Polyzwitterionic copolymers via polymerization induced self-assembly

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Tiivistelmä – Referat – Abstract

In this master's thesis, polyzwitterionic copolymers were synthesized and analyzed with various methods. In the literature part, the theory behind the reactions and results is covered in order to explain the phenomena.

In the literature part of the thesis, articles were used to describe the theory as extensively as possible. The theory elaborates on the most important topics considering the research part. The main topics are reversible addition-fragmentation polymerization (RAFT), polymerization-induced self-assembly (PISA), and polyzwitterions. In the reversible addition-fragmentation polymerization chapter the kinetics and possible monomers and RAFT agents are gone through also considering the pros and cons. Different disadvantages are dealt with as well when talking about RAFT polymerization. In the PISA chapter different possible morphologies and different types of PISA polymerizations are covered, concentrating still on RAFT polymerization. In this chapter also core blocks of PISA were discussed covering the core forming block used in the research, diacetone acrylamide. lastly, polyzwitterions were discussed explaining the theory, possible applications, polyelectrolyte complexes, and thermoresponsivity of polyzwitterions. Also, in this part polysulfobetaines were covered since it is the zwitterionic block in the copolymer synthesis.

In the experimental part, PSBMA-PDAAM diblock copolymers were synthesized and studied with different methods. Different lengths of block copolymer were synthesized and they were studied with the most common characterization methods. Thermoresponsivity, morphology, and also the effect of the solids content of different block lengths were studied. Measurements turned out to be a success since many different morphologies were witnessed and the thermoresponsive behavior of this copolymer showed interesting results.

Avainsanat – Nyckelord – Keywords
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The experimental part of this thesis was done in the University of Helsinki at the Polymers and colloids research group.

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List of Abbreviations

ACVA   4,4-azobis(4-cyanovalenric acid)

ATRP   Atom transfer radical polymerization

CADB   1-cyanopentanoic acid dithiobenzoate

CTA   Chain transfer agent

D_2O Deuterium oxide

DLS   Dynamic light scattering

DMAPMA   N-(3-(dimethylamino) propyl) methacrylamide

DMSO   Dimethyl sulfoxide

DP   Degree of polymerization

DSC   Differential scanning calorimetry

GPC   Gas permeation chromatography

GTP   Group transfer polymerization

ITP   Iodine-mediated polymerization

LCST   Lower critical solution temperature

MeO_2MA 2-(2-methoxyethoxy) ethyl methacrylate

MeOD   Deuterated methanol

NIPA   N-isopropyl acrylamide

NMR   Nuclear magnetic resonance spectroscopy

NMP   Nitroxide mediated polymerization

OEGMA   Oligo(ethylene glycol) methyl ether methacrylate

PDAAM   polydiacetone acrylamide

PDMA   Polydimethylsiloxane

PEC   Polyelectrolyte complex

PEEP   Poly(ethyl ethylene phosphate)

PEO	Polyethylene glycol
PITSA	Polymerization induced thermal self-assembly
PMMA	Poly(methyl methacrylate)
PMPC	Poly(2-methacryloyloxyethylphosphorylcholine)
PNIPA	Poly(N-isopropyl acrylamide)
PSBMA	Polysulfobetaine methacrylate
RAFT	Reversible addition-fragmentation chain-transfer polymerization
PSPP sulfonate	poly(3-[N-(3-methacrylamidopropyl)-N,N-dimethyl]ammoniopropane
TEM	Transmission electron microscopy
UCST	Upper critical solution temperature
UV/Vis	Ultraviolet-visible spectroscopy

Introduction

The interest of polymerization induced self-assembly (PISA) has been growing among scientists since it is efficient way to form block copolymers with different morphologies. It is sufficient way to form the wanted morphologies than post polymerization techniques since it doesn't make the solution more diluted, so high solids content can be achieved 5-50 w%.[1] The first time PISA synthesis was introduced in literature was in 2009 so it is quite new approach but has been growing ever since.[2] Many different nano-objects can be formed via PISA and it is usually done in water using RAFT polymerization but also other solutions and polymerization techniques can be used. PISA can be performed in emulsion or dispersion. In dispersion the monomer is soluble in the solvent and formed polymer chain is not soluble. PISA can be done for almost any monomers, media and solutions, and PISA also forms huge variety of nanoparticles so no wonder the interest has been growing.[3] Polyelectrolytes are important in PISA synthesis because they have ionizable groups, so they have many potential areas in technology and applications. Polyelectrolytes are used in biomedicine, cosmetics, water treatment, food, and paper industries and in chemical engineering. Polyelectrolytes are usually surfactants or coating materials since they can swell in different medias. Any polymer that dissociates as a highly charged molecule when placed in ionizing solvent forming positive, negative or both is a polyelectrolyte.[4] Zwitterionic polymers have positive and negative charges in the same monomer unit, and they have been previously used as a sterical-stabilizers in diblock copolymer particles. Polyelectrolytes are often also thermoresponsive because of the charges.

In the research part zwitterionic copolymer will be investigated and synthesized via polymerization induced self-assembly. Many characterization techniques are used and the thermoresponsive behavior of the copolymer of different solids contents and different block chain lengths are researched. Since there are no articles on this copolymer, it is great importance to researchers since it could be used in many applications. Also, the morphology of the formed copolymers is researched by changing the solids content, PDAAM chain length and PSBMA chain length. One of the most important application fields is biomedicines since the stabilizer block is zwitterionic and biocompatible. Overall, more information about zwitterionic copolymers and their properties is needed because of their many possible application areas.

I Literature part

1. Reversible addition-fragmentation polymerization

Reversible addition-fragmentation chain transfer (RAFT) process is living/ controlled free radical polymerization. Other free radical polymerization techniques are nitroxide-mediated polymerization (NMP) and atom transfer radical polymerization (ATRP). In all of these, the polymer chains reversibly switch between active (propagating) and dormant (non-propagating) states, so that at any moment a vast majority of the chains are in the dormant state. If the reagents provide deactivation, the kinetics should be similar to nitroxide mediated polymerization. Pros in RAFT polymerization is that many different monomers, solvents, and conditions can be used but unfortunately not many reagents are commercially available. PISA with RAFT polymerization is preferred when possible since water is green solvent with high boiling point and polarity and it is nontoxic.[5] RAFT was reported for the first time in 1998 and ever since it has been one of the best polymerization techniques.[6,7,8]

Living polymerization is popular because it has many benefits like control over molecular weight and polydispersity and with it some block copolymers can be polymerized that are not possible in any other way. There are also some problems with living polymerization, it can be done only with limited amounts of monomers, reagents might be difficult to remove or expensive and they might need high temperatures or sensitive to the change in environment.[6]

1.1 Kinetics

RAFT rely to kinetic strategy for controlling the molecular weight and architecture of the produced polymer. With RAFT, the aim is to get as close as possible to a living polymerization which gives narrow molar mass distribution and lower dispersity. Initiation it is quicker than propagation and termination must ne gotten under control.[5] The mechanism is based on the equilibrium between active and dormant species.

Thiocarbonylthio compounds are used as chain transfer agents (CTAs) and can be divided to four subcategories: dithioesters, trithiocarbonates, xanthates and dithiocarbamates. The radical-radical termination is not eliminated completed, but it is minimized. Termination is under control by

decreasing the concentration of the radical species by 10-fold, we can decrease the termination by 100 fold.

$$v_p = k_p[p \cdot][M] \; vs. \; v_t = k_t[p \cdot]^2$$

If we assume that all CTA reacts and termination is not present, the theoretical number average degree of polymerization of a polymer synthesized with RAFT can be calculated

$$DP_{theor} = \frac{[Monomer]}{[CTA]} \cdot conversion$$

where $[Monomer]$ and $[CTA]$ are the concentrations in initial reaction mixture and conversion is the number of reacted monomers. The theoretical degree of polymerization, which can be calculated from NMR spectra, can be used to calculate theoretical number average molecular weight with equation

$$M_{n,theor} = DP_{theor} \cdot M(Monomer) + M(CTA)$$

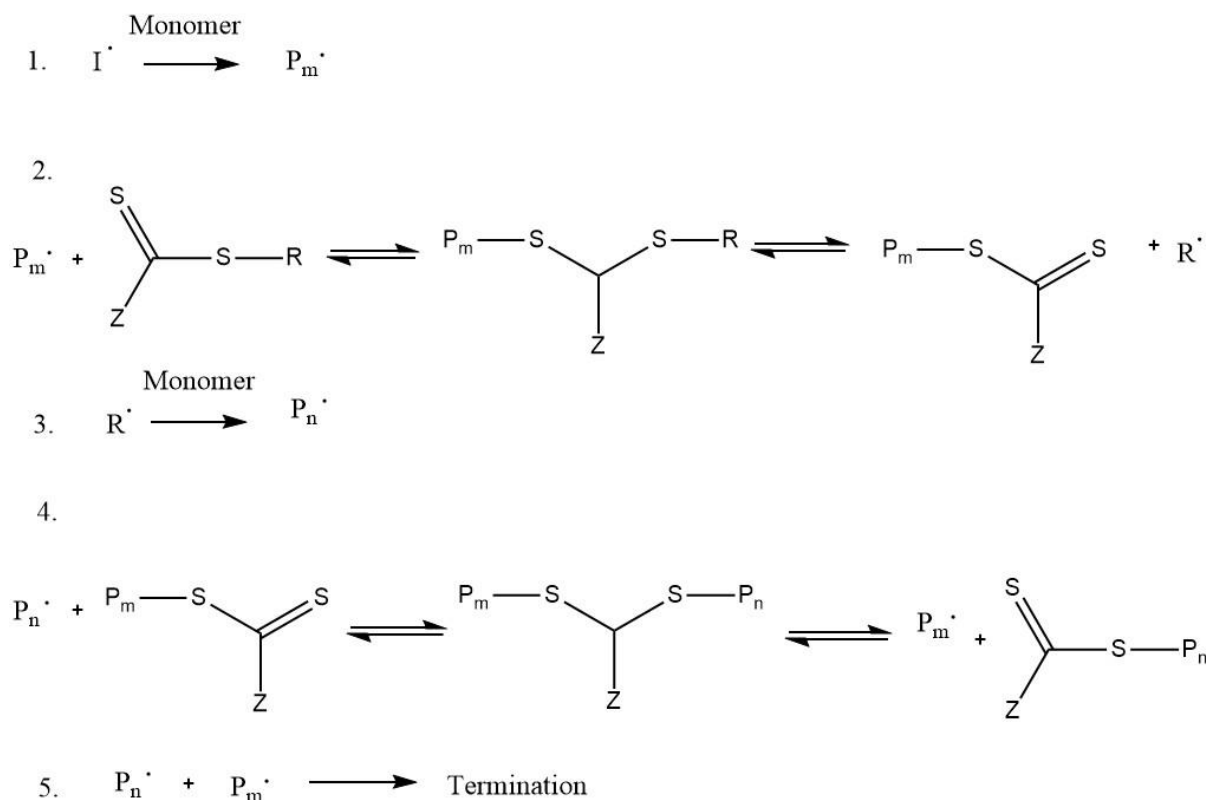The accuracy of the method is determined by the accuracy of the initial molecular weights, and they can be determined.[5]

Figure 1. Mechanism of RAFT polymerization.

The propagating radical adds to the thiocarbonyl sulfur center of the dithioester to produce an intermediate carbon-centered radical. That radical can undergo β-scission, either re-forming propagating radical or liberating new carbon-centered leaving group. In the mechanism picture the first stage is generating radical which works just like normal radical polymerization. Second step is forming growing chain and then it adds to CTA, which is known as fragmentation, it is also possible that the original initiating radical reacts directly with CTA without any propagation. The intermediate radical in step 3 may release either the original growing radical or a new initiating species R, which then initiates a new growing chain (step 4). After consumption of all free CTA, the main equilibrium of RAFT is established (step 5). In the main equilibrium all the chains switch between active (radical) and dormant (macro-CTA) states, which allows equal probability for all the chains to grow, leading to narrow polydispersities.[5]

To achieve control, balance of the rates of these reactions is needed so the dormant species must be a bit bigger in concentration than active species but still the exchange has to be quick. By studying the rates and equilibrium experimentally is difficult, because the experimentally observable properties of the process are not the rates and equilibrium constants of the individual reactions but more like overall polymerization rate. For that reason, to get individual rate and equilibrium constants one must assume a kinetic scheme and make simplifying assumptions, such as steady state assumption.[5]

1.2 Monomers and RAFT agents

In RAFT many monomers can be used if they are available to free radical polymerization. Vinyl monomers can be divided to two different groups based on the reactivity: the more activated with double bond, a carbonyl group, acrylates, aromatic ring, or nitrile and less conjugated with double bond adjacent to oxygen, halogen, sulfur lone pairs, nitrogen, or saturated carbons. If the monomer has nucleophilic substituent like primary or secondary amines, they are more challenging for RAFT.[9] Monomers can be thought to belong to two different classes depending on their reactivity. With terminal of more activated monomer the propagating radicals are less reactive in radical addition and with less-activated monomer the propagating radicals are highly reactive. The less activates group includes monomers where the double bond is attached to saturated carbon, to the heteroatom of heteroaromatic ring, oxygen, or nitrogen lone pair. Examples of the less activated group monomers are vinyl acetate, N-vinylpyrrolidone, N-vinylcarbazole and diallyldimethylammonium chloride. More activated monomers have double bond attached to

carbonyl group, nitrile, or aromatic ring. Examples of highly reactive monomers are acrylamide, methyl methacrylate, methyl acrylate, acrylonitrile, styrene and vinylpyridine.[10]

RAFT agents should have low likelihood of going through side reactions, good solubility to the reaction medium and also the concentration of transfer agent should be optimal. Also, the end group should be optimal for the product and when doing NMR characterization, it should be easily recognizable. When choosing the RAFT agent, it must be appropriate for the monomer or monomer used in the reaction since it plays an important role on the outcome of the polymerization. The RAFT agent should block purity, it should end up product with low polydispersity which is achieved when RAFT agent has high transfer agent concentration, and it should form product with wanted molar mass.[10]
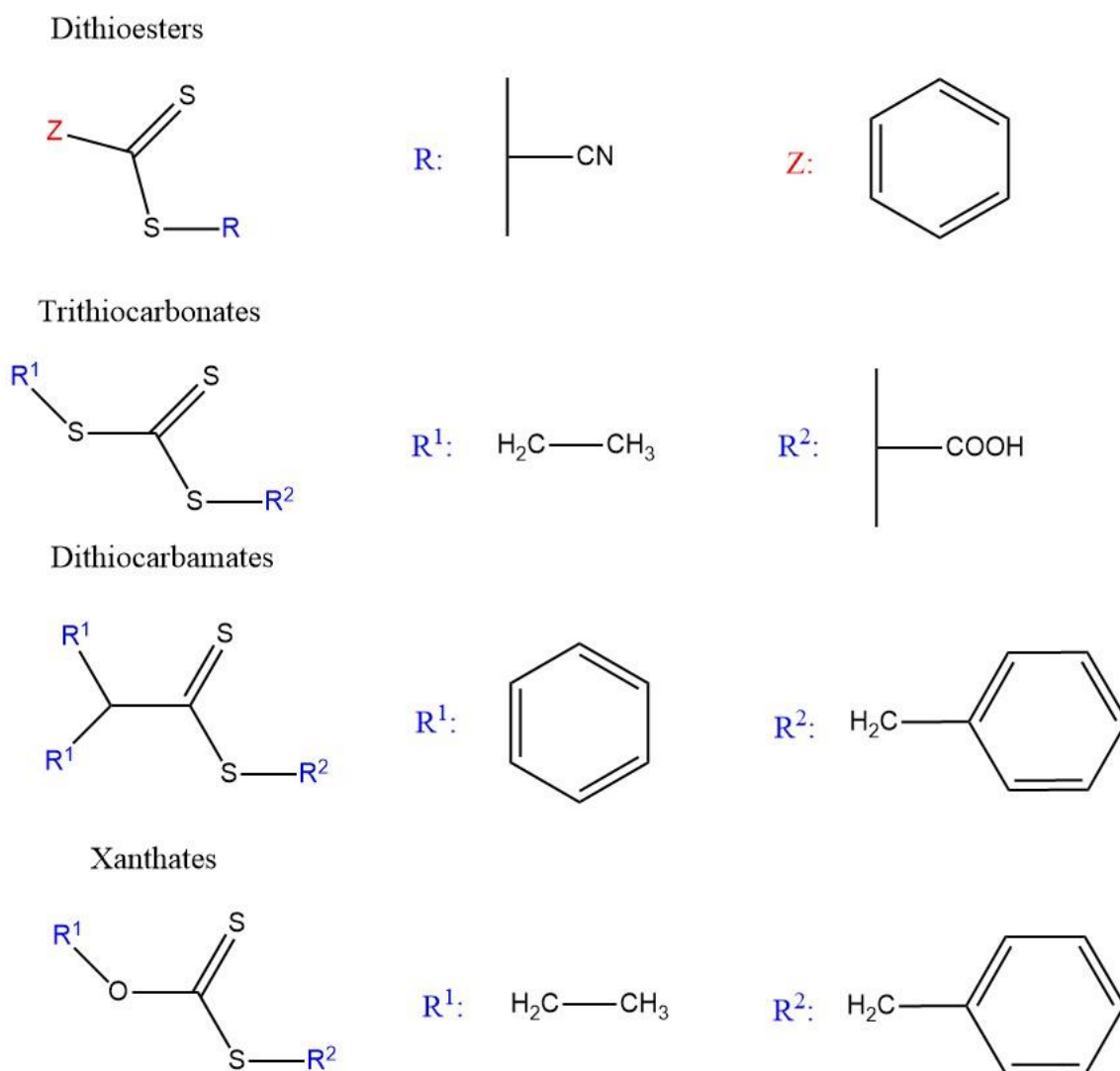

Figure 2. Types of RAFT agents and examples of different Z and R groups.

