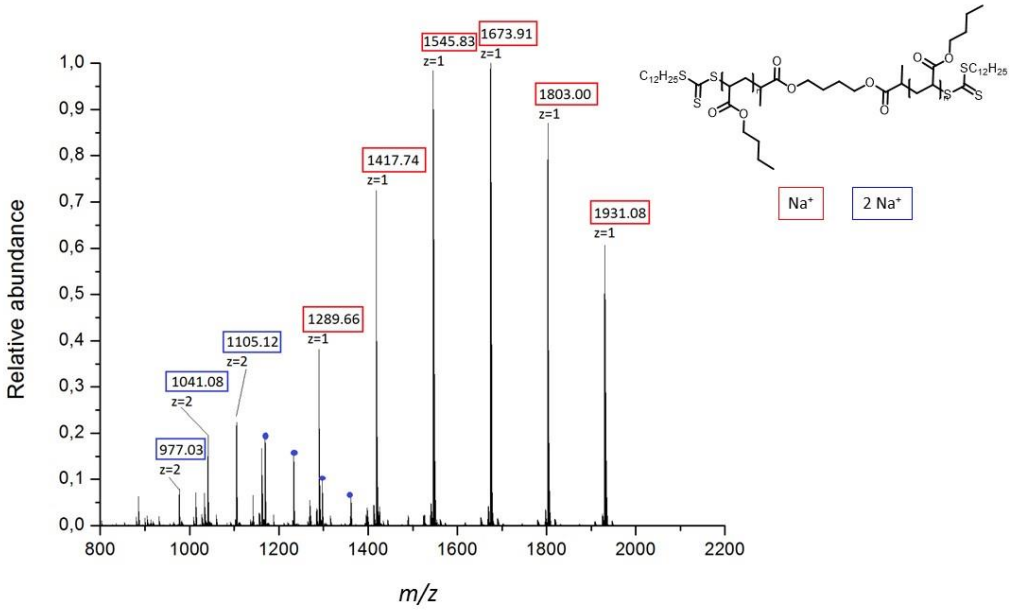


Figure 3: ESI-MS spectrum of RAFT-PBuA made with 10 eq. of monomer and a reaction time of 90 minutes. The red marks indicate the single charged PBuA RAFT polymer with the RAFT agent present at the end on both sides. The blue marks indicate the double charged homotelechelic RAFT polymers.

Poly (isobornyl acrylate) (P*i*BoA). For the synthesis of P*i*BoA next to 10 eq. of monomer also 20 eq. were tested with the same reaction time of 90 minutes. The 90 minutes reaction time was based on the most successful procedure to synthesize PBuA since both *i*BoA and BuA are acrylate monomers and thus should have similar reaction rates. The average size of the polymer made with 20 eq. of *iBoA* monomer was out of range concerning ESI-MS measurements since the M_n values were around 4500 g·mol^{-1}. For the reaction with 10 eq. M_n values were obtained around 3300 g·mol^{-1} with a Đ of 1.12 (Figure 4). Also for P*i*BoA the

homotelechelic bifunctional nature was confirmed via ESI-MS with the RAFT-agent residing on both sides of the polymer chain (Figure 5).

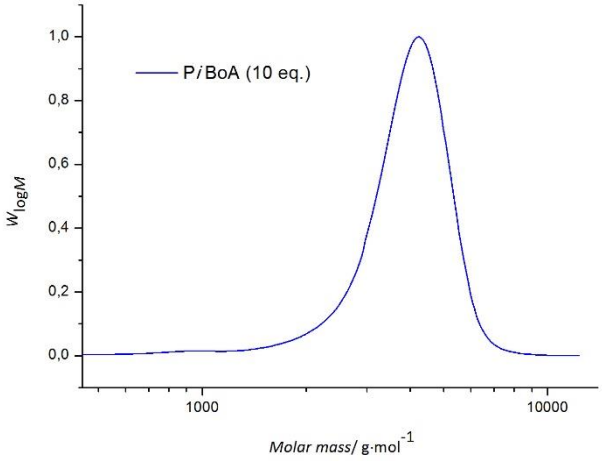


Figure 4: GPC elugram of PiBoA made with 10 eq. of monomer via RAFT polymerization. The distribution had a M_n of 3300 g·mol^{-1} and a $Đ$ of 1.12

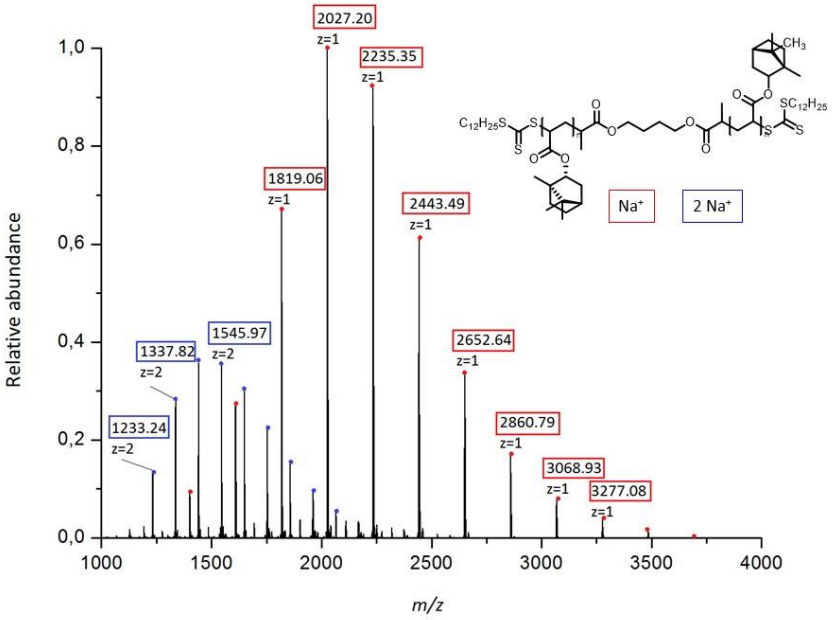


Figure 5: ESI-MS spectrum of BiDopAT-PiBoA. The red marks indicate the single charged RAFT polymer with the RAFT-agent residing at both ending. The blue marks indicate the double charged homotelechelic RAFT polymers.

Poly(N-isopropyl acrylamide) (PNIPAM). Due to issues concerning the presence of monomer in click reactions of acrylates (see step growth polymerization section) the aim of optimization was rather an as high as possible conversion[24] then be able to control the products end group purity via ESI-MS. The obtained GPC results for PNIPAM obtained after 3.5 hours and 6 hours reaction time did not show any significant progression concerning polymer size (Figure 6). This means polymer size is reaching its

maximum length and so is indeed the conversion^{NMR} (98 %). For the 6 hours reaction an M_n of 3900 g·mol^{-1} was obtained with a $Đ$ of 1.16.[13]

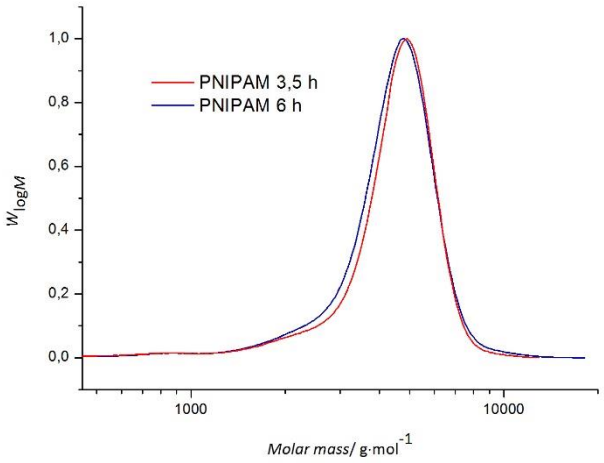


Figure 6: Overlay of GPC elugrams of RAFT PNIPAM made with 20 eq. of monomer after 3.5 and 6 hours reaction time. For the 6 h reaction an M_n 3900 g·mol^{-1} and a $Đ$ of 1.16 was obtained.

RAFT-polymerization with BiDTB

Poly (methyl methacrylate) (PMMA). Polymers with two different sizes were prepared. For the polymer with low molecular weight and thus the smallest M_n of around 2000 g·mol^{-1} and a $Đ$ of 1.25[14], 20 eq. of monomer were used. This polymer has the desired size for ESI-MS measurements. The other polymer with 40. eq of monomer had a M_n of 4300 and a $Đ$ of 1.31. This polymer was used for thiol-ene reactions since its higher molar mass favours precipitation in methanol, which makes purification more feasible. It also is later used for a chain extension polymerization with polystyrene. GPC spectra of both polymers were presented in overlay in Figure 7. ESI-MS of the BiDTB PMMA reveals that a significantly high amount of polymer chains contain an initiator fragment on one end of the chain (Figure 8). These polymers are unable to undergo step growth polymerization. Since the relatively high presence of these by-products this will have a negative influence on the step-growth results. For future studies, this problem might be solved by using a lower amount of initiator or by trying to limit the polymerization to lower conversions. In Vandenbergh et al.[25] is stated that a higher percentage of living chain ends (RAFT agent as end group) is observed when a lower concentration of AIBN (initiator) is used.

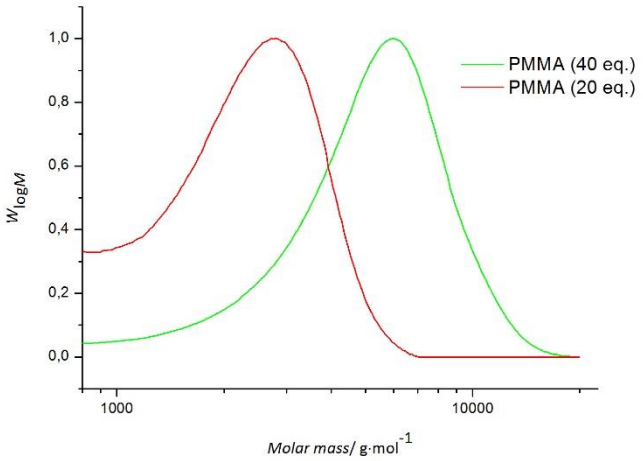


Figure 7: Overlay of GPC elugrams of PMMA made by RAFT polymerization using 20 and 40 eq. of MMA monomer with a reaction time of 3 hours.