1.3 Z and R groups

The stability of the RAFT agents and poly-RAFT agents are affected by Z group, and it is important for the outcome of the reaction. Z and R substituents effect on the transfer constant of the RAFT agent. Fragmentation of the intermediate radicals is affected by the stability of the R group. The agent should have reactive C=S bond to able high kinetics but also it should have weak intermediate radical S-R bond so that it is able to react quickly to reignite the reaction. The radical should be better homolytic leaving group or as good as propagating radical derived from the monomer being polymerized. Z group shouldn't stabilize the intermediate radical more than necessary, but it has to be stable enough to allow the addition of the propagating radical.[5] Through manipulation of Z group, the rate constant of the polymerization can be manipulated. Electron withdrawing Z groups and its capacity to stabilize radical center are enhancing the transfer constant but at the same time it increases the possibility of side reactions. Z group shouldn't cause any side reactions, for example with Z=OR' it is important that the R' group is poor homolytic leaving group otherwise irreversible chain transfer will compete with the wanted reaction.[10] Lone-pair donor Z group like OR, SR or NR_2 can enhance stability by delocalization of electron density. Also, RAFT agents are destabilized by σ-withdrawal and it can be seen with agents that have CN or CF_3. The incorporating the nitrogen lone pairs into an aromatic system reduces the capacity for electron delocalization. If the RAFT agent has poor leaving group, it can induce strong inhibition phenomena.[5] When controlling more activated monomers, RAFT agents like dithioesters or trithiocarbonates are suitable and when controlling less activated monomers, RAFT agents with xanthates, N,N-dialkyl- or N-alkyl-N-aryldithiocarbamates are good Z-group groups. When RAFT agents suitable for more activated monomers are used on less suitable monomers, they tend to be ineffective and the other way around.[11]

R group should be good homolytic leaving group so that the polymerization is well controlled. the radical formed has to be good at reinitiating the polymerization and also the stability of the radical is important to the rate of fragmentation. Steric factors of R groups affect on the control of polymerization. When there is electron withdrawing groups on R the addition to the thiocarbonyl group decreases and fragmentation rate is increased.[10]

1.4 Side reactions

When talking about RAFT polymerization, there is multiple possible side reactions that are complicating the reaction mechanisms and the understanding of the reaction kinetics. Even though

RAFT is controlled method, some side reactions take place in the reaction mixture. Such as hydrogen abstraction reactions can be a problem so by comparing their rates with the normal β-scission reaction it is possible to estimate if there are some side reactions. There are also some possible reversible and irreversible termination reactions of the RAFT-adduct radical. Another side reaction that is possible is the attack of the propagating radical at the carbon center of the C=S bond to produce a sulfur-centered radical. RAFT polymerization is extremely air sensitive because both propagating radicals and intermediate radicals react with oxygen. Also, it is possible that the RAFT agent starts to copolymerize when the intermediate radical propagates. It can be divided into two different reactions, where the other is irreversible and other is reversible. Previously mentioned β-scission involving cleavage of a bond with Z group. Termination of the intermediate radical is also one possible side reaction.[5]

2. Polymerization induced self-assembly

Amphiphilic diblock copolymers undergo self-assembly in aqueous environment due to different behavior of the hydrophilic and hydrophobic blocks. It is called polymerization induced self-assembly (PISA) and it forms tunable dimensions range of different morphologies, which can be tunable such as vesicles, micelles, lamellae, and worm-like particles.[12] Even more complex structures can be generated with more blocks of differently interacting polymers. Hydrophobic interactions are energetically unfavorable so in order to prevent this, the hydrophilic parts of the copolymer interacts with water.[13] Radical polymerization techniques are used in dispersions and emulsions to induce self-assembly of the copolymers but dispersions are more used since they are more easily tunable.[14] PISA offers multiple advantages to block copolymer self-assembly, since the traditional way usually leads to really dilute dispersions. In the traditional way the selective solvent is added after polymerization process, so the number of steps is higher. PISA approach gives also high conversion and often narrow molar mass distribution.[15]

When forming AB block copolymer via PISA, the first block is macro-CTA, and it works as a stabilizer since it is water-soluble. The monomers of the second block are water soluble but the growing polymer chain becomes water insoluble which forms nanoparticle that self-assembles.[16] Also, with thermoresponsive polymers polymerization induced thermal self-assembly (PITSA) is possible, when polymer forms a new morphology for example in higher temperatures than its LCST.[15]

Transitions between different morphologies can take place in solution. When spheres fusion is happening, they collide and form worms. Worms are formed only in high polymer concentrations and at low concentrations the spheres grow in diameter when the DP of the core-forming block increases. Also, temperature can effect on forming the speres-worms morphology change when talking about temperature responsive polymers.[17] Worms can also transit to vesicles when the stabilizer block DP is short. When the ratio between core forming block is adjusted the vesicle diameter can be changed and this has been thought to be because of the packing parameter.[18]

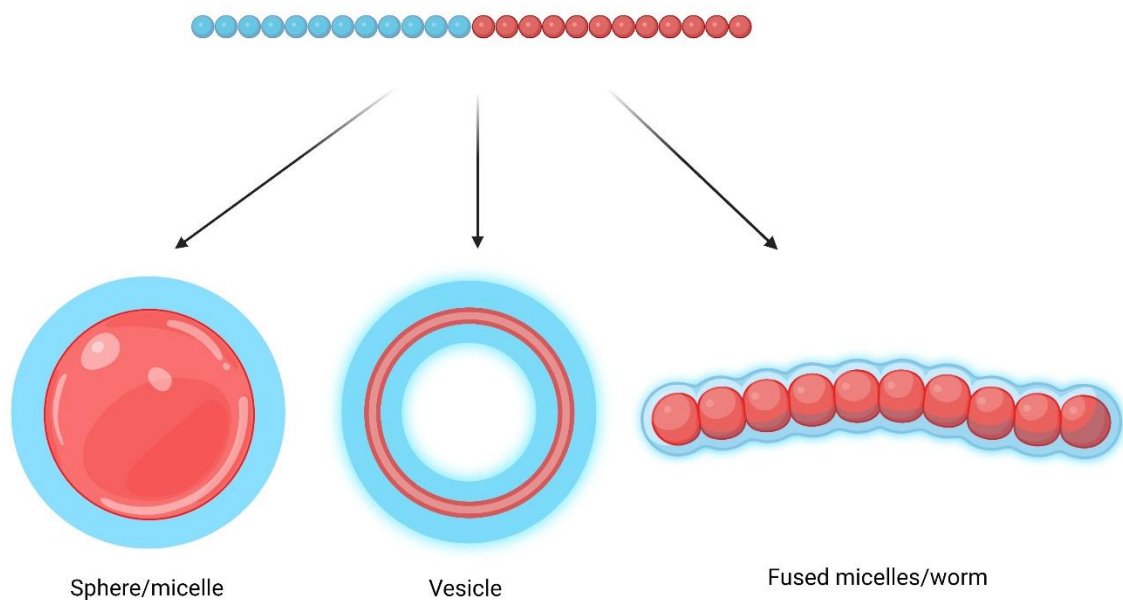| Sphere/micelle | Vesicle | Fused micelles/worm |

Figure 3. Different morphologies formed with PISA approach. There are other possible morphologies but with PISA Sphere, vesicle and worm are the most common ones. Other possible morphologies are fibers, cylinders, lamellae, films, and multilayer nano-objects. This is limited in PISA since the monomer or polymer might not be soluble.

2.1 Polymer morphologies

The morphology of the AB block copolymers depends on the ratio of the hydrophilic and hydrophobic chain lengths, solvent, temperature, and the structure of the blocks. Polyelectrolyte

effect, pH and salt concentration influences also to the forming morphologies. The morphology of the copolymer can be changed by adjusting the reaction parameters.[14]

Spherical structures are usually formed when the polymer has polyelectrolyte chains that can stabilize the structure. Charges are affecting to the possible morphologies since there is repulsion in the chain. High-order morphologies can be formed by reducing the charge repulsions with stabilizers.[14]

Block copolymer vesicles or polymersomes, have excellent mechanical and physical properties compared to liposomes. Higher molar weights lead to increasing membrane thickness and to rigid morphologies. Also, polarity of the hydrophobic membrane affects to the membrane permeability.[13] Also, chain extension of the AB block copolymer vesicles is possible with hydrophobic monomers, and it produces triblock copolymer with adjustable surface hardness.[19]

Packing parameter p defines the nanostructure that is formed:

$$p = \frac{v}{a_o l_c},$$

where a_o is the optimal area of the head group, l_c is the length of the hydrophopic tail and v is the volume of the hydrophopic chains. Head group in this case is hydrophilic and interacts with polar solvent. Spherical micelles are favorable when $p \leq \frac{1}{3}$, vesicles when $\frac{1}{2} \leq p \leq 1$ and cylindrical micelles when $\frac{1}{3} \leq p \leq \frac{1}{2}$.[6]

The core-forming block can be adjusted by copolymerization with different functional groups and structures. Chain mobility is also important for the particle fusion to achieved high-order morphologies with PISA.[15]

2.2 Controlling PISA

In PISA usually reversible deactivation radical polymerization techniques are used such as atom transfer radical polymerization (ATRP), nitroxide-mediated radical polymerization (NMP) and reversible addition-fragmentation chain transfer (RAFT), which is the most used one.[15] Also iodine-mediated polymerization (ITP) has been used for alcoholic solution synthesis.[20,21] In addition to these controlled polymerizations also other initiation mechanisms have been used such as microwaves, enzymes, electrochemistry, ultrasound and visible light. Photopolymerizations can be used in mild reaction conditions and natural sunlight, or light emitting diode can be used. ATRP have

been used by initiating the reaction with ultrasound by employing piezoelectric nanoparticles or forming hydroxyl radicals for either RAFT polymerization or ICAR ATRP.[22]

2.2.1 RAFT PISA

With RAFT polymerization different solvents can be used such as organic solvents and alcohols, ionic liquids, mineral oil, and water. Changing the block volumes, the desired polymer morphology can be achieved, and this can be seen from the PISA phase diagrams in case it is available. Usually, particles formed in nonpolar media have upper critical solution temperature (UCST) because they get progressively plasticized during heating.[17] This is because of the worms which are prepared, have gelation properties, and get entangled. Joseph R. L. and all suggested that percolation theory could explain the behavior of the copolymer worms. They suggested that 3D gel network forms via multiple contacts between neighboring worms and not from worm entanglements.[23] Non-ionic diblock copolymers can have pH-responsive behavior when the worms can convert to spheres on increasing the pH. This is reversible reaction and happens because of the ionization of terminal carboxylic acid end group.[24]

RAFT polymerization via PISA has been popular in recent years since it is nontoxic, it has high polymerization rate, low cost and can lead to various morphologies with choosing the right copolymers. Premise to conduct PISA is that the other block has to be insoluble to the solvent so that it forms a core and self-assemblies. The list of matching copolymer pairs is short and for that reason it is important to study new copolymers for PISA. The first studies focused to prepare thermoresponsive cross-linked nanogels with lower critical solution temperature (LCST). One disadvantage of RAFT-PISA is that the polymerization process needs quite high temperatures because the initiators are thermal which can also effect on the morphologies formed.[17]

2.2.2 ATRP PISA

ATRP is other widely used technique in PISA synthesis, but it has some disadvantages. It needs copper catalyst which makes it toxic, and it is not suitable for biomedical applications. Copper removal is also possible but it's an extra step. Also, when using metal catalyst, oxidation has to be taken into account. The control on molecular weight and morphology is not as good as with RAFT polymerization. The morphology in ATRP PISA is dependent on solids content and the degree of polymerization of blocks just like in RAFT-PISA. In higher concentrations worms are obtained and in low concentration only spheres occur. Formation of worms has been suggested to happen because

of sphere-sphere fusion.[17] Pan and co-workers reported the first PISA trough ATRP polymerization in 2007.[25] PMPC nanolatexes have been formed via ATRP with PEO-based initiator. When dialysis is done against water it becomes swollen microgel and still has narrow size distribution.[26]

2.2.3 NMP PISA

Nitroxide-meditated polymerization has shown good results in PISA polymerization. Spherical dispersion polymerization particles have been formed[27] and later it was shown that cross-linked particles can be formed. In this approach the polymer has temperature responsive microgel core and high solids content can be achieved.[28] Different morphologies of block copolymers have been produced with NMP PISA at the surface of silica particles in the presence of aqueous suspensions of colloidal silica.[29] The silica particles were coated with many different block copolymer varieties.

2.3 Core forming block in PISA

Core block in PISA synthesis determines what morphology occurs and the properties that effect on it are T_g and the polymerization temperature, core volume and the solubility of the core to the solvent. When choosing the core forming block, monomer has to be soluble to water and the forming block has to be hydrophobic.[30] The monomer solubility and the hydrophobic moiety of the core effects on the copolymer morphology.[31] Higher order morphologies can be achieved by increasing the alkyl chain length and the DP of the core. Usually is an advantage if the polymer has T_g lower than the polymerization temperature but with PMMA it has been shown that higher morphologies can be achieved and this can be explained by the high solubility in water.[30] Different functionalities of the core forming blocks haven't been researched that much. Functionalized core could be advantageous for many applications.[32] Sometimes the core can encapsulate while polymerizing so in those cases they can't be used in catalysis. The most used ones for core forming monomers are N-Isopropylacrylamide, 2-hydroxypropylmethacrylate, 2-methoxyethyl acrylate and oligo(ethylene glycol)methacrylate. Diacetone acrylamide is quite new in PISA research, but the interest has been growing since its commercially available and is used in beauty products.[33]

2.3.1 Diacetone acrylamide

Diacetone acrylamide (DAAM) is a core forming monomer in PISA synthesis, since the monomer is water soluble, but the polymer is insoluble to water. This is because there are intramolecular hydrogen bonds and for that reason it forms the core block in copolymer PISA synthesis. DAAM is an acrylamide with ketone group, and it has both hydrogen-bond accepting and donating

properties.[15] That gives it an opportunity to synthesize with thermoresponsive polymers and to form different morphologies. With DAAM at relatively low temperatures, it is hard to form higher morphologies such as worms and vesicles unless the solids content is higher because of the repulsion of the counterions. PDAAM can change its morphology at low temperatures after the polymerization, called post polymerization transition. It forms reversible temperature worm/spheres transition when cooling down.[15]
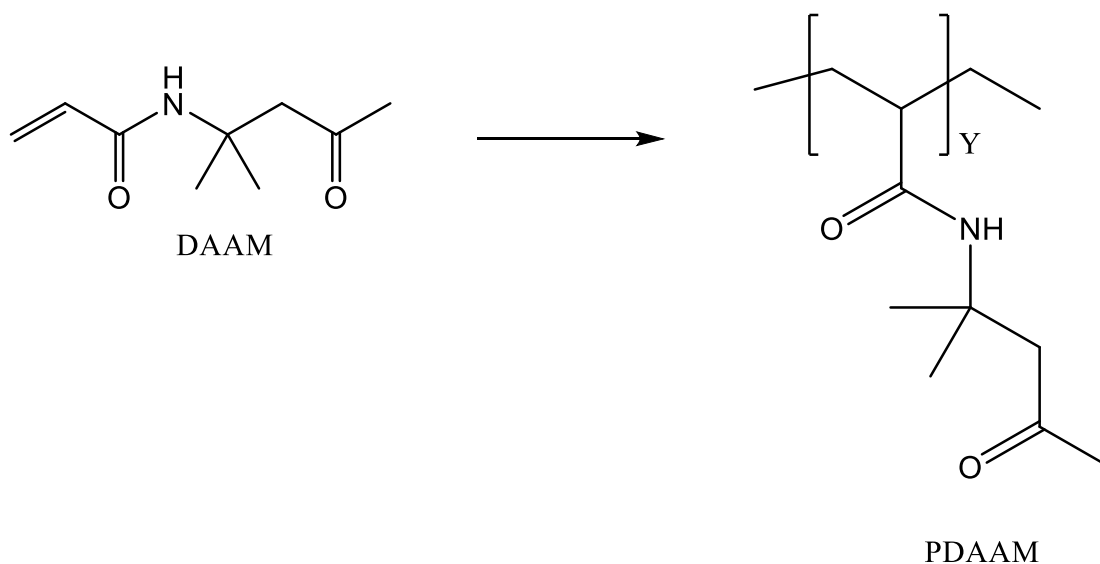

Figure 4. DAAM and PDAAM.

PDAAM with ketone functionalized core forms nanospheres and vesicles that can be adjusted with solids content and degree of polymerization. The ketone group can be functionalized quite easily or also crosslinked. Also, by lowering the polymerization temperature and the concentration of the initiator, vesicles were formed because the polymerization rate was decreased because the chains have more time to adjust and change the morphology.[32] Usually PDAAM is RAFT dispersion synthesized at 70 °C but also it is possible to synthesize at 25 °C ultrafast (30 minutes) under visible light irradiation.[33] PDAAM has been copolymerized for example with N,N-dimethylacrylamide (DMA). With PDMA it is possible to form worms, vesicles, and spheres, but worms were synthesized only with one copolymer composition and solids content. The worms were temperature responsive and also exhibited pH sensitive behavior. PDAAM with using $PDMA_{30}$ formed lamellae structure and asymmetric crosslinker was used to crosslink block copolymers during synthesis. Formed lamellae structures were able to have morphological changes to spheres and worms when cooling down.[34]

3. Polyzwitterions

Polyzwitterions are group of polyelectrolytes where are ionic and cationic groups in the same monomer unit. Polyelectrolytes have either negative or positive charge in their monomer units making the polymer either positively or negatively charged. Polyampholytes on the other hand have charged groups on different monomer units. Different groups of polyzwitterions are polycarbobetaines, polysulfobetaines and polyphosphobetaines. Polyzwitterions are biocompatible, anti-fouling agents and they form electrosterically stabilized colloids since they electrical charge is zero. They are used in drug delivery applications since they are biocompatible.[35] They are thermoresponsive and have upper critical solution temperature and/or lower critical solution temperature.[35] Polyzwitterions can be in the solution neutral, anionic or cationic depending on the environment. Charged polymer solutions consist charged polymer chains, counter ions, solvent molecules and salt ions.[36] When developing ionic materials, the lifetime of the ionic interaction is one of the key parameters. Polyelectrolytes can be classified as weak or strong depending on how they interact in solution. When various combinations of ionic strengths are combined in the material, strong materials are formed. When there is polymer solution of differently charged polymers, they form polyelectrolyte complexes which is elaborated later.[37]

Polyzwitterion is an important feature of many important biopolymers, such as DNA and proteins are polyelectrolytes. Polyzwitterions are formed when ions in the chain, counterions, breaks off from the chain and gives the chain a charge. Polyelectrolyte has an electrical charge and al the counterions are free. When the counterions are attached to the polymer backbone, it is called ionomer.[38] It forms ion pairs, dipoles inside the chain. When many monomers dissociate in water solution, the solution has high dielectric constant. Polyelectrolytes forms gels which swell, due to extra pressure of the counterions that makes the network swell. Polyelectrolytes are also called super absorbents because of this unique feature.[38]

Polyelectrolytes can be classified in many ways and from different perspectives. They can be classified by their charge and position of ionic groups in the polymer chain. Based on the origin they can be synthetic, semi-synthetic like xanthan gum or natural like proteins. Based on the composition they can be copolymers or homopolymers and they can also be linear or crosslinked/ branched. The shape can be rigid or spherical like in globular proteins. Nevertheless, the type can be determined on the charge, if its negative or positive or polyzwitterion and the charge density if its weakly pH-sensitive or strongly pH-sensitive.[4]
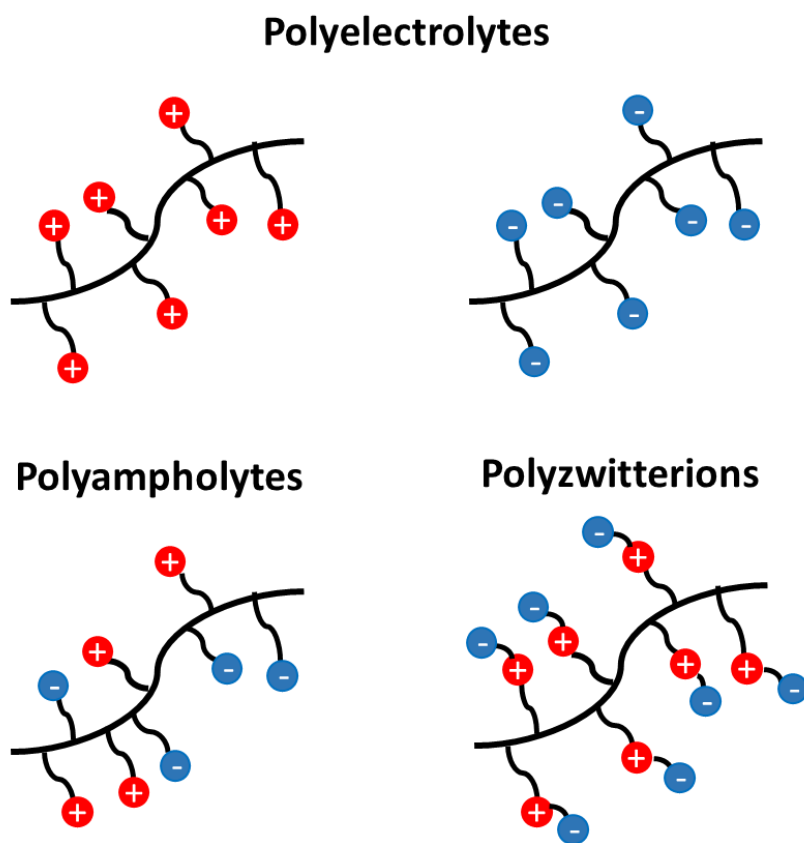
Figure 5. Schematic picture of polyelectrolytes an its subgroups. In polyelectrolytes there is only cations or anion in the monomer units. Polyampholytes have negative and positive charged monomer units and polyzwitterions have the same amount of cationic and anionic functions in the same monomer units.

3.1 Applications

Since polyelectrolytes have many different properties, they have wide range of applications. These applications include biomedical applications, soft matter robotics, water treatment, catalysis and energy storage. In water treatment they can be used as leveraging electrostatic interactions to remove metals or other particles. Polyelectrolyte membranes are also used in electrochemical devices like fuel, batteries ad solar cells.[37] Antipolyelectrolyte effect, also called salting-in effect is formed when the chain attraction of the opposite charges is reduced and the polymer chain forms stretched conformation in salt solutions. The antipolyelectrolyte effect can be used to retain saline water, thicken, or stabilize salty media.[39]

Polyzwitterions are highly biocompatible which gives many possible uses in the field of biomedical applications. Sulfobetaines and carbobetaines have possibility to use as a zwitterionic materials in biofouling and biomedical applications since they have been shown to have low protein adsobtion. Sulfobetaine methacrylate and its hydrogels have low protein adhesion and low cell adhesion. Polysulfobetaines could be used as drug carriers, bioconjugates and coating for blood contact devices. That's because they can avoid the immune system to recognize themselves and have delayed blood clearance.[35]

3.2 Ionic interactions

There are many interactions to take into account, such as monomer-solvent interactions, the interactions between charges and solvent molecules, polymer and salt concentrations and the electrostatic interactions between the charges. The solvent affinity of the polymer backbone is controlled by monomer solvent interactions.[36] Dynamic properties have an effect on the behavior of the material, and these are ionic strength, polymer concentration, temperature and diffusion coefficient. Addition of the salt can increase the diffusion coefficient and lower the energy barrier. Strong ionic interactions have high activation energy and weak ionic interactions have low activation energy, so it describes the ionic strength.[37] When polyelectrolytes are dissolved to water, they have in dilute concentrations extended conformation and the counterions start to form ion-bridging and the polymers remains in solution. With high salt concentrations polyelectrolytes precipitate because of strong charge screening and with increasing the salt concentration more the polyelectrolytes will start dissolving again because of the high-charges. This can be explained by scaling model approaches and Flory theory.[4]

Two different basic interactions between ionic molecules and materials are attraction and electrostatic repulsion. Cationic and anionic pairs have attraction and the same charged repulse each other's. Because of the repulsion same charges have, polyelectrolytes act as a rigid rod if there are not opposite charges around. When adding salt to these systems, it forms attraction between the charges and the polymer chain is not anymore rigid since the charges balance each other's allowing the chain to collapse due to charge screening mechanism. On the other hand, polyzwitterions and polyampholytes are the opposite from this, because of their intra chain attraction known as the anti-polyelectrolyte effect. The addition of low molecular weight salt increases the viscosity in anti-polyelectrolyte effect.[37]

In dilute salt-free polyelectrolyte solutions the distance between chains is bigger than their size so the electrostatic interactions inside the chain dominates. At low polymer concentrations almost all counter ions are distributed outside the volume that the chai occupies. In this case the electrostatic interactions between charged groups and chains elasticity control the chain dimensions. The number of possible conformations decrease when end-to-end distance increases and then chain conformational entropy decreases. Monomers which are charged, move further away from each other when chain size is bigger because of the electrostatic charges.[36]

3.3 Polyelectrolyte complexes

If there are more polymer chains of differently charged polymers, they tend to form complexes with oppositely charged groups. It is formed because of the electrostatic interactions between polyanions and polycations when mixing the oppositely charged polyelectrolytes. Complexes occurs between polyelectrolyte-polyelectrolyte, polyelectrolyte-nucleic-acid, polyelectrolyte-surfactant and polyelectrolyte-drug. The interactions are physically crosslinked which makes then sensitive to changes in environment and makes them easier to use in pharmaceutical industry. They are not soluble in most solvents, and they can be rubbery in wet conditions and brittle and hard in dry conditions.[4] Polyelectrolyte complexes can be classified to three groups colloidally stable, soluble and coacervated complexes.

Many things effect on the properties of polyelectrolytes like chain flexibility, pH, temperature, ionic strength, molecular weight, concentration, chain stiffness, strength of the ionic groups and the nature of those.[4] The formation of PECs is in three stages, first the form primary complexes where the bond formation is random. Second step is the formation of secondary complexes where intracomplex rearrangement of the bonds takes place where orderly complex is formed and finally the step three is intercomplex aggregation where hydrophobic interactions between the complexes cause the aggregation. Polyelectrolytes can be divided into two different groups by their structure: scrambled egg structure and ladder-like structure. Scrambled egg structure is formed when there is high number of chains, and it is intermixed and ladder-like structure on the other hand is when there is single-stranded hydrophilic and hydrophobic segments from weak electrolytes and they form double stranded structure.[40]

During polyelectrolyte complex formation adding salt influences on the process. Adding NaCl decreases the level of aggregation which gives possibility to control mass and size of the complex

particles. Adding more salt causes secondary aggregation and flocculation because of the screening of the electrostatically stabilized shell around the particles.[41]

3.4 Polysulfobetaines

Polysulfobetaines have an alkyl sulfonate group with cationic repeating unit. Polysulfobetaines have high polarity and high hydrophilicity, stimuli-responsiveness, and anti-polyelectrolyte effect. They are chemically stabile against oxygen and other oxidants, and for that reason better antifouling agents compared to polyethylene oxide for instance.[42] Combining betaines with different hydrophilic and hydrophobic fragments, different morphologies are achieved such as capsules, spheres, thin films, or structured hydrogels. Betaines can be formed through polymerization of zwitterionic monomers or by zwitterionic functionalization of reactive precursor polymers. Sulfobetaines are usually synthesized by alkylsulfonatior or a monomeric or polymeric tertiary amine with sultones. Other way is to react tertiary amines with haloalkylsulfonate. Often polymerization is done through controlled radical polymerization, either ATRP or RAFT method. Narrow molecular weight distribution can be achieved and also block copolymers can be synthesized trough these methods. The success of RAFT polymerization depends on the chain transfer agent. Also, one route to synthesize sulfobetaine block and homopolymers is the functionalization of precursors prepared by group transfer polymerization (GTP).[39]
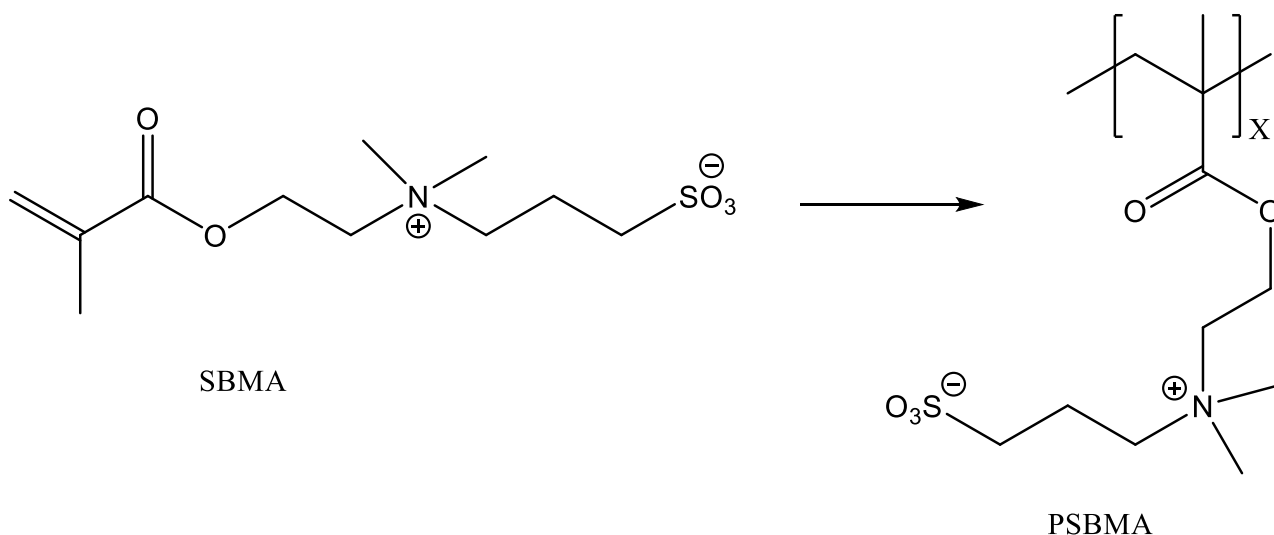

SBMA

PSBMA

Figure 6. Example of sulfobetaine, sulfobetaine methacrylate and its polymer.