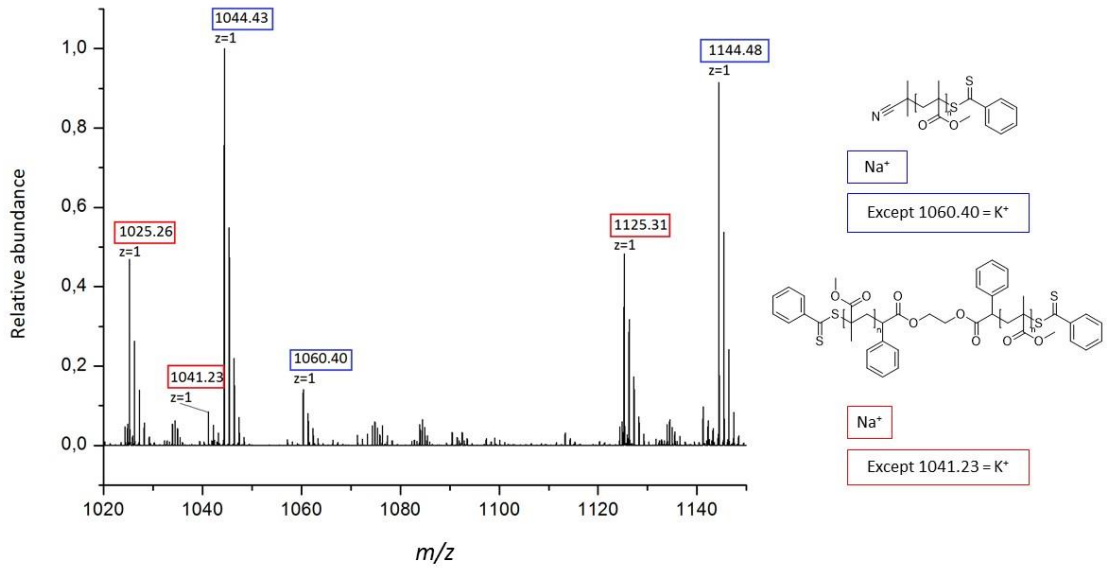


Figure 8: ESI-MS spectrum of PMMA made with 20 eq. of monomer. The peaks indicated by a blue rectangle display the RAFT polymers, which obtain a AIBN fragment. The red rectangles display the polymers, which have both RAFT end groups. Both Na^+ as well as K^+ ionized species are observed.

Polystyrene (PS). Polymerization with BiDTB as the RAFT-agent proceeded slower than the PS synthesized with BiDoPAT. After 6 hours a M_n of 536 g·mol^{-1} and a $Đ$ of 1.24 was obtained with a conversion^{NMR} of 16 % (Figure 9), which means a short oligomer compared to the polymerization with BiDoPAT (PS: 2300 g·mol^{-1}) is formed. Since such long reaction times are needed to obtain only a very short polymer, it can be stated that severe inhibition and retardation is taking place. BiDTB as such is therefore not well suited to control

the polymerization of styrene. Therefore, this oligomer was not further used in any aminolysis/ thiol-ene reactions.

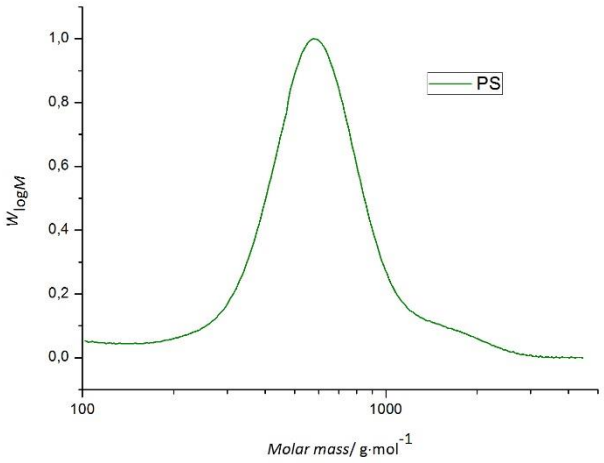


Figure 9: The GPC elugram obtained for polystyrene made by RAFT polymerization with 40 eq. of styrene monomers and a reaction time of 6 hours using BiDTB as the CTA.

Chain extension. To see whether certain side reactions can be avoided (see section aminolysis on PMMA) the PMMA polymer is chain extended with styrene monomers. The results from the chain extended polymer showed a minor shift of the distribution in GPC compared to the PMMA starting material. The PMMA with a M_n around 4300 g·mol^{-1} is used as macroRAFT agent. The obtained polymer after chain extension had a M_n of 4500 g·mol^{-1} and a $Đ$ of 1.34, so only a few styrene units have been built in, which corresponds to the earlier findings concerning the troublesome polymerization of styrene using BiDTB.

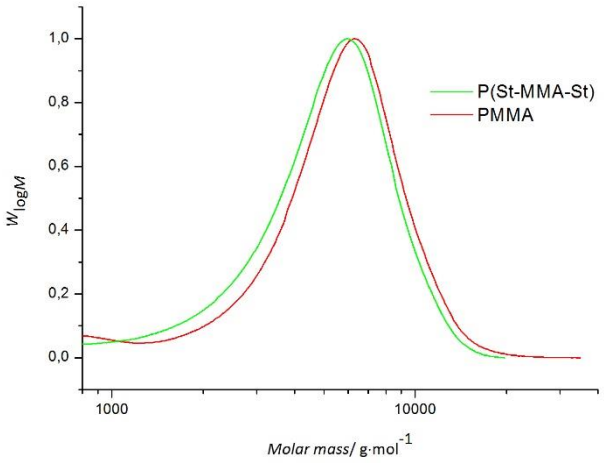
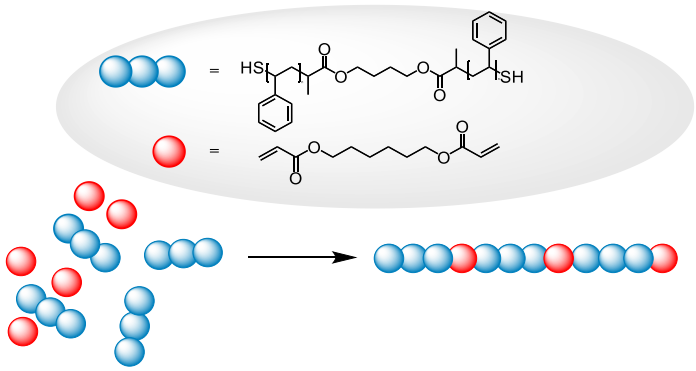


Figure 10: Overlay of GPC elugrams of PMMA and styrene extended PS-b-PMMA-b-PS. M_n was increased from 4300 to 4500 g·mol^{-1} after chain extension. The obtained Đ was 1.34.

3.2 Aminolysis and (in-situ) step growth click polymerization of BiDoPAT polymers



Scheme 14: Schematic representation of thiol-ene click step growth polymerization of polystyrene

Once all bifunctional RAFT polymers were synthesized, the aim was to convert the thiocarbonyl thio end groups via aminolysis into thiols. The so-obtained dithiol polymers can then be used in a thiol-ene step growth polymerization by reacting with a diacrylate (Scheme 14). Both reactions can either be performed in-situ (one-pot), or by separate aminolyis, isolation of the polymer and subsequent thiol-ene polymerization. Both systems display different advantages and disadvantages, as explained in the following sections.

BiDoPAT-Polystyrene

Aminolysis and in-situ thiol-ene click (one-pot) reaction. As a first study, the aminolysis and the thiol-ene step growth polymerization were performed in-situ. This means aminolysis and thiol-ene addition were carried out in one vial without the purification of the aminolysis product in between both steps. This method has the interesting feature and advantage of avoiding the unpleasant smell of thiols, which are formed during aminolysis. Even more importantly, the thiol-end capped polymers will be able to react instantaneously with the diacrylate moieties, thereby avoiding the chance of reacting with themselves into undesired disulphide side products. For the initial tests with varying acrylate/thiol ratios, 3eq. of hexylamine were used. In all cases, only minor shifts of the SEC elugrams towards higher molar masses were observed. The reason for failure of reaction can be explained by the low amount (3eq.) of hexylamine used for aminolysis and thiol-ene step growth. In theory when performing aminolysis on one trithiocarbonate moiety 2 molecules of hexylamine are needed. Since a bifunctional RAFT-agent is used, at least 4 eq. is consumed for the entire aminolysis of RAFT polymer. So in order to be able to perform both aminolysis and subsequent thiol-ene reactions, at least 4.1 eq. of hexylamine should have been used. The only information that could still be gathered from these experiments, was the influence of oxygen on the aminolysis reaction. For the reaction in ambient atmosphere a clear shoulder arises towards a higher molar mass (Figure 11). This difference is explained by the effect of oxygen, which induces formation of disulphide bridges between the thiol-PS chains. For the reaction in inert atmosphere the shoulder is absent.