Polybetaines have different properties in solution, in condensed state, and gel state because they polyzwitterions. Betaines are able to form zwitterionic fragments to form a cyclic conformation of

the anionic and cationic groups of intragroup or intrachain within single macromolecule and interchain ion contacts between neighboring macromolecules and these forms cross-linked networks. They are soluble in salt containing solutions and have gel-like characteristics. Low molecular weight zwitterions can fold intramolecularly to loop conformation and this can be varied by the linker length and flexibility. The intramolecular association of betaines is pH dependent and by increasing the pH of the solution the mobility decreases because of the increased ionization of the carboxylic groups. With increasing salt concentration, the solvent quality increases. With increasing salt concentration, the second virial coefficient, intrinsic viscosity, radius of gyration and hydrodynamic radius increases. In solid state polybetaines can form clusters or ionic aggregates. Because of the sulfonate groups, there is electrostatic repulsion so in hydrogels they are swelling at pH 2-3.[39]
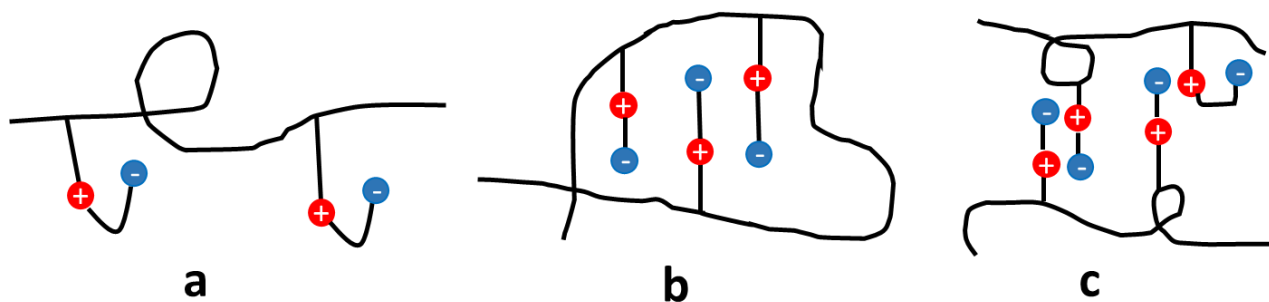

Figure 7. Interactions of sulfobetaines. Intragroup (a), intrachain (b), and interchain (c) salt bonds.

The difference on different monovalent cation with Cl has been studied by Afan Hamzah et all. The study shows that the UCST increased and then decreased when the salt concentration increased. Salts used with water solution were LiCl, KCl and NaCl. The polysulfobetaine gel adsorbed with the amount of adsorption tendency of LiCl<NaCl<KCl. This can be explained by the hydration force of Li$^+$ and also Hofmeister series explain this where properties of hydrating cations.[43]

Polysulfobetaine methacrylate (PSBMA) is zwitterionic polymer which has upper critical solution temperature behavior in aqueous solutions. Properties depend on molecular weight, concentration and salt concentration and also branching of the polymer. The properties like architecture of the polymer, affects to phase separation temperature, cloud point. Low molecular weight PSBMA polymers have not been detected to have any UCST behavior with or without salt, but when copolymerizing, the properties change even with shorter chains. When sodium dodecyl sulfate is added to the solution, micelles are formed to the polymer chains with high concentrations. When

adding bigger amounts of Sodium dodecyl sulfate crosslinking nodes are formed to the hydrophobic tails. Because of the counterions, PSBMA has negative net charge in saline solutions.[44] PSBMA contains a large number anionic SO_3^- groups which makes grafting with peptides easier because they improve selective cell adhesion. It also has been proved that the increase of SBMA concentration can improve the comprehensive strength of hydrogels.[45]

3.5 Thermoresponsivity

Zwitterionic polymer happens to have often thermoresponsive behavior but unfortunately studies haven't been focused to this behavior that extensively until the past decade. Different stimuli-responsive polymers can be sensitive to light, pH, electro-stimuli, magnetic fields, and temperature. Many of temperature responsive polymers have changes in their solubility when temperature changes and that's why its important thing to consider when dealing with polymers. Because water is the cheapest, safest and best solvent for environment, temperature dependent polymers are important application in wide areas of biochemistry. Only small part of the publications from thermoresponsive polymers have UCST behavior instead of LCST behavior.[46] They have either lower critical solution temperature (LCST) where polymer is soluble in lower than its critical temperature and becomes insoluble above that, or upper critical solution temperature (UCST) wherein polymer is soluble above the critical temperature and becomes insoluble below that. The transition stage in UCST/LCST type polymer is concentration dependent and for that reason it is not absolute. Polymers that have LCST it is soluble because of the hydrogen bonding's intra- and intermolecularly and with water, and while heating the bonds between polymer and water disrupts and the solubility changes. When increasing the hydrogen bonding with water, hydrophilicity, the transition state increases to higher temperatures. Adding salt to this system changes the properties and also, the critical solution temperature.[47] Polymer and solvent interactions influences the transition and by adding salt, surfactant and co-solvent changes the temperature or can even make it disappear.[48]
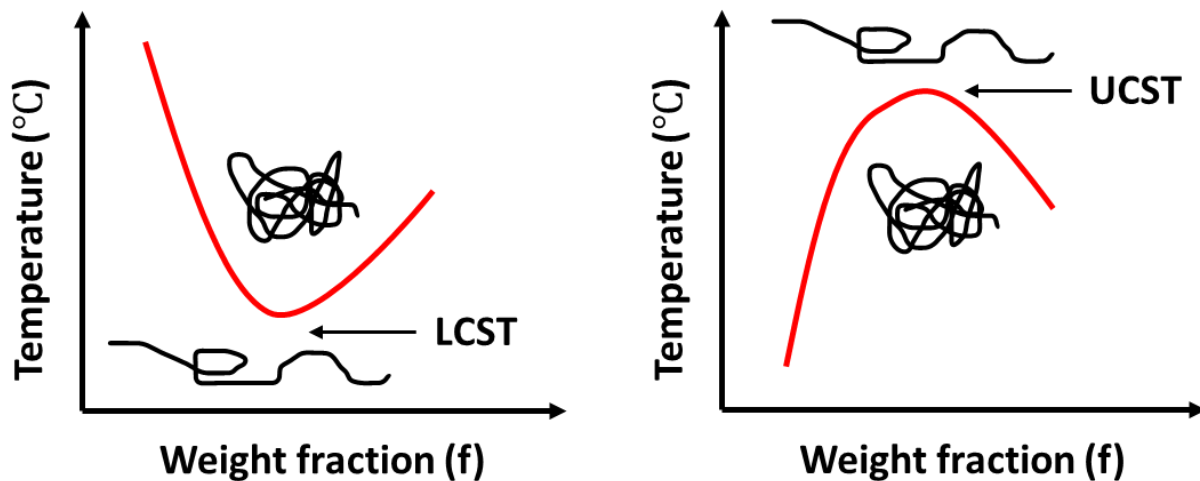
Figure 8. Scheme of LCST and UCST type behavior when changing weight fraction and temperature.

Process in solution must be thermodynamically favorable in order it to be spontaneous and this is explained with Gibbs free energy

$$\Delta G = \Delta H - T \cdot \Delta S,$$

where T is temperature, ΔS is the change of entropy and ΔH is enthalpy. For spontaneous dissolution enthalpy and entropy should be negative of the polymer and solvent interactions. Positive value of Gibbs free energy means that the process is not energetically favorable and negative that it is spontaneous. If the polymer is not soluble to the solvent, Gibbs free energy is positive. For LCST polymers hydration process has negative enthalpy for the dissolution process since the molecules from the solvent forms a thin layer to the hydrophilic part of the polymer. Negative value of entropy can be explained by extensive hydrogen bonding between the polymer and surrounding water molecules. When the temperature is changed hydrogen bonds break when it collapses and entropy and enthalpy increases, which gives positive value for Gibbs free energy. The polymer collapses and inter-molecular aggregation happens, and conformation change takes place.[49]

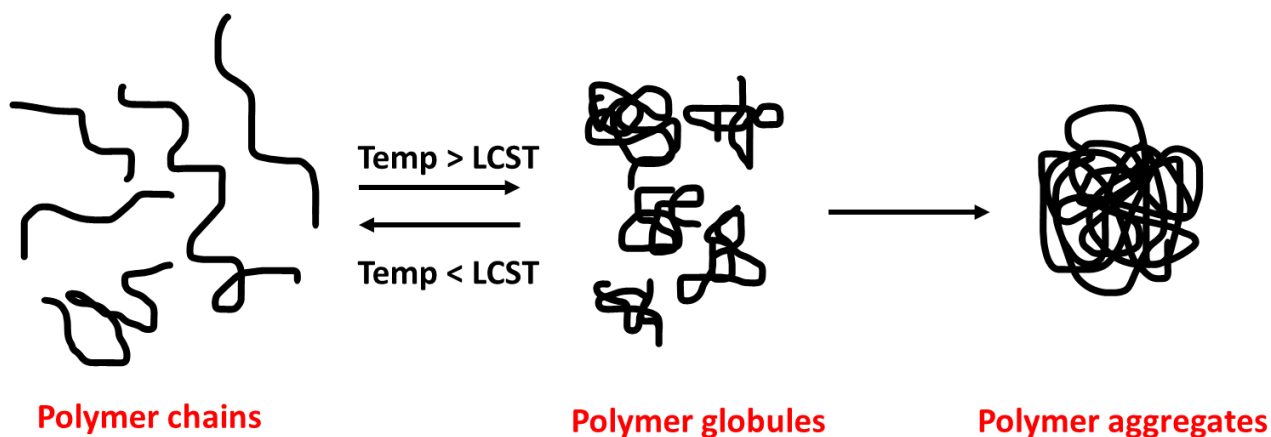**Polymer chains**   **Polymer globules**   **Polymer aggregates**

Figure 9. Interactions of LCST type polymers in a solvent. When temperature is lower than transition temperature, chains are free in the solution and when temperature is higher than transition temperature, chains forms polymer globules and finally polymer aggregates.

There are plenty of polymers that have LCST type behavior, and they are also studied a lot in the field of biomedical applications. One of the most studied polymer group is Poly(N-alkyl (meth)acrylamide)s and especially poly(N-isopropylacrylamide) PNIPAM is another studied thermoresponsive polymer that has aroused great interest. It has transition stage at around 32 °C and it is easily adjustable with different copolymer blocks. Other studied groups are Poly(N-vinylalkylamide)-based polymers, Poly(alkyloxide) copolymers, lactam/pyrrolidone/pyrrolidine based polymers and Poly(dimethylaminoethyl methacrylate). Poly(ethylene glycol), PEG is studied polymer since it has many biomedical applications. Poly(oxazoline)s are also studied group in the biomedical field.[47]

The UCST transition temperature increases when increasing the chain length because the entropy of mixing is decreased. Also, molar mass distribution has to be taken into account since polymers are never exactly uniform and it's a mixture of different molar masses. The highest value of the UCST curve is not the critical point because it is found from higher concentrations. Many polymers show UCST and LCST in certain solvent because of the temperature dependence and interaction parameter. For zwitterionic polymers, saline solutions decrease the Debye length, so salt enhances the solubility and lowers UCST.[46]

UCST type polymers have not been studied that extensively, but the interest is growing. Polybetaines have been detected to have UCST behavior because of the ionic interactions. Studied UCST type polymer is Poly(N-acryloyl glycinamide) PNAGA with terminal amide functional group. It

shows UCST transition in pure water and in saline environment because of the thermally reversible hydrogen bonding. but the solution and polymer must be pure. In dilute solutions it doesn't show gelation but forms still aggregates.[50] Those aggregates are thermosensitive but not thermoresponsive. Even though PNAGA was found in 1964, UCST behavior wasn't studied until the last decade because ionic groups were introduced by acrylate impurities in the monomer, hydrolysis of the side chains or usage of CTAs or ionic initiators.[51] Also, copolymers of NAGA and butyl acrylate with low acrylate loading showed UCST behavior.[47] Polysulfobetaines are known group which have UCST behavior because of their ionic interactions and polyzwitterionic properties. Adding salt to polysulfobetaine solutions decreases the UCST temperature because of the better solubility. Increasing molar mass, the transition temperature also increases so molar mass is affecting strongly to its UCST.[52] The chemical backbone of polysulfobetaine is affecting the UCST behavior and methacrylate derived polymers are the most common but also styrene backbone polysulfobetaines have UCST.[53]

Polymers which have both LCST and UCST have been interested researchers. Poly(3-dimethyl(methacryloyloxyethyl) ammonium propane sulfonate) and poly(N,N-dimethylacrylamide) have been synthesized trough RAFT polymerization and it has been detected to have both solution temperatures. Different blocks were altered for each transition and micelles were formed during heating or cooling the opposite way forming micelles and reverse micelles.[54] Diblock copolymer of poly(N-isopropyl acrylamide) (NIPA) and poly(3-[N-(3-methacrylamidopropyl)-N,N-dimethyl]ammoniopropane sulfonate) (SPP) via RAFT process was synthesized. Copolymer shows UCST and LCST behavior since the blocks have different behavior in water, PSPP has UCST and PNIPA has LCST. With long chain lengths copolymer stays diluted to water in whole range 0-100 °C.[55] Tunable properties are great interest and with for example poly(ethyl ethylene phosphate) (PEEP) has higher LCST if molecular weights are smaller and when it has more hydrophobic components, LCST is lower and sharper.[56] Hai-Ying Tian and coworkers detected copolymer which has tunable UCST and LCST blocks. 2-(2-methoxyethoxy) ethyl methacrylate (MEO$_2$MA), N-(3-(dimethylamino) propyl) methacrylamide (DMAPMA) and oligo(ethylene glycol) methyl ether methacrylate (OEGMA) via RAFT polymerization. LCST behavior can be tuned by changing the fraction of OEGMA and the UCST can be tuned by degree of quaternization of DMAPMA.[57]
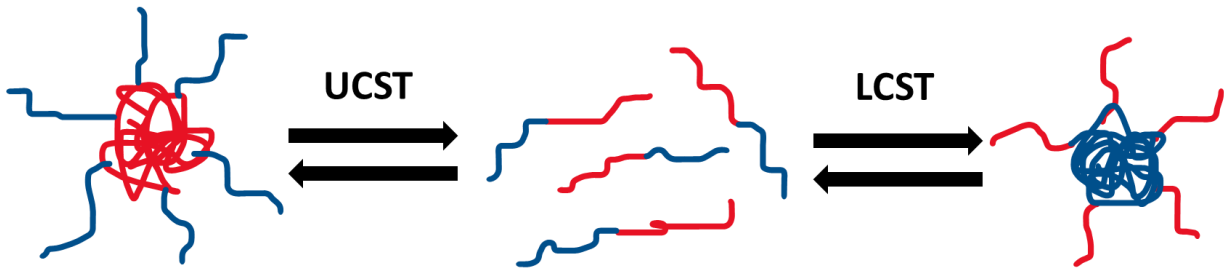
Figure 10. Illustrating picture of copolymer which has both UCST and LCST behavior in different temperatures.