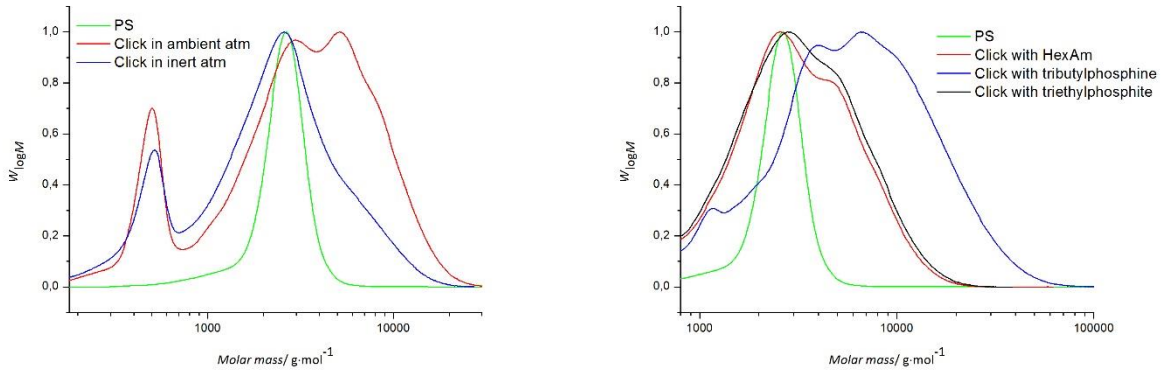
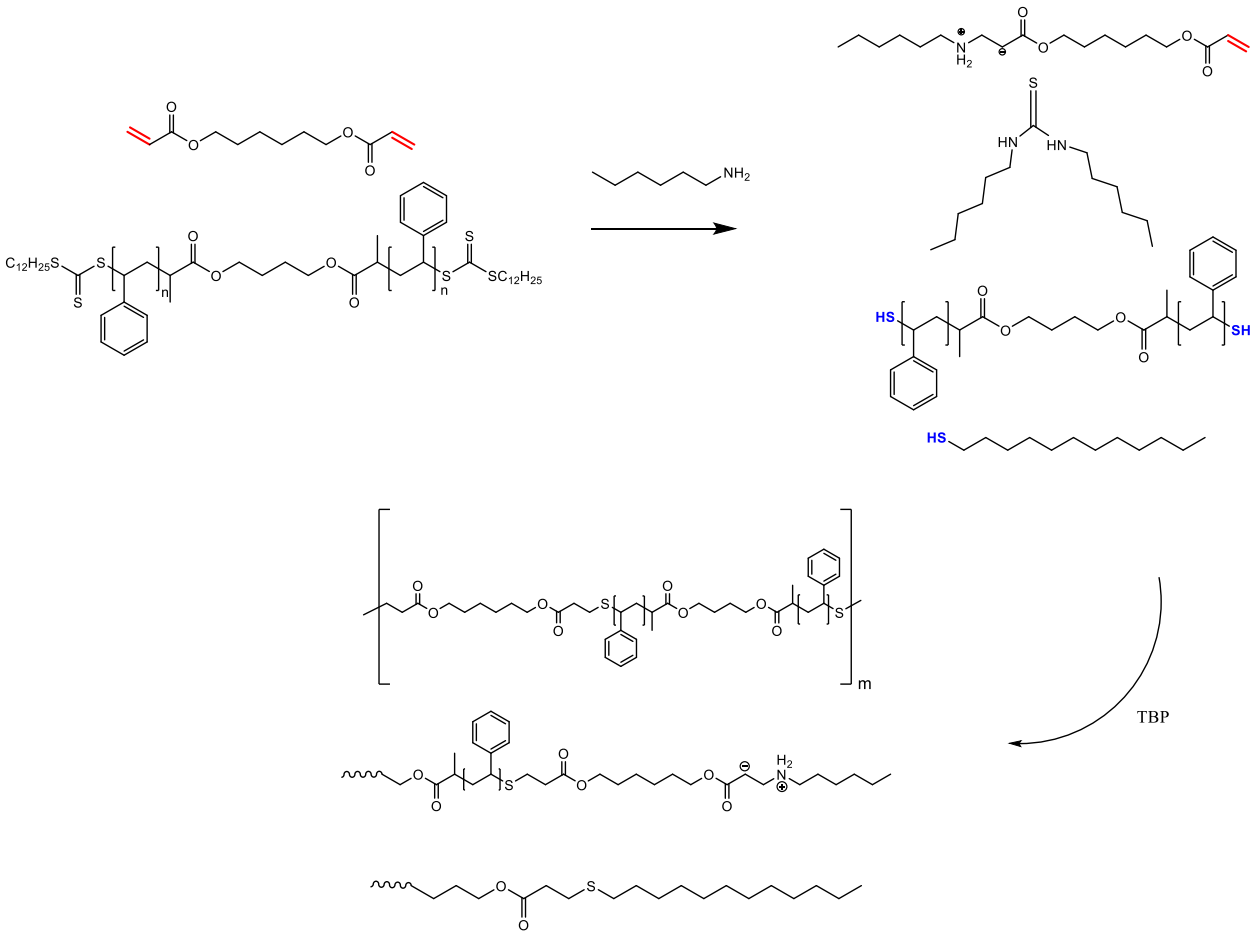


Figure 11: Left figure shows an overlay of GPC elugrams that display the difference between the influence of an in situ reaction in inert or ambient atmosphere under given conditions. The right graph shows an overlay of GPC data displaying the difference between various catalysts in inert atmosphere.

In the next series of experiments, 5 eq. of hexylamine were used and an extra reducing agent (TBP or TEP) was added to avoid formation of disulphides after aminolysis. Furthermore, the reducing agents are also nucleophilic catalysts, which are able to execute a thiol-ene reaction. The reactions were carried out in inert atmosphere. For the reaction with hexylamine/TBP the result displays a clear shift towards higher molar masses. This indicates a successful stepwise polymerization and possible catalysis by the phosphine on top of the amine. Up to four dithiol RAFT-polystyrene building blocks are conjugated. The size of the polymer is likely limited due to the interference of the dodecanethiol tails, which are also produced during aminolysis (Scheme 15). This means that for one pot reactions the equivalent of acrylates actually must be doubled to obtain an equimolar condition. Furthermore, the monofunctional dodecantehiol tails are able to end cap the growing chain and thus limiting the size of the step growth polymer. Also an excess of the amine (which is preferred for aminolysis) might interfere with the in-situ step growth reaction. In excess the amine can attack the unreacted acrylates to form a zwitterion, which is also able to end-cap the polymer[(9)].



Scheme 15: Schematic representation of products formed during aminolysis and step growth polymerization using BiDoPAT as RAFT-agent. The monofunctional thiol and acrylate molecules end cap the step growth process. Every blue denoted thiol functionality can react with every red denoted activated alkene.

For the reaction with TEP only one shoulder in the GPC elugram with a relatively small shift was observed. However, this was also the case for the blank containing only hexylamine. For both these reactions assumedly disulphides have formed instead of thiol-ene click product. Important to note is that although the reactions were carried out in inert atmosphere, the resulting products were measured in a THF-GPC, which also can induce disulphide formation during analysis (see section aminolysis of PS). So it may be assumed that the disulphides were not formed during reaction, but only appear during analysis.

In general, it can be stated that the use of a two-step reaction is recommended. This to purify the aminolysis product from the dodecanethiol side products and to limit the amount of hexylamine required for the click reactions.

Aminolysis. The shift towards slightly lower molar mass in the GPC spectra indicate successful aminolysis based on the mass of the thiols side products, which is 488.26 g·mol^{-1} for both side chains of the RAFT polystyrene. This is clearly visible for al aminolysis reactions. Some shifts, however, are accompanied by another shoulder towards a higher molar mass. As mentioned earlier the dithiol polymers tend to form disulphides. The formation of disulphides is enhanced when performing the aminolysis reaction in THF. This is caused by presence of peroxides in the THF. For aminolysis in DCM or DMF this shoulder is less

pronounced. The usage of the THF-GPC has to be taken into account in this situation since the disulphides are slowly formed in the GPC columns as well. To compare and to demonstrate this a sample was also measured in a DMF-GPC and a chlorobenzene GPC (Figure 12). As expected no shoulder was observed. Furthermore, when adding TBP as reducing agent after an aminolysis reaction, any formed disulphides can be cleaved again[26].

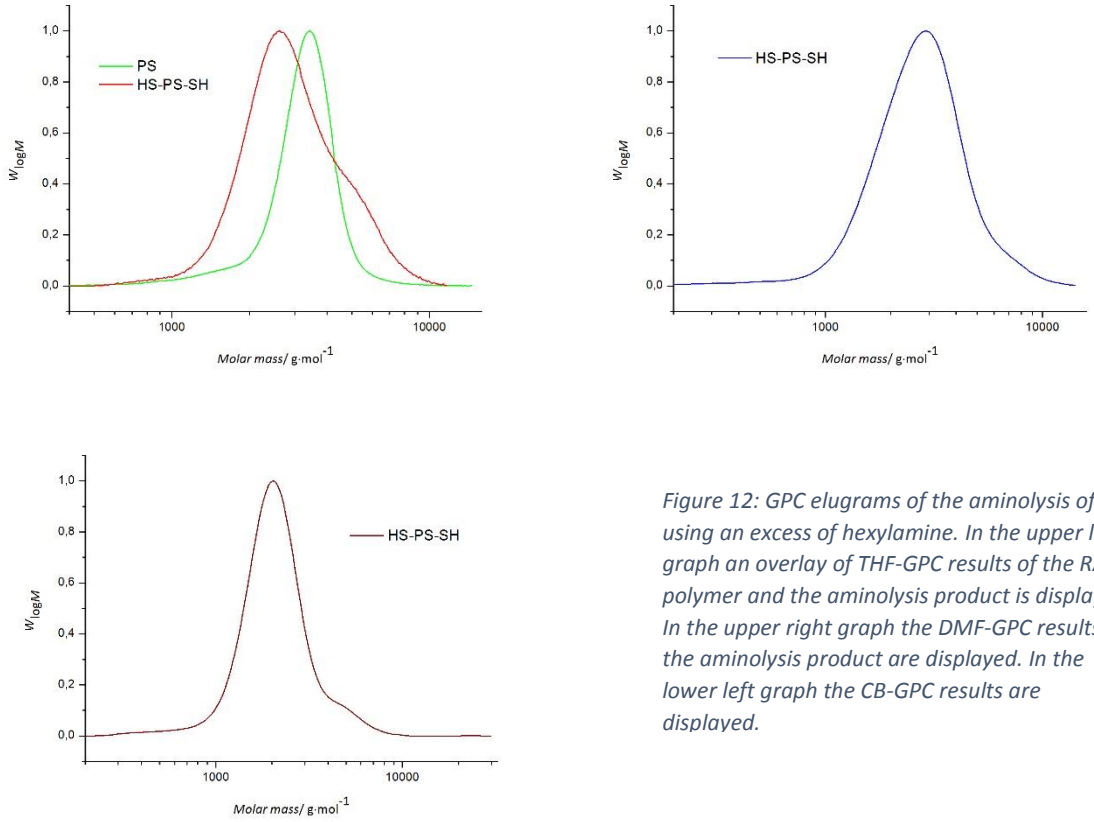


Figure 12: GPC elugrams of the aminolysis of PS using an excess of hexylamine. In the upper left graph an overlay of THF-GPC results of the RAFT polymer and the aminolysis product is displayed. In the upper right graph the DMF-GPC results of the aminolysis product are displayed. In the lower left graph the CB-GPC results are displayed.