II Experimental part

4. Introduction

In this experimental part PSBMA$_x$PDAAM$_y$ diblock copolymers are studied with different variations of block chain lengths and solids content to study the properties of the block copolymer and individual polymer chains. Synthesis was performed via reversible addition fragmentation chain transfer (RAFT) aqueous dispersion polymerization. PSBMA is thermoresponsive stabilizer with UCST behavior, which gives interesting properties to its copolymers. Characterization was done utilizing many different methods such as NMR, GPC, DSC, DLS and TEM. To understand more about the thermoresponsive behavior UV/Vis, DLS, rheology and micro-calorimetry was used. Changing the solids content of PISA can change the morphology of synthesized particles so this was studied, and the particles were imagined with TEM. From all these different methods, results were put together and analyzed to understand the behavior of this copolymer.

5. Aim of the research

To study the thermoresponsive behavior of PSBMA and PDAAM copolymers. More importantly, the copolymer particles via PISA synthesis of these two polymers is studied. Self-assembly to different morphologies is investigated and the thermoresponsive behavior of the particles with different morphologies was studied.
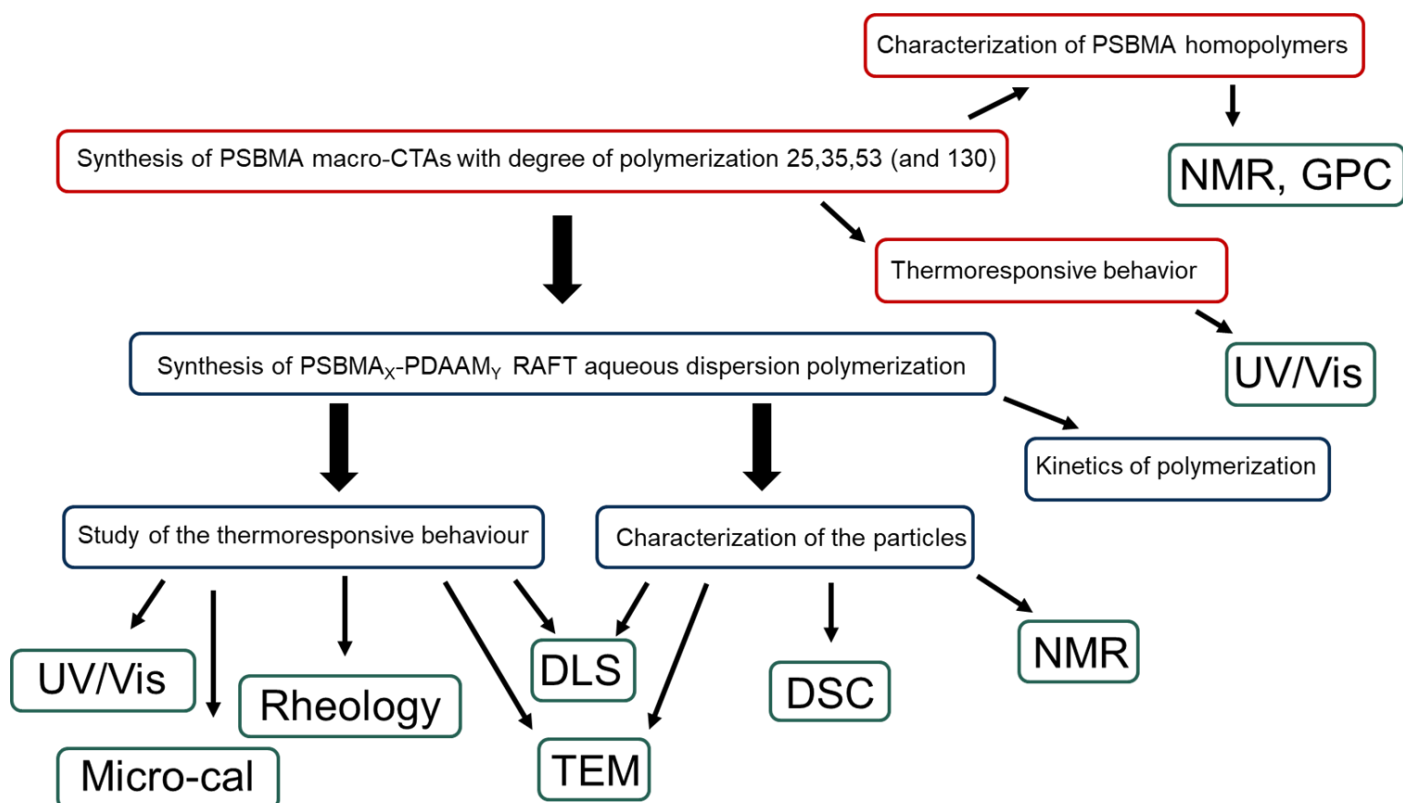
Figure 11. Schematic overview of the study.

Particularly the study concentrated to the following points:

1. **To study the PISA synthesis of PSBMA-PDAAM particle and plan the synthetic procedure. Also, reaction kinetics was studied briefly in this part.**
2. **Characterize the particles with multiple different methods. Aim is also to learn how to use these methods and understand the results from the experiments.**
3. **Study the thermoresponsive behavior of the particles by dividing the results to three different aspects: chain length of PSBMA, chain length of PDAAM and the solids concentration of the particle dispersions.**

6. **Experimental**

   6.1 NMR

The nuclear magnetic resonance (NMR) spectra were recorded using a Bruker Avance III 500 MHz spectrometer. [1]H spectra were recorded from the conversion samples and from dried samples at 21 °C. Conversion samples were recorded with 32 scans and dried products with 62 scans. Macro-CTA conversion and product were measured in D_2O, since both monomer and polymer are soluble in water. 20-50 mg/ml of the purified polymer was used in the NMR experiments

6.2 GPC

The molar masses and polydispersities were measured with a Waters Acquity APC-system equipped with Acquity APC XT 200 Å columns and UV- and RI- detectors were used. Molecular weight distributions were determined by using a phosphate buffer eluent (0.08 M Na2HPO4; adjusted to pH 8.9 using NaOH) at a flow rate of 1.0 ml min^{-1}. The number average molecular weight (M_n) and polydispersity (Mw/Mn) were calculated using a series of near-monodisperse poly(ethylene oxide) calibration standards.

6.3 Dynamic light scattering

For DLS studies, aqueous dispersion samples were prepared directly from the reaction mixtures by diluting them to 0.1% w/w. The size distribution of the nanoparticles is collected at 25°C with a Brookhaven instrument equipped with BI-200SM goniometer, BIC-TurboCorr digital pseudo-cross-correlator, and a BI-CrossCorr detector including two BIC-DS1 detectors. The light source was 25 mW Coherent Sapphire 488 nm blue laser. The temperature in the goniometer was controlled with a Lauda RC 6 CP thermostat. CONTIN was used to obtain hydrodynamic radii of the particles. Measurements were conducted from three different angles, 40 °, 90° and 140° and the average of these is determined.

For the study of thermoresponsive behavior, Malvern instruments ZetaSizer Nano-ZS equipment with 4mW He-Ne laser at 633 nm was used. Samples were put to capillary cuvettes for the measurements. Back scattering technology was used in the measurements, since the samples were so turbid. Procedure used in the measurements is cooling from 70 to 10 °C and then the size of the particles is followed 10 hours. Measurement is 5 °C step measurement and the measurements are done only to some of the polymers synthesized. With PSBMA$_{53}$PDAAM$_{400}$ also reproducibility and the effect of concentration was studied.

6.4 TEM

JEM-1400, Jeol Ltd, transmission electron microscope was used operating at 80-120 kV and fitter Orius SC1000B bottom mounted CCD-camera were used to image the nanoparticles. Images were taken at concentration of 0.1% w/w and the samples were the same than the DLS samples. Carbon coated copper TEM grids were used. The dispersions 50 μL were placed to the grid and for getting better contrast, 9 μL of 3 % uranyl formate was added to the sample. Excess sample was removed with blotting paper and the sample was let to get dry.

6.5 UV/ Vis Spectroscopy

UV–vis spectrophotometer, JASCO V-750 equipped with a JASCO CTU-100 water jacketed Peltier thermostat system was used for all the transmittance measurements. The transmittance curves for diluted dispersions were collected at 600 nm wavelength as a function of temperature. The cooling and heating rates were 1°C min^{-1}. The samples of different concentrations were stabilized at starting temperatures for 10 minutes in both cooling and heating runs.

6.6 Differential scanning calorimetry

DSC measurements were conducted with TA-Instruments DSC Q2000. Samples were saturated and prepared into hermetic aluminum pans. PSBMA homopolymers are measured with DSC and couple of copolymers to see if there are any changes in results. The amount of PSBMA$_{53}$ sample in aluminum pan was 5.1100 mg, PSBMA$_{53}$PDAAM$_{400}$ amount was 4.9200 mg and for PSBMA$_{53}$PDAAM$_{100}$ 5.0900 mg.

6.7 Rheology

TA Instruments DHR2 stress-controlled rheometer with a 20 mm steel plate-plate geometry and a Peltier heated plate were used for rheological measurements. The linear viscoelastic region for measurements was confirmed with strain sweeps. Temperature sweep and flow measurement were performed using an oscillation frequency of 1.0 rad/s. Temperature sweep was performed cooling from 20 ℃ to 1 ℃ at 10 1/s. Cooling rate used was 2 ℃/min. Flow measurements were done for each polymer studied, at temperature 20, 15, 10 and 5 ℃.

6.8 Micro-calorimetry

PSBMA$_{53}$PDAAM$_{100}$ was studied with micro-calorimetry at concentration 0.1 w%. Malvern MicroCal PEAQ DSC microcalorimeter was used to collect the thermograms. The same samples prepared for DLS were used in these experiments. The measurements were done by heating the cells from 2 ℃ to different temperatures to see how the polymer behaves. Heating rate used is 10 ℃/hour and cooling at rate 20℃/hour. Heating was performed by heating the sample three times from 2 ℃ to 100 ℃ at heating rate 10 ℃/hour and cooled down at rate 20℃/hour. Between new heating cycle, sample was let to recover for 10 minutes. In the second experiment heating was performed to 50, 60, 70, 80, 90 degrees and two times to 100℃ using the same reaction parameters.

7. Materials

Sulfobetaine methacrylate (97 %), 4,4-azobis(4-cyanovalenric acid) (≤98 %) and 1-cyanopentanoic acid dithiobenzoate (97 %) were received from Sigma Aldrich. 1M NaOH was from VWR Chemicals. Diacetone acrylamide was produced by a member of the research group. Sodium chloride was from Fisher Scientific (≤99 %). SpectraPor membrane, MwCO=1 kDa used in dialysis was from Spectrumlabs. Deionized water was from ELGA pure lab ultra-purification system.

8. Results and discussion

8.1 Synthesis

PSBMA macro-CTA is first synthesized and then copolymerization of $PSBMA_x$-$PDAAM_Y$ was done. In macro-CTA synthesis reaction solution is adjusted to pH 7.0 to facilitate dissolution of the RAFT agent. Electrolyte is also added to mixture because it enhances the water solubility of PSBMA. Series of different chain lengths of copolymer is synthesized by polymerization- induced self-assembly. $PSBMA_x$ is used in PISA as a chain transfer agent for the RAFT aqueous dispersion polymerization of PDAAM in deionized water. The total solids content used in the PISA formulation is 10 % w/w to 20 % w/w. Solids content is calculated from the sum of the solid starting compounds and when the conversion is over 99 %, it can be said that all of the solids are now particles, and the total solids can be thought to be the same.
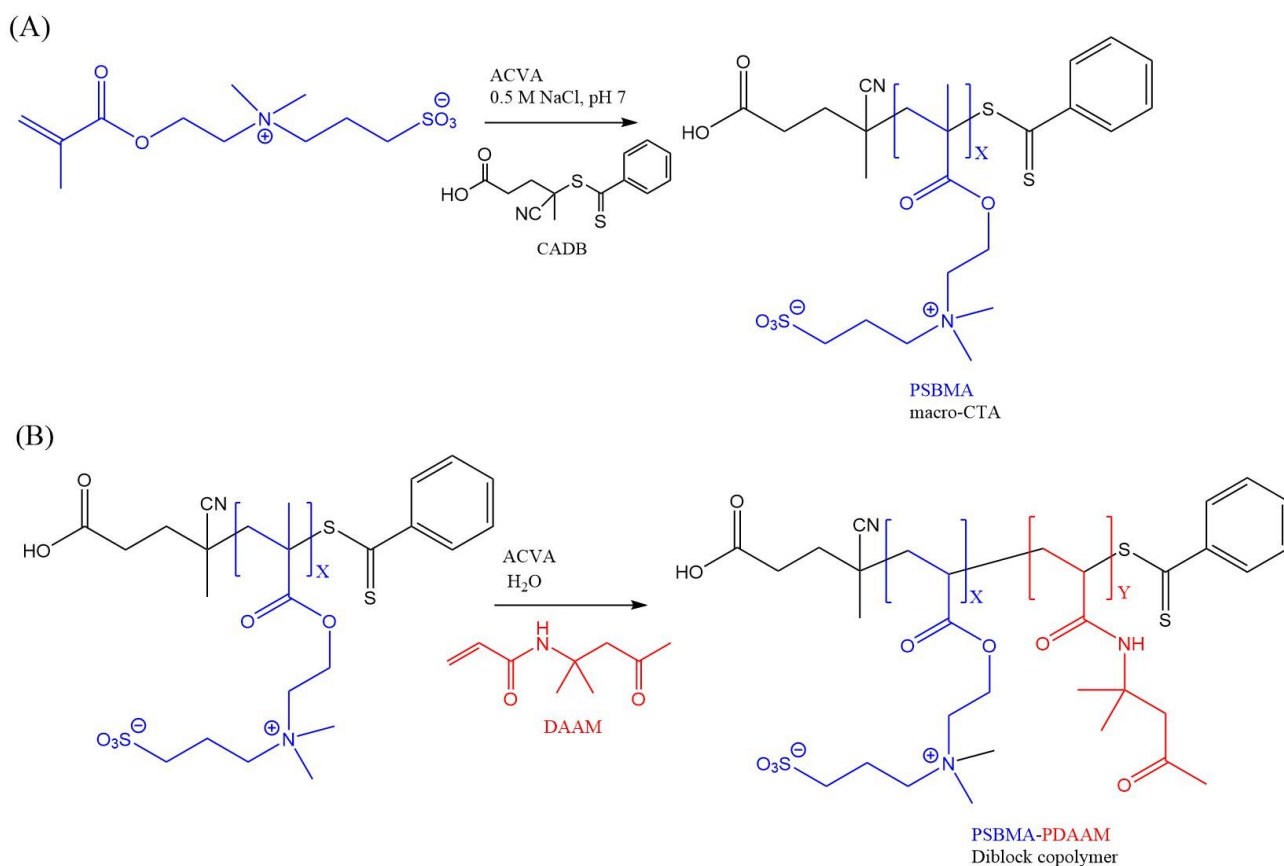
(A)

(B)

Figure 12. Mechanisms of the two reactions. In the part A macro- CTA is synthesized in pH 7 0.5 M NaCl solution and the reaction time is 3 hours. The second part of the reaction is synthesizing the copolymer via PISA. Certain lengths of PDAAM are added to three different degrees of polymerization of the PSBMA macro-CTA chains.

8.1.1 Synthesis of PSBMA$_{35}$ macro-CTA

Goal degree of polymerization used was 40, and the reaction is stopped at 88 % conversion. CADB (1-cyanopentanoic acid dithiobenzoate) RAFT agent (0.0125 g, 0,045 mmol), ACVA (4,4`-azobis(4-cyanovalenric acid) (2.52 mg, 0.009 mmol) and SBMA monomer ((2-methacryloyloxy)ethyl dimethyl (3-sulfopropyl) ammonium hydroxide)) (0.5 g, 1.8 mmol) are weighted to 20 ml reaction flask with stirring bar. CTA/ACVA ratio used in the synthesis is 5. 3 ml of 0.5 M NaCl solution is added to make the SBMA solubility better, and pH is adjusted to pH 7 using 1 M NaOH. Solution is sealed with a rubber septum and purged with nitrogen for 30 minutes. Oil bath is pre-heated to 70 ℃ and flask is immersed to it. After reaction time 2 h, polymerization is terminated by rapid cooling with liquid nitrogen when the monomer conversion is around 90 %. Conversion 88 % was detected with NMR end group analysis. The product is purified by dialysis against water and freeze dried. Different chains of PSBMA are formed by following lengths 25, 35 and 53 and these are calculated from

conversion analysis by calculating the heights of the peaks, since the samples has the same volume, they can be compared. From each of these lengths, series of different PDAAM lengths are synthesized.

8.1.2 Synthesis of PSBMA₃₅-PDAAMₓ RAFT aqueous dispersion polymerization

PDAAM target chain lengths formed are x=100, 150, 200, 250, 300 and 400. As an example procedure, target degree of polarization for this typical protocol of PDAAM is 200. $PSBMA_{35}$ (0.1737g), ACVA (0.993 mg) and DAAM (0.6 g) were weighted to reaction flask and 3 ml of water was added. CTA/ACVA molar ratio also in this procedure is 5. The solution is purged with nitrogen for 30 minutes and oil bath is heated to 70 ℃. Reaction is left for overnight and stopped by exposure to air and rapid cooling in cold water. After the reaction time conversion is more than 99 %, so the final lengths of the PDAAM chains are the same than target degree of polymerizations. Conversion is calculated from the end group analysis.

Number	macro-CTA	[M]:[CTA]:[I]	solids (w/w %)	Conversion %	M_n (theor.) (g/mol)
1	$PSBMA_{25}$	100:1:0.2	12.4, 20	>99	24 200
2	$PSBMA_{25}$	150:1:0.2	11.3	>99	32 650
3	$PSBMA_{25}$	200:1:0.2	10.7, 20	>99	96 850
4	$PSBMA_{25}$	250:1:0.2	10.5	>99	119 300
5	$PSBMA_{25}$	300:1:0.2	13.2, 20	>99	141 750
6	$PSBMA_{25}$	400:1:0.2	12.8	>99	186 700
7	$PSBMA_{35}$	100:1:0.2	19.2, 15	>99	27 000
8	$PSBMA_{35}$	150:1:0.2	17.2	>99	35 450
9	$PSBMA_{35}$	200:1:0.2	20.5	>99	99 600
10	$PSBMA_{35}$	250:1:0.2	19.8	>99	122 000
11	$PSBMA_{35}$	300:1:0.2	19.3	>99	144 550
12	$PSBMA_{35}$	400:1:0.2	18.6, 15	>99	189 450
13	$PSBMA_{53}$	100:1:0.2	18.4, 13.4, 15	>99	32 000
14	$PSBMA_{53}$	150:1:0.2	16.0	>99	40 450
15	$PSBMA_{53}$	200:1:0.2	14.7, 18	>99	104 650
16	$PSBMA_{53}$	250:1:0.2	14.0	>99	127 100
17	$PSBMA_{53}$	300:1:0.2	13.4, 20	>99	149 550
18	$PSBMA_{53}$	400:1:0.2	12.8	>99	194 500

Rows 1–6: $PSBMA_{25}$-PDAAMᵧ
Rows 7–12: $PSBMA_{35}$-PDAAMᵧ
Rows 13–18: $PSBMA_{53}$-PDAAMᵧ

Table 1. Synthesized polymers with different solids contents listed. Different solids contents per the same copolymer chain lengths are separated with comma. From every chain length of PSBMA, 6 different PDAAM block chain lengths are synthesized.

8.1.3 Kinetics of the dispersion polymerization

Kinetic studies were conducted from dispersion copolymerization to understand better the properties of the polymerization process. RAFT aqueous dispersion polymerization of DAAM was conducted using a PSBMA$_{35}$ macro-CTA in deionized water at 70 ℃ and 16 % w/w solids. PDAAM chain length was 400 and CTA/ACVA molar ratio used was 5. The same reaction conditions were used than in part 7.1.2 and reaction was conducted similarly.
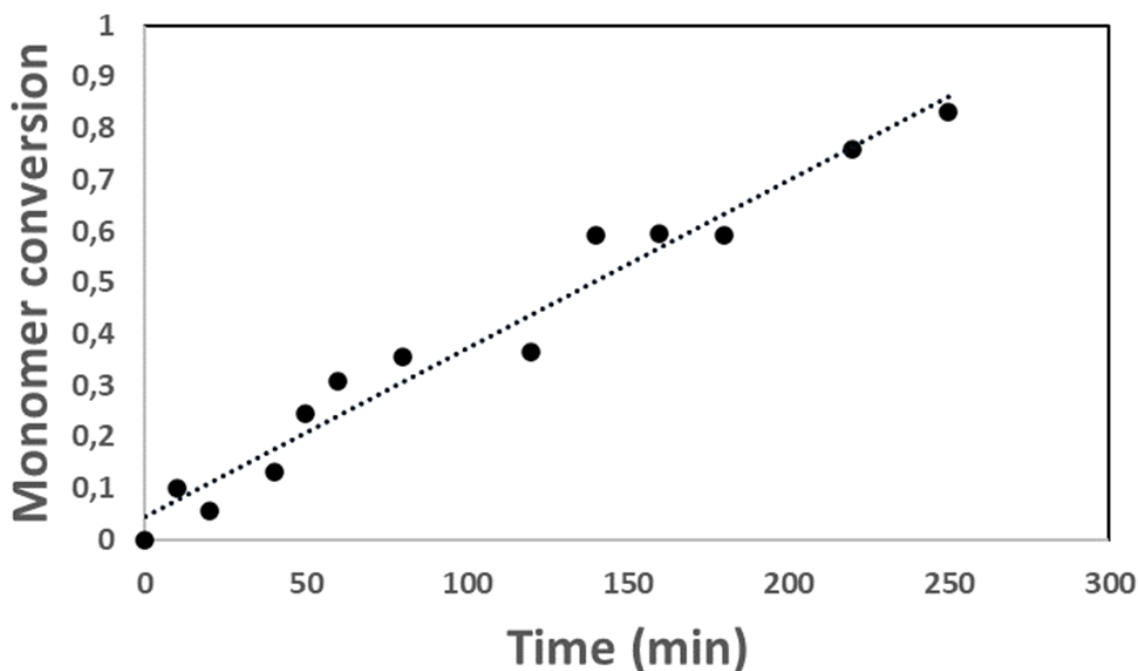

Figure 13. Kinetic plot for the RAFT aqueous dispersion polymerization of DAAM using PSBMA$_{35}$ macro-CTA in deionized water at 70 ℃ and 16 w/w % of solids.

Plot shows linear kinetics, which is usually not the case in PISA synthesis. After 4 hours the conversion is 83 % and since the plot is exponential, >99 % conversion can be expected from all the reactions because they were conducted over the night. NMR solvent used is DMSO-d$_6$ and the conversion is calculated by using DMSO peak as a reference and monitoring the change of the monomer peaks. Concentration in the NMR measurements is 0.1 ml in 1 ml of DMSO.

9.1   Characterization

In the characterization, NMR, GPC and DLS are used to determine the succession of the synthesis and to determine the size of the particles and also successful synthesis. In the characterization part, the size of the particles with DLS is recorded at 25 ℃ with Brookhaven instrument.
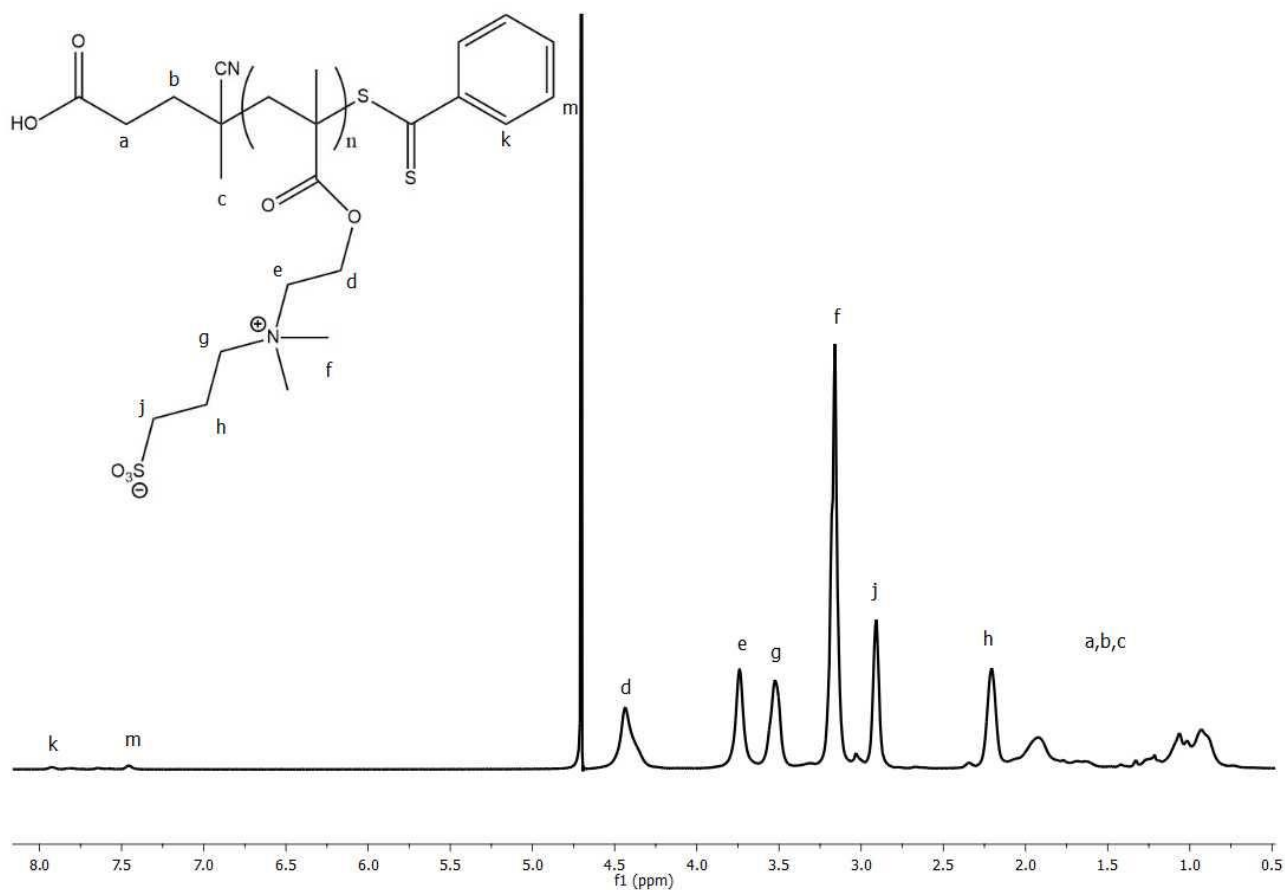
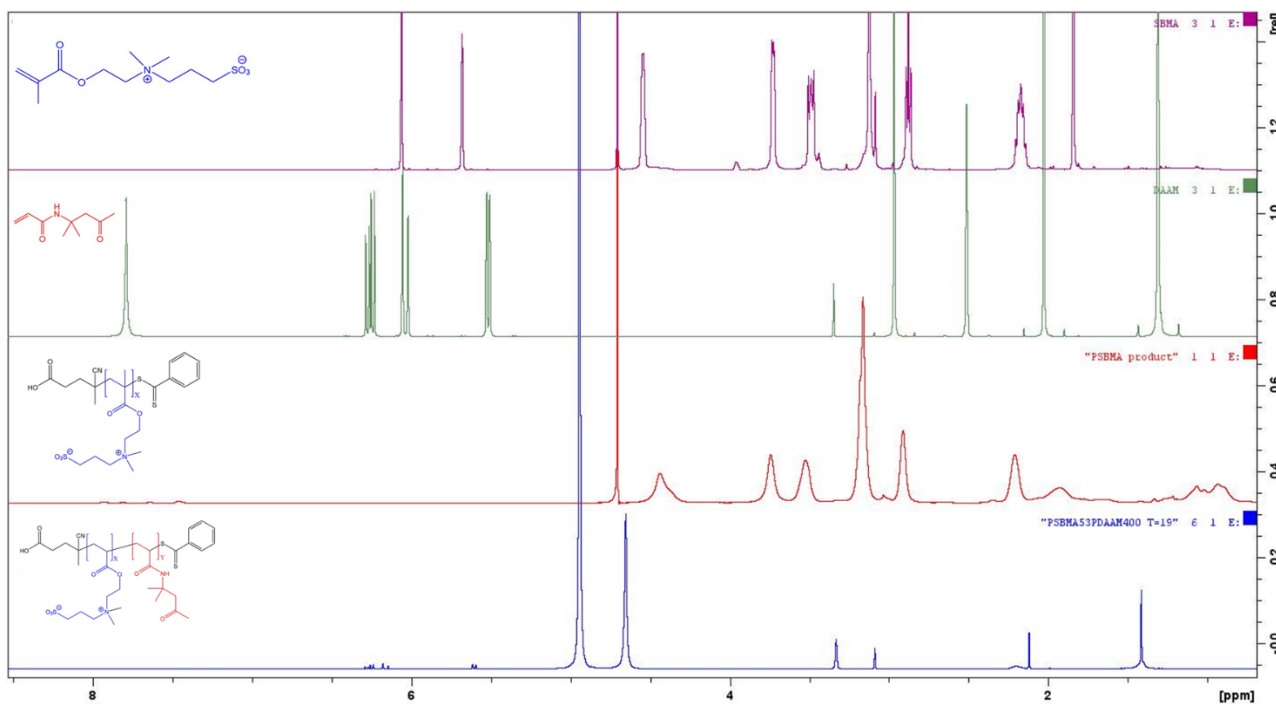*Figure 14. ¹H NMR spectrum recorded from PSBMA macro-CTA in D₂O. 64 scans were recorded at 21 ℃.*

Figure 15. NMR from monomers, PSBMA homopolymer and PSBMA-PDAAM copolymer. No monomer peaks can be seen in the copolymer NMR spectra, so all the monomer has been reacted and conversion is high.