To purify the sample from all the remaining reagents and from the side products polystyrene was easily precipitated in cold MeOH and isolated via decantation. The precipitate had a rather white colour unlike the RAFT-polymer, which displayed a distinct yellow colour. The colour change from yellow to white also indicates successful removal of the trithiocarbonate groups into thiols by aminolysis.

Thiol-ene click step growth. The effect of two different catalysts namely hexylamine and TBP was compared. Differences are already displaying after two minutes. For the reaction with the amine catalyst only one shoulder is emerging towards higher molar masses, not making many progress with longer reaction times. This shoulder is likely due to disulphides formed during the GPC measurement, which is possible when assuming the click reaction did not occur. For the reaction with phosphines after 2 minutes multiple peaks are visible in the GPC elugrams, which is characteristic for a step growth polymerization. When prolonging the reaction time, peaks gradually emerge towards higher molar masses (Figure 13). The M_p of a chosen peak happens to be the sum of two preceding peaks, which again indicates successful step growth polymerization. For reaction times longer than 60 minutes not much improvement is observed. The size of the polymer was limited to a M_n of 7000 g·mol^{-1}, a maximum peak of around 20000 g·mol^{-1} and tailing towards 100000 g·mol^{-1}. This polymerization was successful when using only minimal amounts of

phosphine (0.2 eq.). When using more phosphine there is a higher tendency to attack the acrylates and therefore to quench the reaction since there are too many active centres. The nucleophilic attack is necessary for the catalysis but the active zwitterion has to be formed in situ in a controlled fashion. To assure this the phosphine and acrylates should be added together at the last moment. The presence of this zwitterion is indicated by a red colour.

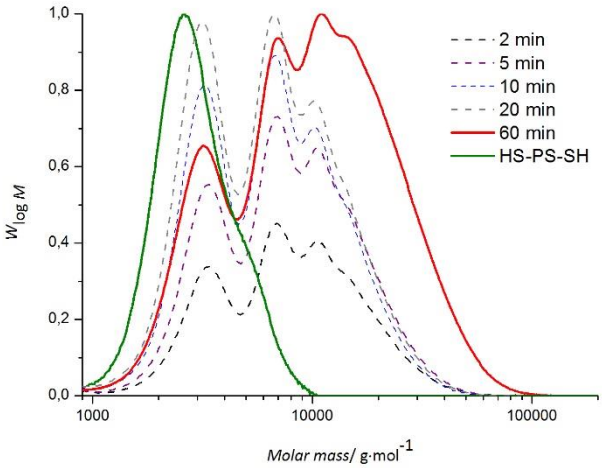


Figure 13: Overlay of GPC results displaying the influence of reaction time on thiol-ene click step growth polymerization of PS. Step growth polymerization was performed with 0.2 eq. of phosphine and with equimolar amounts of diacrylate (HDDA) and dithiol PS. The reaction time was varied between 2 and 60 minutes.

When using a certain percentage of branched acrylates (TAEIC or PETA) in addition to the diacrylate, tailing towards higher molar masses is observed compared to the linear step growth polymerization (Figure 14). This effect is more pronounced when using the four arm star PETA. The effect of step growth polymerization displayed in the multiple peaks is still visible in the GPC elugrams. When using a higher percentage of branched acrylates the tailing is more prominent. GPC data could not be obtained from the reactions with only branched acrylates (no linear acrylates) since dense insoluble networks were produced.

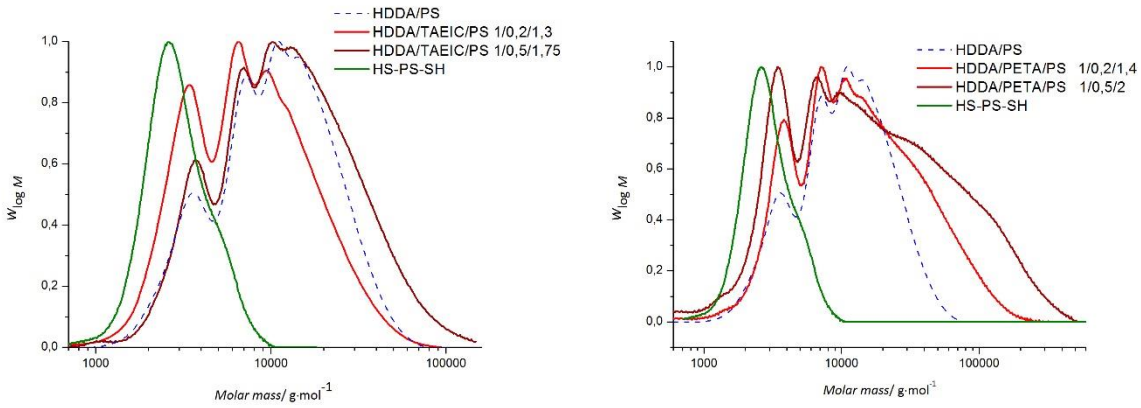


Figure 14: GPC results of step growth polymerization of PS with different ratios of multifunctional acrylates. In the left figure TAEIC is used as the acrylate. In the right figure PETA is used. The reaction was catalyzed with phosphine (0.2 eq.) and stirred for 2 hours.

BiDoPAT-poly(butyl acrylate) (BuA) and BiDoPAT-poly(isobornyl acrylate) (PiBoA)

Aminolysis and in-situ thiol-ene click (one-pot) reaction. The protocol for PBuA was based on the most successful formula used for PS. Poor results were obtained since there was only one shoulder observed towards a higher molar mass, which possibly indicates the formation of disulphides and thus unsuccessful click reaction (Figure 15). Since the reaction was not performed in THF but in acetone in presence of a reducing agent it is more likely the disulphides are formed during the THF-GPC measurement. To isolate the products a rotavap was used. PBuA is very difficult to precipitate and is therefore difficult to separate from side products and remaining reagents. That way the earlier mentioned dodecanethiol, which is also formed after aminolysis is able to end-cap the growing step growth polymer. The extra peak in the GPC elugram around 500 g·mol^{-1} is most likely a collection of side products formed by the reaction of one or two dodecanethiols or with unoccupied diacrylates. As explained in the following section also monomer leftovers might have reacted with these thiols.

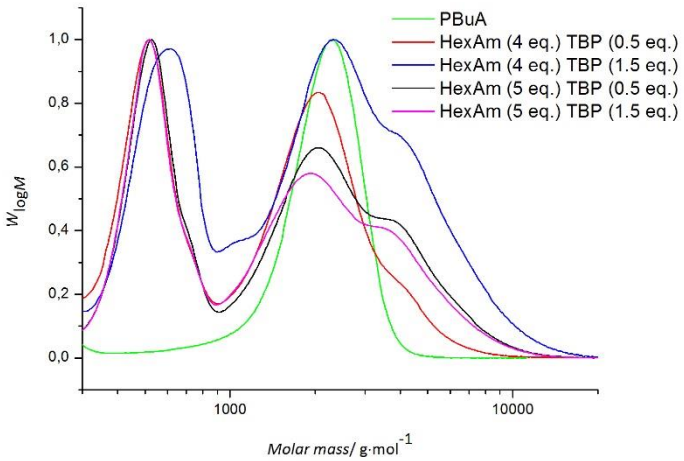


Figure 15: Overlay GPC elugrams of PBuA after in situ click reactions with varying conditions concerning hexylamine and TBP. PBuA and HDDA were used in equimolar amounts. The reaction was stirred overnight.

Aminolysis. When using acrylate polymers instead of PS, a new problem arises. After RAFT polymerization, usually a few percentages of monomer are trapped inside the polymer matrix. In the case of PS the monomer (styrene) does not contain an activated ene moiety. In the case of PBuA or P*i*BoA leftovers of BuA or *i*BoA monomers will remain in the matrix, which later on will interfere with the desired diacrylate during the thiol-ene reaction. The acrylate monomer leftovers will therefore also endcap the thiol(-ene) polymers. As a solution for this problem all PBuA and P*i*BoA RAFT polymers were purified over a Biobeads-SX1 column, which separates the polymer from the monomer leftovers. Subsequently, aminolysis was carried out. For both PBuA and P*i*boA results were poor after isolation of the aminolysis product. Only undesired disulphide formation likely caused an extra shoulder in the GPC spectra. Since no precipitation is possible for dithiol PBuA, dodecanethiol side products are assumed to be in the mixture. As mentioned earlier these thiols might endcap the click reaction and therefore give poor step growth results. P*i*BoA on the other hand can be precipitated in cold MeOH when the molar mass is around 4000 g/mol^{-1} or larger, thereby removing the dodecanethiol side products.

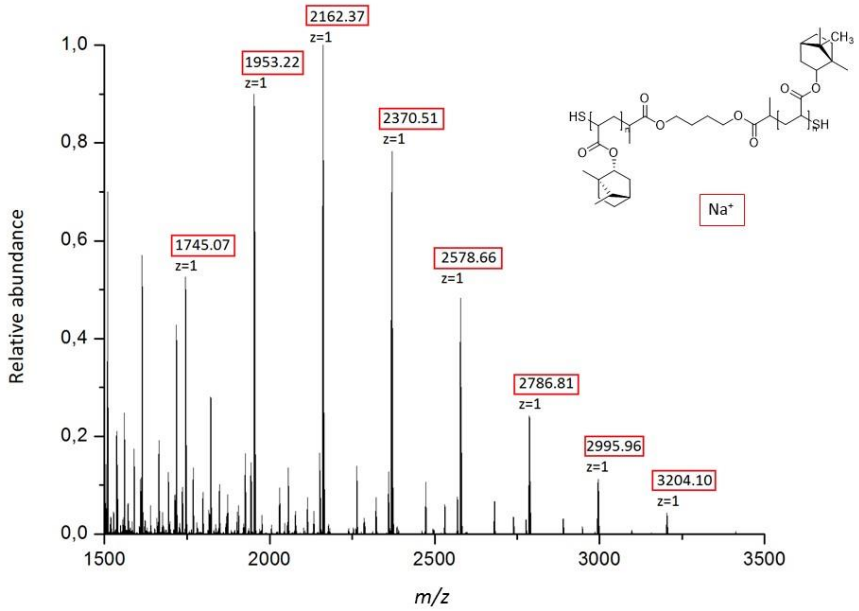


Figure 16: ESI-MS spectrum of PiBoA with dithiol end groups obtained after aminolysis in an excess of hexylamine . The red marks represent the single charged homotelechelic dithiol PiBoA

Thiol-ene click step growth. When the precipitated dithiol P*i*BoA was used in a thiol-ene step growth reaction no satisfying shift in molar mass was observed. This indicated that precipitation of the polymer is insufficient as purification method. Therefore, another batch of dithiol P*i*BoA obtained after aminolysis was purified using recycling GPC. This is a technique by which molecules can be separated based on their size (molar mass). The ESI-MS spectrum of the purified aminolysis product of P*i*BoA confirmed the homotelechelic nature of P*i*BoA with both thiols as end-groups (Figure 16). The thiol-ene reaction after purification with recycling GPC showed promising results (Figure 17). Also precipitation of the resulting thiol-ene product was easier compared to products from unsuccessful reactions.