Initiator area in NMR spectra is 8-7 ppm, and it is used to calculate the degree of polymerization from the products. DP is calculated by integrating initiator area 8-7 ppm and d group from the polymer (*Figure 14.*) in area 4.5-4.3 ppm. After this the initiator area integrate is determined to be 5 since the aromatic ring has 5 hydrogens. The final degree of polymerization we get by dividing the value with hydrogens the polymer group has which is 2. Conversion is calculated from the monomer peaks which are decreasing during time and reactions are thought to be done after there is no monomer peak or at the case of PSBMA homopolymer when the conversion is around 88-90 % the reaction is stopped.

Macro-CTA	M (Theo.)	M_n(NMR)	M_n(GPC)	M_w(GPC)	M_w/M_n
PSBMA$_{25}$	8 680	7 250	2 516	3 455	1,37
PSBMA$_{35}$	11 480	10 000	4 830	6 227	1,29
PSBMA$_{53}$	14 300	15 050	7 479	9 501	1,27

Table 2. Molar masses of homopolymer PSBMA chain lengths from different characterization methods.

Molar masses used in the study are from NMR, since it turned out to be the most reliable in comparison to GPC because of the calibration standard used. Theoretical molar masses are the goal degree of polymerizations so as the conversion is around 90 %, the real molar mass is not theoretical one. It is common that molar masses from GPC have lower molar weight than from NMR. Also, the calibration standard is not the best one for this homopolymer, so the molar mass is not reliable from GPC. Polydispersity and the distribution from GPC are more important and the polydispersity is low, and distribution is narrow which means that the polymerization is successful, and the product is not a mix of different lengths. Copolymers wasn't studied with GPC.
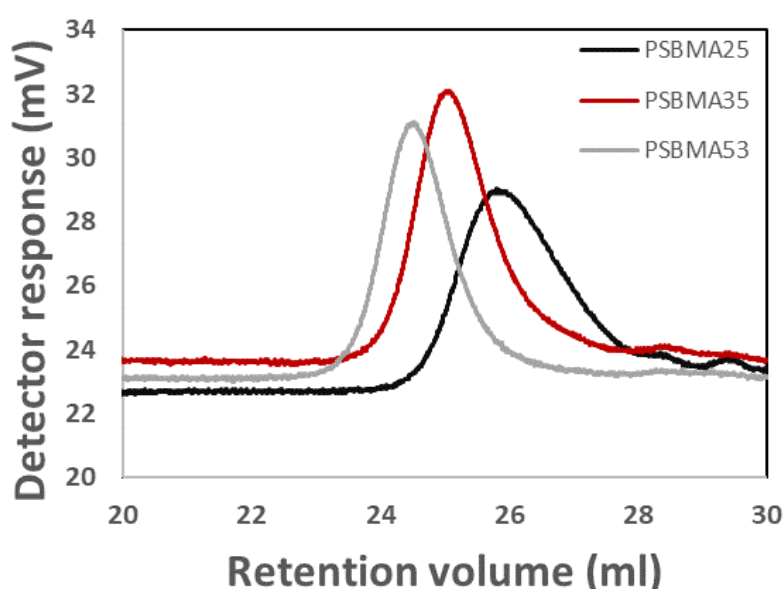
Figure 16. GPC spectra from different chain lengths. From the figure it can be seen that the longest chain comes first and the shortest last.

Sample	DP of PDAAM	90°	140°	40°	Average
PSBMA$_{25}$-PDAAM$_y$	100	792,7	275,7	1069,4	712,6
	150	677	545,5	773,4	665,3
	200	900,8	1155,5	826	960,7667
	250	497,4	798,7	740,3	678,8
	300	121,1	121,1	117,4	119,8667
	400	93,5	93,5	121,8	102,9333
PSBMA$_{25}$-PDAAM$_y$ 20 w%	100	332,4	258,3	358,7	316,4667
	200	205,3	252,3	250,5	236,0333
	300	203,1	265,7	220,8	229,8667
PSBMA$_{35}$-PDAAM$_y$	100	85,4	84,2	89,3	86,3
	150	55,2	55,3	56,4	55,63333
	200	643,5	823,3	205,3	557,3667
	250	126,2	127,4	111,9	121,8333
	300	107,8	108,7	105	107,1667
	400	74	75,4	64	71,13333
PSBMA$_{53}$-PDAAM$_y$	100	17,4	19,4	17,7	18,16667
	150	24,1	27,5	20,5	24,03333
	200	33	38,7	27,2	32,96667
	250	40,8	46,6	30,3	39,23333
	300	49,2	55,2	35,4	46,6
	400	73,7	73,6	53,8	67,03333

Table 3. Results from the DLS measurements from three different angles. Values marked with red are problematic because the value from different angles differs.

From the DLS, it can be estimated that there are different morphologies, but also strong aggregation is possible which will be studied later in this research.
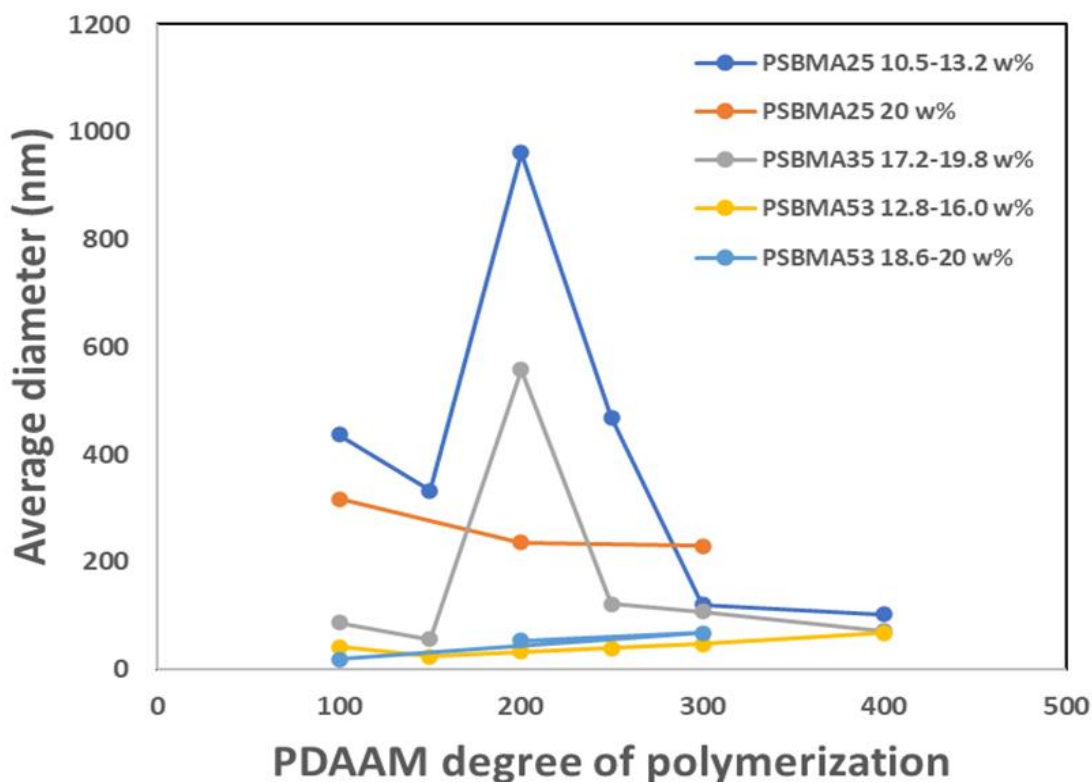
Figure 17. Figure formed from the average of the three different angles. Aqueous dispersion samples were prepared directly from the reaction mixtures by diluting them to 0.1% w/w. The size distributions of the nanoparticles were collected at 25°C with a Brookhaven instrument.

If we compare PSBMA$_{25}$-20 w% sizes to the lower solids content polymers with same PDAAM chain length, higher solids content ones are 2-3 times smaller. Also, copolymers from PSBMA$_{53}$ are clearly smaller than from shorter chain lengths. At this point, different morphologies such as worms and vesicles are expected.

9.2 Measurements

Different properties of copolymers were measured and analyzed. Especially temperature dependance was studied to see if the copolymer has different properties at low and high temperatures. In this part of the research, DLS, TEM, DSC, rheology, and Micro-Calorimetry are used.

9.2.1 Morphology
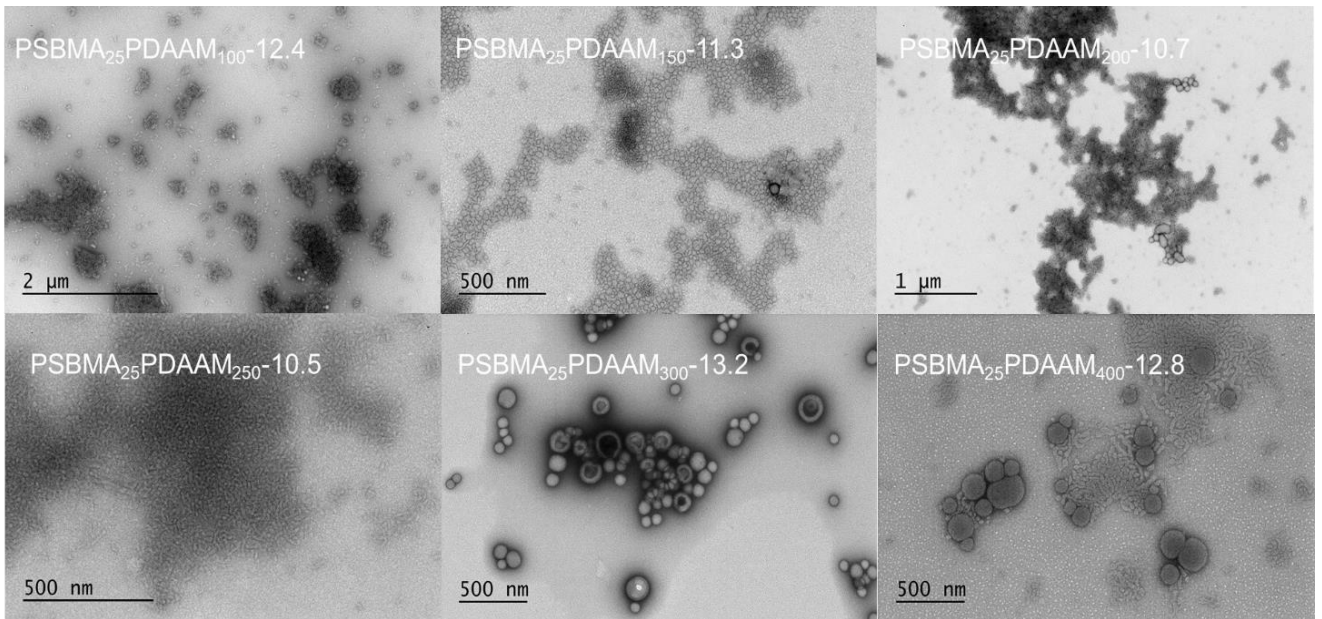
Figure 18. TEM pictures from PSBMA$_{25}$PDAAM$_y$. Concentration of the samples is 0.1 % w/w.

Strong aggregation can be seen from the pictures and some small networking in PDAAM chain length 200 sample. PDAAM chain length 300 forms already some micelles. Networking and aggregation could explain why light scattering sizes are quite large.
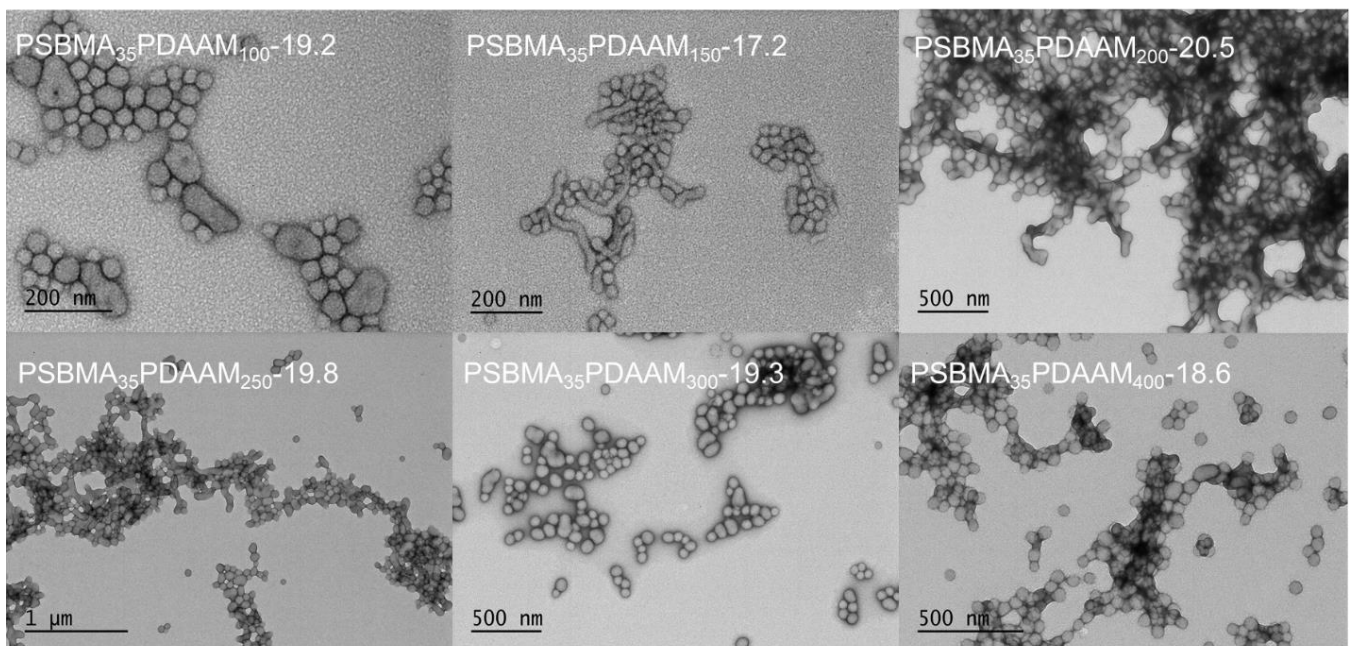
Figure 19. TEM pictures from PSBMA$_{35}$PDAAM$_y$. Concentration of the samples is 0.1 % w/w.

Mostly spheres and small associated worms can be seen. Also, at PDAAM length 200 and 250 networking in a small scale can be observed.
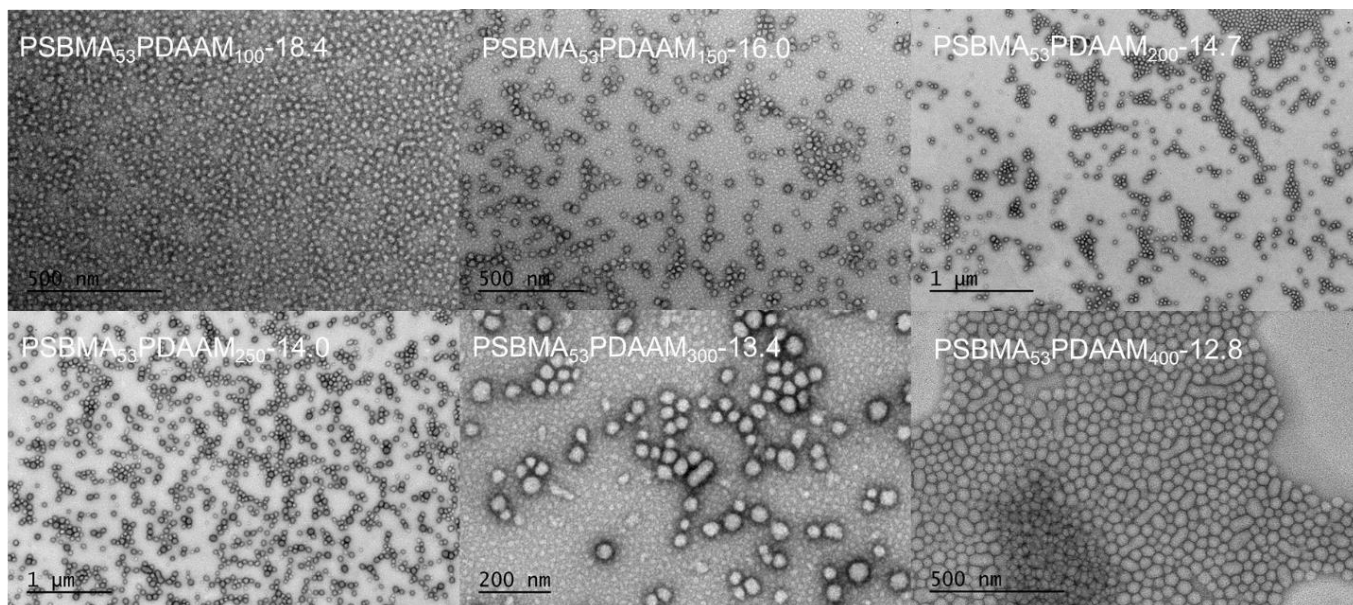
Figure 20. TEM pictures from different chain lengths of PDAAM with macro-CTA chain length 53.