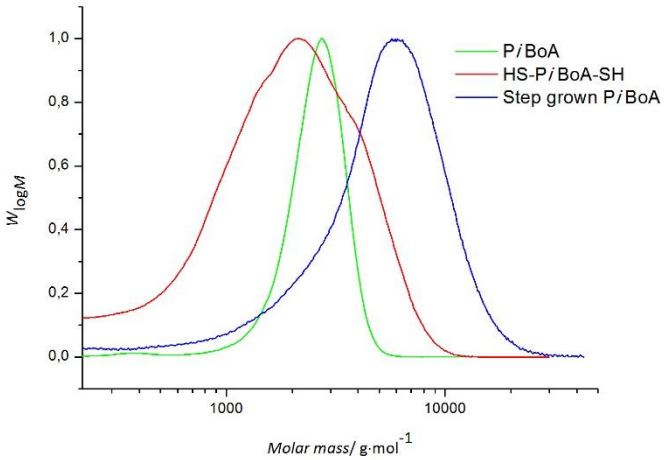
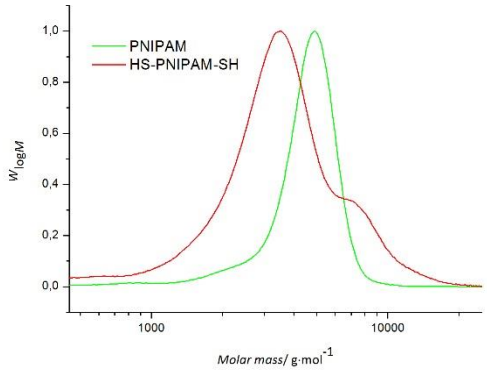
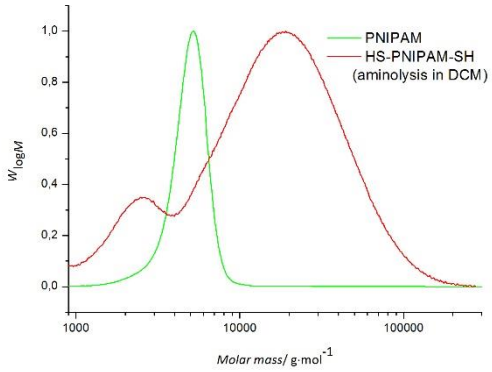


Figure 17: Overlay of the GPC results of PiBoA RAFT polymer, aminolysis product obtained after using an excess of hexylamine and step growth polymer made with dithiol PiBoA – after purification over a recycling GPC- and HDDA, catalyzed with 0.2 eq of TBP .

BiDoPAT-poly(N-isopropyl acrylamide) (PNIPAM)

Aminolysis. For PNIPAM, the RAFT polymer was first purified over a Biobeads® column in order to remove any monomer leftovers, since acrylamides are also susceptible to thiol-ene reactions. Afterwards, aminolysis was carried out in 2 solvents, either acetone or DCM. Resulting products were precipitated in cold hexane. The upper left figure shows the SEC profile after aminolysis in acetone (Figure 18). The shift towards lower molar mass indicates successful aminolysis, along with a shoulder corresponding to some disulphide products formed in-situ during the SEC measurement. The right figure shows the SEC result for aminolysis carried out in DCM. When performing the aminolysis in DCM instead of acetone a very broad peak with a huge shift was observed with an M_P of 22000 g·mol^{-1}. A certain amount of TBP (> 1 eq.) was added to eliminate the chance of excessive disulphide formation. However, this did not seem to have a significant influence on the result. Up until now no reasonable explanation was found for this phenomenon.





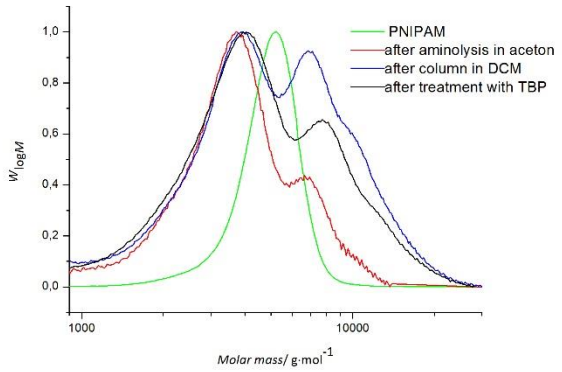


Figure 18: Upper left graph shows overlay GPC results of PNIPAM before and after aminolysis in acetone. Upper right graph shows on overlay of GPC results from PNIPAM before and after aminolysis in DCM. Lower left graph displays on overlay of GPC results of PNIPAM before and after aminolysis in acetone, after the subsequent Biobeads® column in DCM and after the subsequent treatment with TBP.

Thiol-ene click step growth. The dithiol PNIPAM obtained after aminolysis in acetone was subjected to a thiol-ene click polymerization with BDDA. However, after reaction there were no visible indications of a successful step growth present in the GPC elugram (Figure 19). The changes in the elugram are minor. The precipitation procedure to purify the dithiol PNIPAM is likely insufficient and other methods like using a biobead® column or a recycling GPC could give more promising results. Note, however, that the recycling GPC, which runs on DCM might lead to the formation of a large polymer like in the upper right graph of Figure 18, which cannot be further used in the click thiol-ene process.

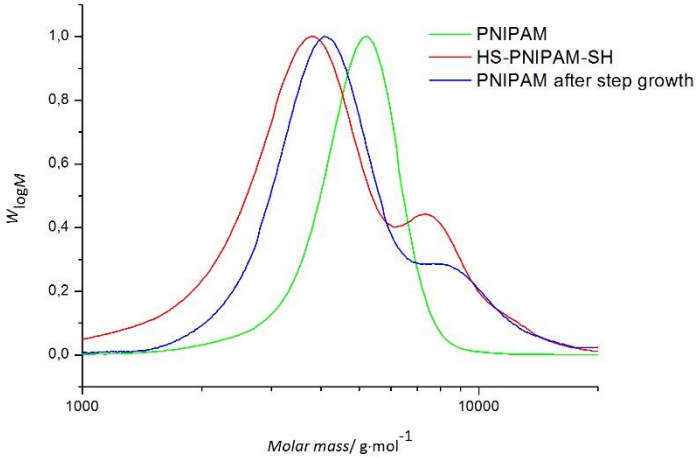
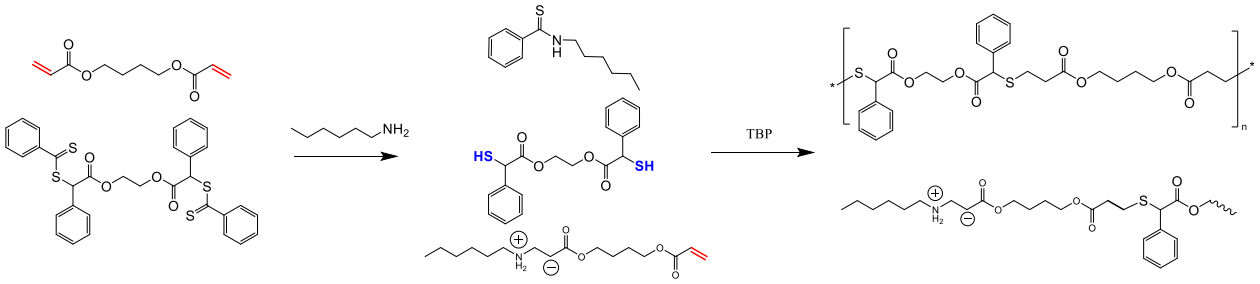


Figure 19: Overlay of GPC data of PNIPAM to compare the results before and after aminolysis to the subsequent step growth polymerization performed with 0.2 eq. of TBP and equimolar conditions concerning acrylates and dithiol PNIPAM.

3.3 Aminolysis and (in-situ) step growth click polymerization of BiDTB RAFT polymers

On BiDTB RAFT agent

Aminolysis and in-situ thiol-ene click (one-pot) reaction. To test whether the BiDTB RAFT agent is suitable for a thiol-ene click reaction pure RAFT agent was linked by BDDA as acrylic linker. In the results obtained from GPC multiple peaks are displayed (Figure 20). These peaks and tailing are an indication of a step wise growing process, which means a successful step growth polymerization might have occurred.



Scheme 16: Schematic representation of products formed during aminolysis and step growth using BiDTB as RAFT-agent. No monofunctional thiol byproducts, which are able to inhibit the step growth process are formed. Every blue denoted thiol functionality can react with every red denoted activated alkene.

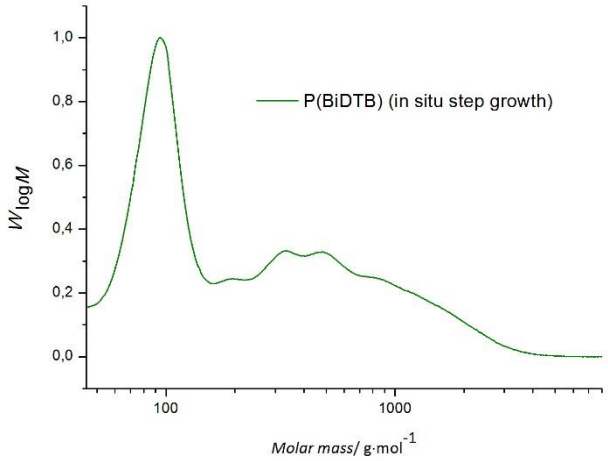


Figure 20: GPC elugram obtained from in situ step growth polymerization of pure BiDTB and BDDA in equimolar amounts catalyzed with 0.2 eq of TBP, aminolyzed with 2.1 eq. of hexylamine and stirred for 2 hours

When the same in situ aminolysis and thiol-ene reaction is carried out on the BiDoPAT RAFT agent, no success was obtained. This result shows the advantage of working with a dithio RAFT agent like BiDTB instead of a trithio RAFT agent like BiDoPAT. During aminolysis of the dithiobenzoate no dodecanethiol side products are produced that can inhibit the thiol-ene step growth reaction by end capping (Scheme 16). This means a one-step reaction might become possible for these kind of polymers. This has the advantage of avoiding unpleasant thiol odours.

BiDTB-poly(methyl methacrylate) (PMMA)

Aminolysis. In the first aminolysis trials of BiDTB-PMMA 0.5 eq. of TBP was used and the reaction was performed in ambient atmosphere. A significant shift towards higher molar masses compared to the initial RAFT-PMMA was observed on the GPC elugrams. This is in contrast with the expected outcome, which is a shift towards a lower molar masses due to the loss of the benzoate groups. A possible explanation for this event is the formation of disulphides.

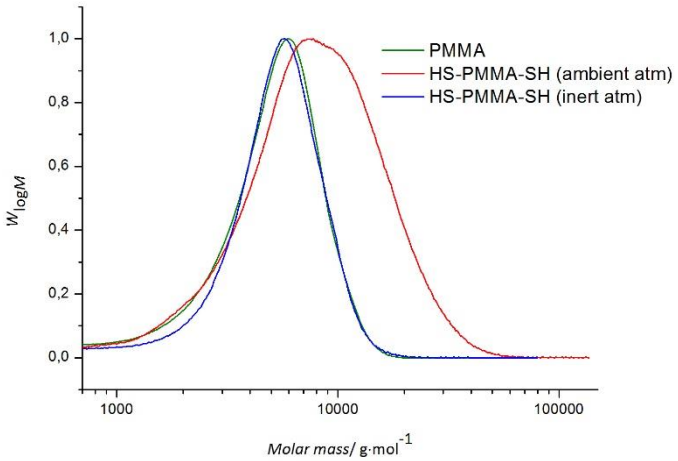


Figure 21: Overlay of GPC elugrams of PMMA before and after aminolysis in an excess of hexylamine performed in inert and ambient atmosphere.

Therefore the reaction was repeated under inert environment and a doubled amount of phosphine was used. The GPC result for this aminolysis displayed a distribution, which was alike the distribution for the initial RAFT-polymer with only an almost negligible shift towards a lower molar mass (Figure 21). The colour of the polymer changed from pink to almost white, indicating successful cleavage of the dithiobenzoate RAFT end groups.

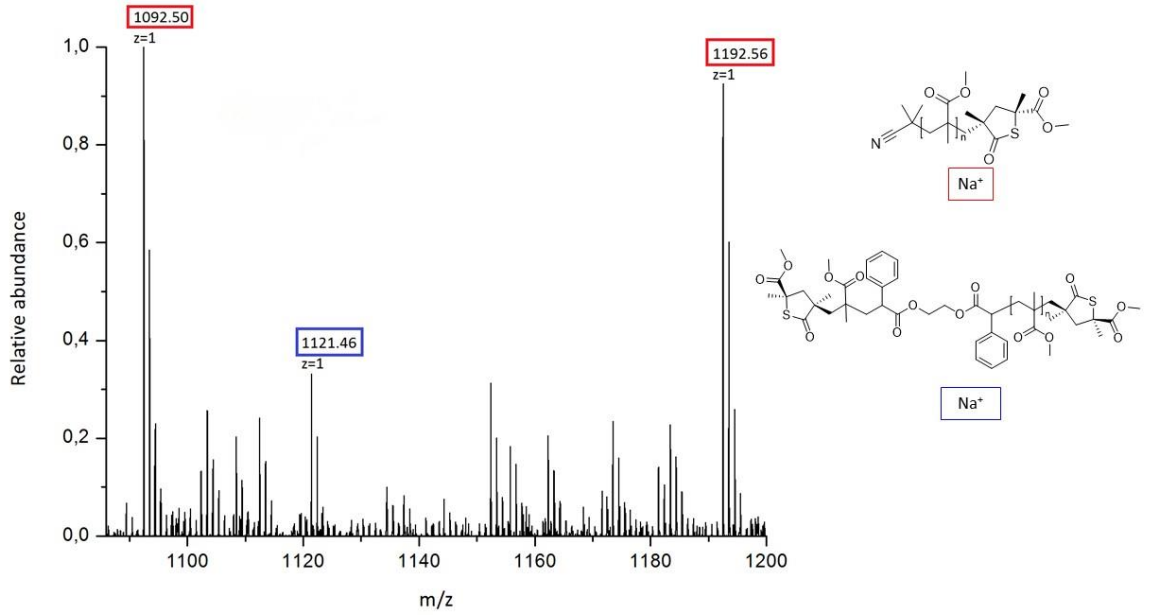


Figure 22:The ESI-MS spectrum of PMMA after aminolysis. The red marks indicate a PMMA polymer containing a initiator fragment on one side and a thiolactone on the other. The blue mark indicates a PMMA polymer with a thiolactone on both sides.

When analyzing the obtained ESI-MS data of the thiol product of PMMA unexpected by products namely thiolactone end groups were observed (Figure 22). Apart from the high amount of polymers containing an initiator fragment the thiol end groups had reacted via backbiting to form thiolactone end groups. These end groups are unable to undergo a thiol-ene click reaction and therefore inhibit step growth polymerization. This is a significant problem when using PMMA for thiol-ene click reaction. A solution for this problem could be the chain extension of PMMA with styrene before performing aminolysis (Figure 10). That way backbiting and thus thiolactone formation might be prevented. Furthermore multiple peaks were obtained, which could not be explained or assigned.

Aminolysis and in-situ thiol-ene click (one-pot) reaction. For this reaction the polymer with an M_n around 4300 g·mol^{-1} is used to facilitate precipitation after reaction. The in-situ reaction was carried out in ambient atmosphere. In the obtained GPC elugram shift towards larger molar mass is observed, indicating a successful thiol-ene click reaction (Figure 23). However, since aminolysis alone (in ambient conditions) also results in a shift towards higher molar masses, the result of the in-situ thiol-ene click reaction must be interpreted with some caution. Because during the one-pot reaction any formed thiol polymer will quickly react further with the added diacrylate (through the desired thiol-ene step growth pathway) it is, however, unlikely that the thiol polymer will have the time to undergo disulphide formation. But again this is not confirmed. Step growth polymerization could be limited by the formation of the earlier mentioned thiolactone moieties causing the polymers to lose their bifunctional thiol nature and therefore the capacity to polymerize via step growth. Again this might be partially overcome by performing an in situ reaction. However, this is yet to be confirmed.

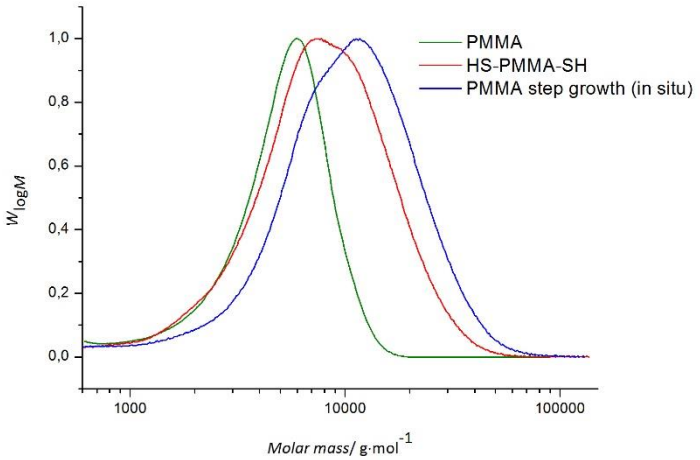


Figure 23: Overlay of GPC elugrams of PMMA before and after aminolysis in an excess of hexylamine and subsequent step growth polymerization catalyzed with Hexylamine (2.1 eq.)

Nevertheless, because the reaction is carried out in ambient atmosphere, the formation of disulphides will be more likely than under inert atmosphere. In the future this phenomenon can be excluded by repeating and testing this reaction in an inert environment with the eventual addition of TBP as a reducing agent.

Thiol-ene click step growth (two-step reaction). This procedure gave similar results as the in situ reaction described above (Figure 24). By first dissolving the aminolysis product in THF together with the phosphine the possible formed disulphides are broken before the click reaction was initiated.

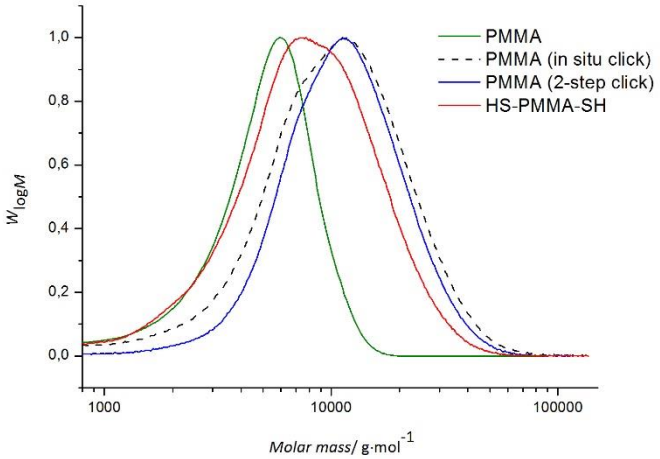


Figure 24: Overlay of GPC elugrams of PMMA before and after aminolysis in an excess of amine and subsequent step growth polymerization, which is catalyzed with TBP (1 eq.). This result is compared to in situ step growth polymerization

3.4 Baylis-Hillman polymerization

Optimization of Baylis-Hillman polymerization

The chosen protocol was based on results of a preceding project. Compared to the thiol-ene click addition the Baylis-Hillman reaction is a very slow reaction exhibiting reaction times of 24 hours or more. Also larger amounts of amine catalyst (1 eq. compared to 0.2 eq. for thiol-ene click) are required. When comparing the catalysts DABCO, 3-HQD and DMAP best results are found for 3-HQD in combination with HDDA. When varying the acrylates HDDA still gave the best results. It is possible that other monomer combinations might also yield satisfactory results. However, this was not tested during this project. The 3-HQD + HDDA GPC elugram had an M_n of 1100 g·mol^{-1} and an M_P of 2200 g·mol^{-1} (Figure 25). This means on average the Baylis-Hillman polymers contained three repeating units. These polymers were used throughout subsequent crosslinking assays.

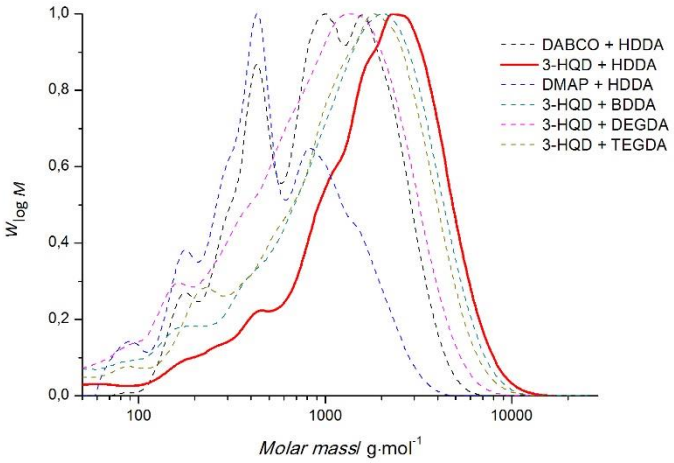


Figure 25: Overlay of GPC elugrams for Baylis-Hillman step growth polymerizations. Catalysts and acrylates are varied and compared. The best results showed a M_n of 1100 g·mol^{-1} and an M_P of 2200 g·mol^{-1}

The ESI-MS data obtained from these Baylis-Hillman step growth polymers synthesized using 3-HQD and HDDA showed that the 3-HQD was able to endcap the polymer by building itself in at the end of the polymer chain (Figure 26). This might contribute to the limited polymer size containing only three repeating units on average.

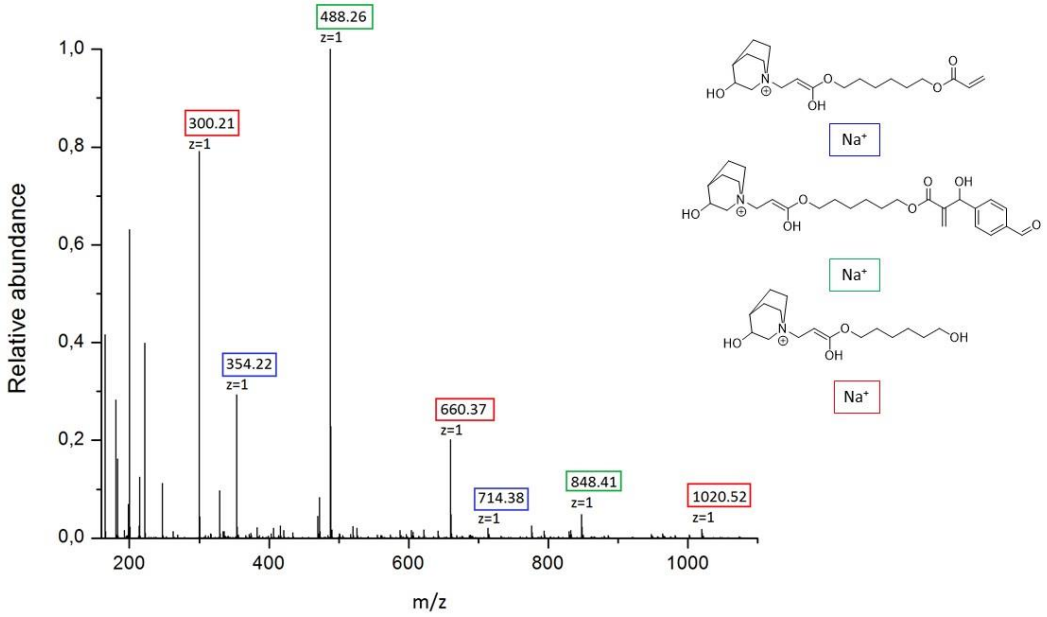


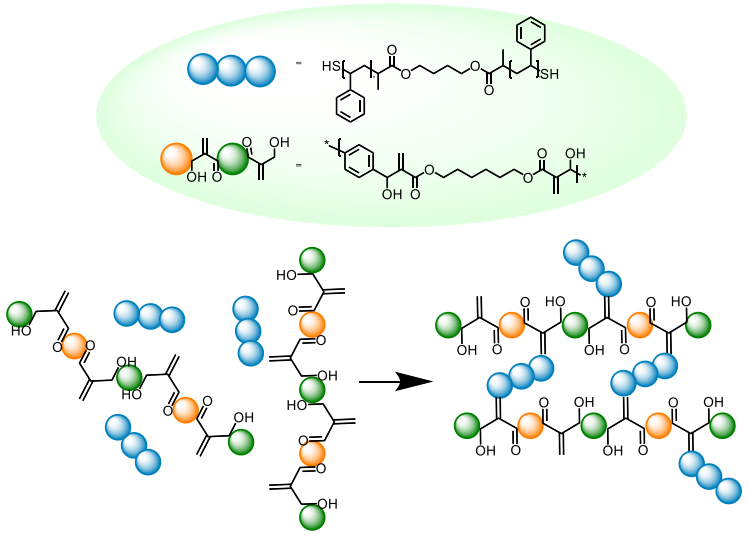
Figure 26: ESI-MS spectrum obtained from the Baylis-Hillman step growth polymers. Red marks represents the molecules, which are end-capped by a monofunctional acrylate impurity and a 3-HQD. The blue marks represent the molecules, which are end-capped with 3-HQD on one side and an unoccupied diacrylate on the other side. The green marks represent the molecules, which are end capped with 3-HQD on one side and an unoccupied terafthalaldehyde on the other side

Crosslinking of Baylis-Hillman polymers

Multifunctional thiols. To crosslink Baylis-Hillman polymers the vinyl groups, which are yielded during a Baylis-Hillman reaction are reacted by using multifunctional thiols. The thiol-ene crosslinking reactions are unlike the Baylis-Hillman polymerization relatively fast. During the polymerization the formed networks precipitated from the solvent (THF), which means the networks became insoluble. This happens within fifteen minutes or less. This counts for both reaction compositions (butanedithiol and PETMP) with the PETMP reaction being slightly faster.

Figure 27: Picture of the insoluble dense networks, which are formed by linking Baylis-Hillman polymers by using PETMP (left vial) and butanedithiol (right vial).

Dithiol polystyrene. Crosslinking Baylis-Hillman polymers with dithiol polystyrene (Scheme 17) catalysed by either hexylamine or TBP gave partly insoluble networks with a gel-like structure. When preparing samples for GPC analysis a certain level of blockage is experienced during filtering, which indicates formation of partly insoluble material. The obtained GPC results coming from the reaction with only TBP provides an indication of crosslinking since there is significant tailing towards higher masses observed (Figure 28). However, this result is hardly representative for the real size distribution since part of the crosslinked network cannot pass the filter.



Scheme 17: Schematic representation of the crosslinking of Baylis-Hillman polymers by using dithiol polystyrene. Unoccupied vinyl groups can be used for further modification of the polymer networks in the future.

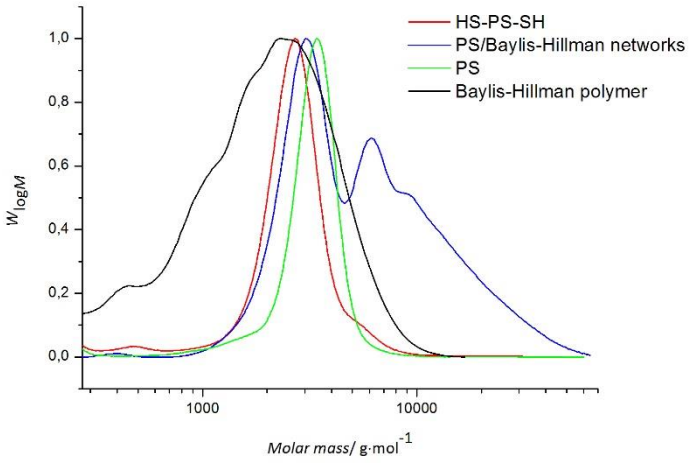


Figure 28: Overlay of GPC elugrams of the initial Baylis-Hillman and PS polymers, the aminolysis product of PS and the crosslinked Baylis-Hillman polymers with dithiol polystyrene.

3.5 Suspension polymerization

The thiol-ene step growth polymerization was also investigated using suspension polymerization in order to produce particles. Since these were the first experiments to perform the reactions in such way simple building blocks like HDDA, PETA and butanedithiol were used. Unlike for the solution thiol-ene click step growth polymerization - where TBP was the preferred catalyst - hexylamine was here the preferred catalyst for the suspension technique. Previous studies compared TBP and hexylamine for the synthesis of particles via emulsion[27]. Hexylamine gave the better results, which is explained by the fact that hexylamine is soluble in water while TBP is not. Therefore hexylamine will have the ability to diffuse from the continuous water phase into the dispersed organic droplet, while TBP will not have that chance. This is required since polymerization takes place in the dispersed monomer droplets. When comparing the reactions with 5 mg/ml PVA and 10 mg/ml PVA as stabilizing agent a clear difference in resulting particle size is observed in images made by optical microscopy (10 X magnification) (Figure 29). The particles from the 10 mg PVA/ml experiment are smaller with cross sections between 30 and 80 µm. The particles from the 5 mg PVA/ml experiment vial have a diameter up to 200 µm.

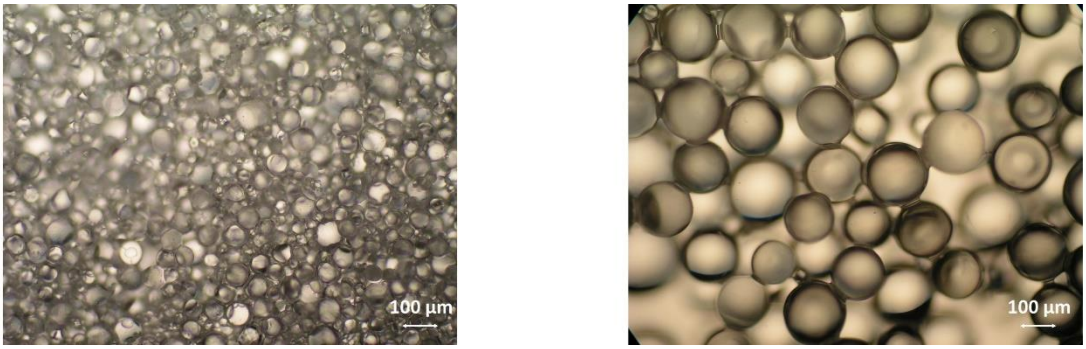


Figure 29: Optical microscopy pictures from particles synthesized using the suspension technique. Magnification is 10X. For the left picture 10 mg/ml PVA is used while for the right picture 5 mg/ml PVA is used.

When a mixture of linear (1 eq.) and branched (0.1 eq.) acrylates was polymerized with a linear dithiol (1.2 eq.), the resulting particles seem to show a certain rate of porosity (Figure 30). Although it is not clearly visible with a standard optical microscope at 10x magnification, SEM will give an improved view. Particles produced from only a linear acrylate (HDDA, 1 eq.) and a tetrafunctional thiol (PETMP, 0.5 eq.) seem to have a more smooth appearance. Apart from the porosity the size of the particles are similar (100-200 µm)

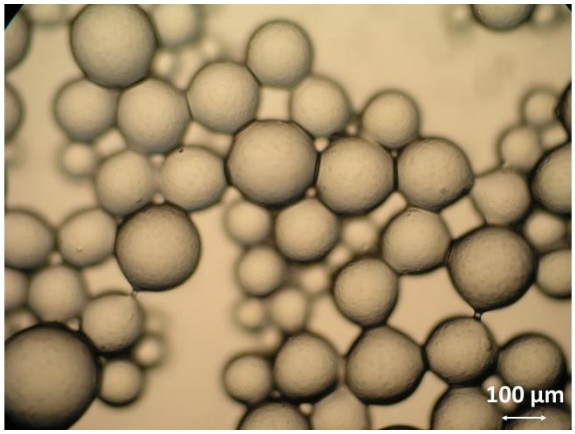


Figure 30: Optical microscopy image from particles synthesized using the suspension technique. 5 mg/ml PVA was used as stabilizer. A mixture of branched (0.1 eq.) and linear (1 eq.) acrylates was used to react with a linear dithiol (1.2 eq.).

Particles produced in this way will in the next step be used for biocompatibility studies (cooperation with UMaastricht, in progress)

4 Conclusion

To be able to click polymers together via step growth polymerization bi-functional RAFT agents were produced namely bis-2-([(Dodecylsulfanyl)carbonothioyl]sulfanyl) propanoic acid (BiDoPAT) and ethane-1,2-diyl bis(2-phenyl-2-((phenylcarbonothioyl)thio)acetate), which is a dithiobenzoate (BiDTB). When using BiDoPAT the desired M_n was obtained after adjusting the procedure depending on the type of monomer. This was successfully done for PS (2300 g·mol^{-1}, $Đ$ = *1*.17), PBuA (1600 g·mol^{-1}, $Đ$ = 1.17), P*i*BoA (3300 g·mol^{-1}, $Đ$ = 1.12) and PNIPAM (3900 g·mol^{-1}, $Đ$ = 1.16). End group fidelity was confirmed by ESI-MS measurements, which ensured the homotelechelic nature of the polymers.

One-step in situ step growth polymerizations on the trithiocarbonate functionalized polymers were challenging and did not yield the desired results. This is because aminolysis of the RAFT end group not only resulted in the desired dithiol telechelic polymers, but also in dodecanethiol side products, which impede proper thiol-ene step growth by end capping reactions. Furthermore, in the case of polyacrylates and PNIPAM, leftovers of monomer trapped inside the polymer matrix are in competition with the intended diacrylates used for the thiol-ene step growth polymerization. The monomer leftovers will also end cap the step growth polymers in a premature state. To this end, all further reactions on BiDoPAT functionalized polymers were carried out in 2 separate reactions: first aminolysis, followed by purification and then the thiol-ene step growth reaction.

For the two-step system, aminolysis and further step growth polymerization for BiDoPAT PS was successful (M_n = 7000 g·mol^{-1}, M_p= 20000 g·mol^{-1}, tailing towards 100000 g·mol^{-1}). The polymer was easily purified by precipitation after the separate aminolysis step and therefore freed from residual monomer and dodecanethiol by-products. The click reaction was executed with TBP, which was able to reduce the eventually formed disulphides and catalyse the Michael addition efficiently. When this 2-step procedure was performed on BiDoPAT functionalized polyacrylates or PNIPAM several difficulties were experienced. PBuA was impossible to precipitate while PNIPAM and P*i*BoA could only be partially purified by precipitation so monomer leftovers stayed trapped inside. To this end, all RAFT polymers were first purified over a BioBeads® column to remove monomer traces. Furthermore, after aminolysis the polymers had to be purified again on a recycling GPC system in order to entirely remove dodecanethiol. Only after this circuitous procedure, some success was obtained in the final thiol-ene step growth reaction for P*i*BoA ($M_{n,\ RAFT\ PiBoA}$ = 2300 g·mol^{-1}, $M_{n,\ click}$ = 3300 g·mol^{-1}, $M_{p,\ click}$ = 5300 g·mol^{-1}). In the case of PNIPAM the results were not explicable at this moment since executing the aminolysis in DCM resulted in a tremendous increase in molar mass. For now, the use of this polymer was abandoned.

To avoid formation of dodecanethiol side products in aminolysis the use of an alternative bis-dithiobenzoate RAFT agent is investigated. For styrene the polymerization was very slow with only very low conversions. This shows the BiDTB RAFT-agent is not the proper CTA for styrene. On the other hand, this RAFT agent successfully polymerized MMA. However, investigation of the end group fidelity with ESI-MS revealed a substantial fraction of chains that carried an AIBN fragment on one side of the polymer chain. These chains are therefore no longer bi-functional substituted and will after aminolysis and thiol-ene click disrupt chain coupling. This will severely limit the molar mass that can be achieved through step growth polymerization. It is believed that the RAFT polymerization of BiDTB-PMMA can be optimized by decreasing the initiator concentration (to about 0.01 equiv. AIBN) and by aiming for lower conversions.

For the step growth polymerization of the current BiDTB-PMMA samples, no clear conclusion can be drawn. After aminolysis in ambient atmosphere the GPC elugram shifts significantly towards a higher (doubled M_n) molar mass, which might indicate disulphide formation. When performing aminolysis in inert atmosphere this shift was absent and aminolysis seemed successful. However, inspection of the end group fidelity with ESI-MS revealed the presence of thiolactone chain ends, instead of the desired thiol end groups. This side reaction has previously been described in literature, and is specific for aminolysis of PMMA. In order to avoid this side reaction, the BiDTB-PMMA was chain extended with a few monomer units of styrene. The aminolysis procedure will have to be repeated in future, in order to see if proper thiol chain ends are obtained.

When performing a step growth polymerization on PMMA the same results were obtained for the in situ reactions and for the two-step reactions. A shift towards a higher molar mass was observed. This indicates at least partial success of the thiol-ene click polymerization. As stated before, the formation of thiolactone together with the presence of initiator fragments, which are incorporated in an unusual high rate, impede the step growth polymerization.

A second research project described the step growth synthesis of Baylis-Hillman polymers using dialdehydes and diacrylates. Baylis-Hillman polymerizations were optimized by screening different amine catalysts and different diacrylates/terefthalaldehyde combinations. The best experiment gave a short polymer containing about three subsequently conjugated repeating units (with the repeating unit consisting of both a diacrylate and a dialdehyde unit). This was achieved by using 3-HQD as the catalyst and HDDA as the acrylate. This polymerization reaction is comparatively slow but gives few by products. In ESI-MS the presence of 3-HQD at the chain ends was confirmed in a significant amount of chains. This might give problems concerning biocompatibility in the future because of its high toxicity. The initial B-H polymers contained dangling activated vinyl bonds, which in a next step were successfully thiol-ene crosslinked using thiols like butanedithiol en PETMP (tetrathiol). The crosslinks were formed within 15 minutes when using TBP as the catalyst. Dense insoluble networks were obtained. Also when dithiol PS was used to crosslink the polymers insoluble gel-like networks were obtained. In a future study, these networks will be thermally characterized by means of (MT)DSC and TGA. This will be done in collaboration with the University of Brussels.

In a third side project, the synthesis of particles via thiol-ene suspension polymerization was investigated. Linear or branched multifunctional acrylates and thiols were used as building blocks. Hexylamine was used instead of TBP since the diffusion of hexylamine from the water phase towards the dispersed monomer droplets was more pronounced for this water soluble catalyst. The biocompatible PVA was used as the stabilizer. Optical microscopy revealed that particles size ranged from 50 to 200 µm, depending on the amount of PVA stabilizer. When a higher linear to branched monomer ratio was used, the porosity of the final particles could be tuned as well. In a next study, the biocompatibility of the particles will be investigated by cell studies, in collaboration with the University of Maastricht.

In general, for all polymers synthesized during this master project, further characterization is necessary. Not only physical characteristics like shear stress but also biological properties like biodegradability and biocompatibility will be targeted in future studies.

As potential outlook, an interesting application for the Baylis-Hillman polymers can be thought of. Since during Baylis-Hillman polymerization pendant vinyl groups are obtained they can be used for further modification. When crosslinking the Baylis-Hillman polymers - upon using a lower ratio of thiols to enes -

a certain amount of unreacted vinyl groups can be retained. If these crosslinked polymers are synthesized via suspension or emulsion techniques, the resulting particles will contain these vinyl bonds on the surface and can be used to graft proteins via thiol-ene coupling with a cysteine aminoacid.

Lastly this step growth method is a method with a lot of potential towards biological or medical applications. However, more optimization is needed to improve the scope of available monomers but also to get rid of possible toxic elements, which give the polymers a higher success rate for biomedical purposes.

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Richting: **master in de biomedische wetenschappen-bio-elektronica en nanotechnologie**
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