Pictures correspond well with the DLS results where each of the series should be under 70 nm. The size of the spheres is increasing when PDAAM length is increasing. At the 300 and 400 DP there are small, combined spheres/ small worms.
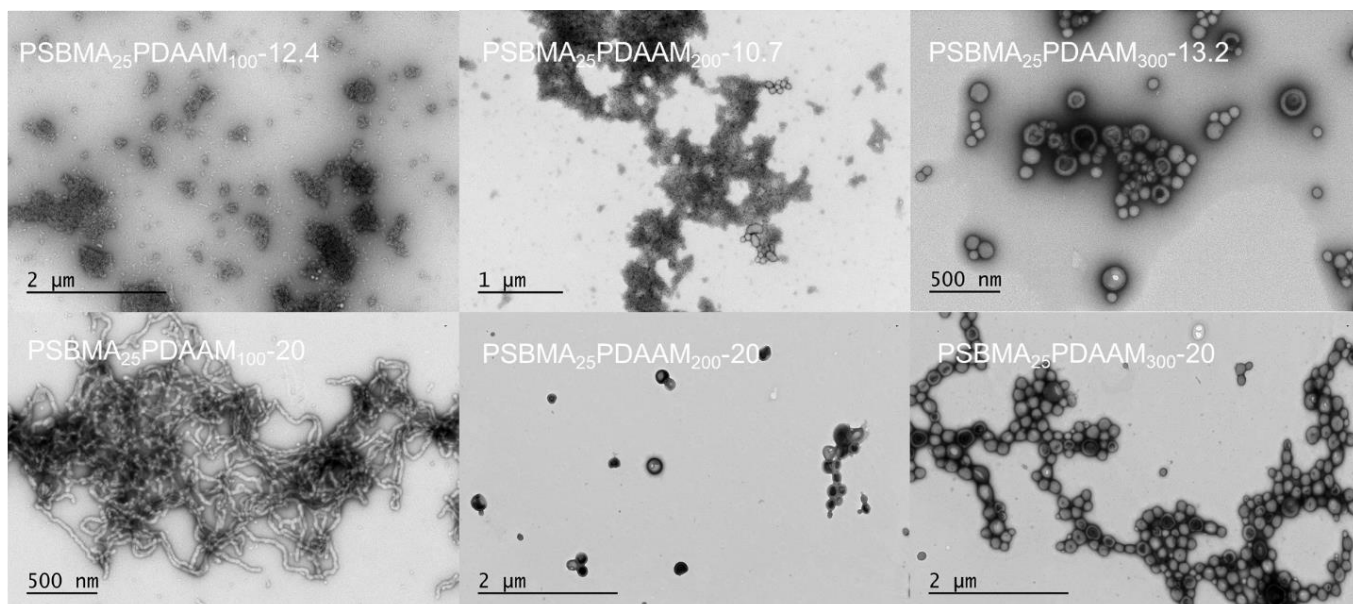
Figure 21. Change in the morphology when the solids content is changed.

In lower solids content mostly spheres are formed but when the total solids content is 20 w% different morphologies can be seen. PDAAM chain length 300 is the only one that doesn't change its morphology when changing solids content. To conclude, morphology changes from spheres to worms and vesicles with short PSBMA chain lengths when changing the solids content to high values.

Because the polymer is thermoresponsive, it was interesting to see if there are any morphological changes when cooling down the sample. TEM imaging was done by keeping sample for 10 hours at 10 °C, diluted quickly and measured immediately so that we could picture the morphology as precise as possible at lower temperature. Procedure in the imagining was the same than with the previous TEM samples.
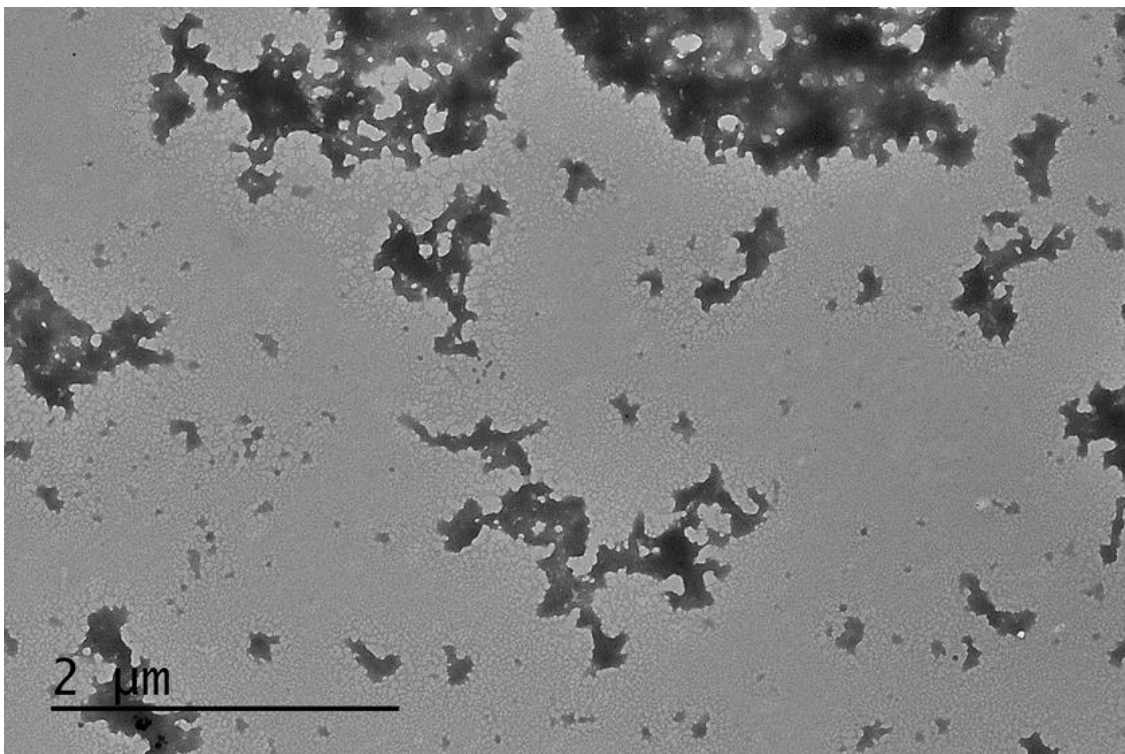
Figure 22. PSBMA$_{53}$PDAAM$_{200}$ 14.7w% sample after 10 hours in 10 degrees. Concentration in the dilute TEM grid system is 0.1 w%.

From the picture it can be seen that there is strong aggregation, and this could explain why the size of the polymer gets bigger in the DLS experiment when temperature is decreased in later studies. When looking closely, the morphology is still same, spheres and small worms so no morphology change can be seen.

9.2.2 Thermoresponsivity

Turbidimetry measurements was done to different chain lengths of PSBMA and to different PSBMA$_{53}$PDAAM$_y$ series. For this, also PSBMA$_{130}$ was synthesized to see possible changes in the turbidity. Copolymer series studied with this method didn't give hoped results, because the solutions were already turbid even at the starting temperature 90 ℃ so back scattering with DLS was used with the copolymers. Also, the effect of salt was studied with couple samples but no changes in the turbidity was noticed.
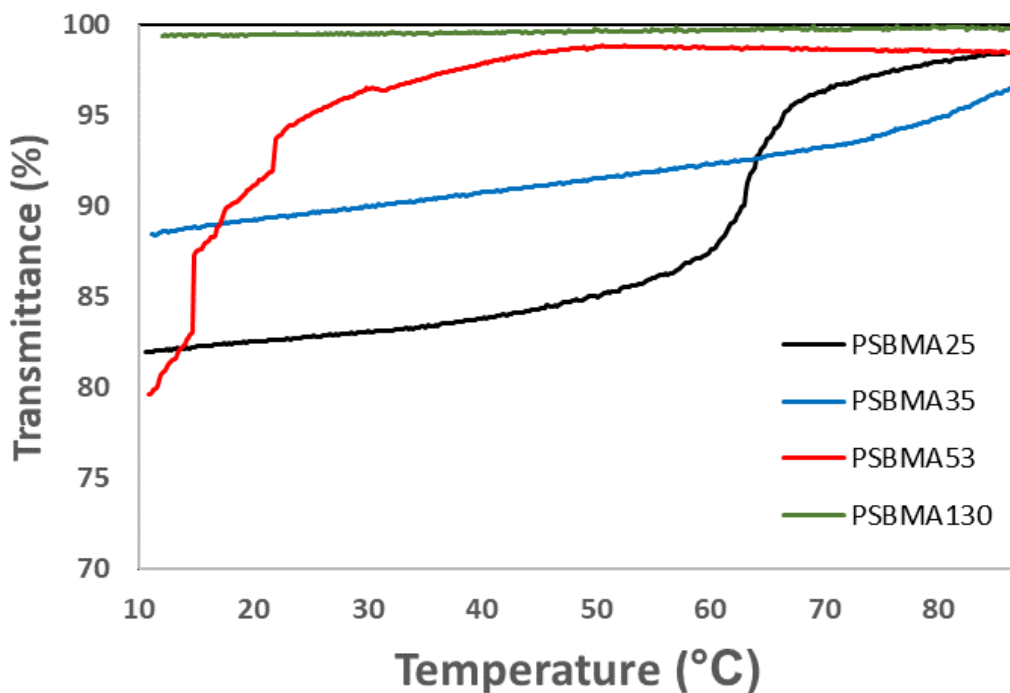
Figure 23. Transmittance curve of synthesized PSBMA chains upon cooling the sample from 90 ℃ to 10 ℃.

Unexpectedly, PSBMA$_{25}$ shows clearest UCST behavior. Also, chain lengths 35 and 53 shows UCST behavior, but PSBMA$_{130}$ doesn't show any change in transmittance. Concentrations used in the transmittance experiments were 0.1 w %. It is possible that it has UCST that is out of the measured temperatures. The transmittance curves of different chain lengths doesn't show any typical behavior.
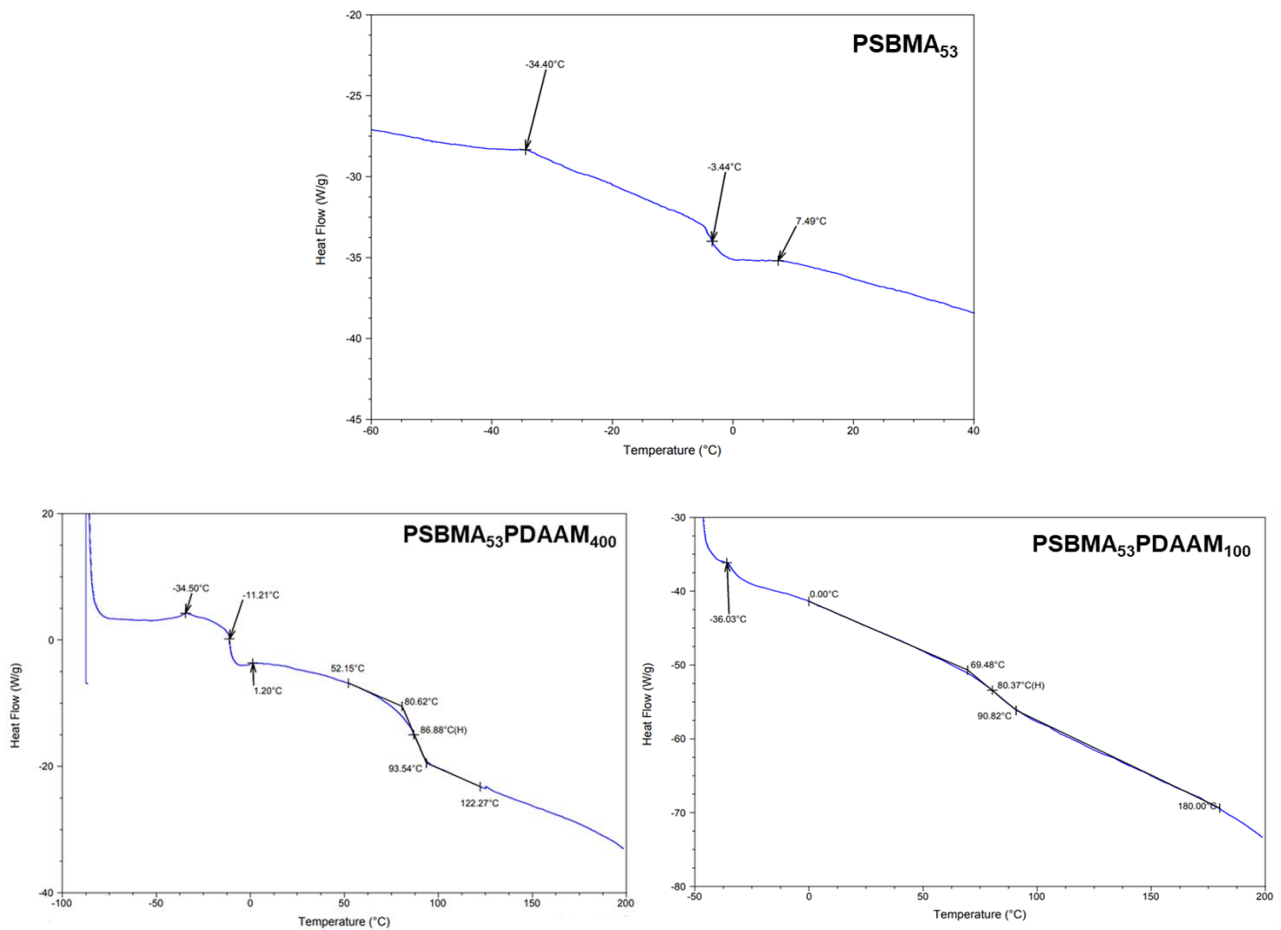
Figure 24. Results from DSC measurements. The amount of PSBMA$_{53}$ sample in aluminum pan was 5.1100 mg, PSBMA$_{53}$PDAAM$_{400}$ amount was 4.9200 mg and for PSBMA$_{53}$PDAAM$_{100}$ 5.0900 mg.

Samples for DSC was dialyzed and then freeze dried. The same PSBMA transitions can be seen in homopolymer and in copolymers, so PDAAM transition is the one not to be seen in the homopolymer figure. Transition in PSBMA is seen around 0 ℃ and around -35 ℃. PDAAM on the other hand gives T$_g$ temperature of 86.88 ℃ with longer chain and with shorter chain 80.37 ℃.
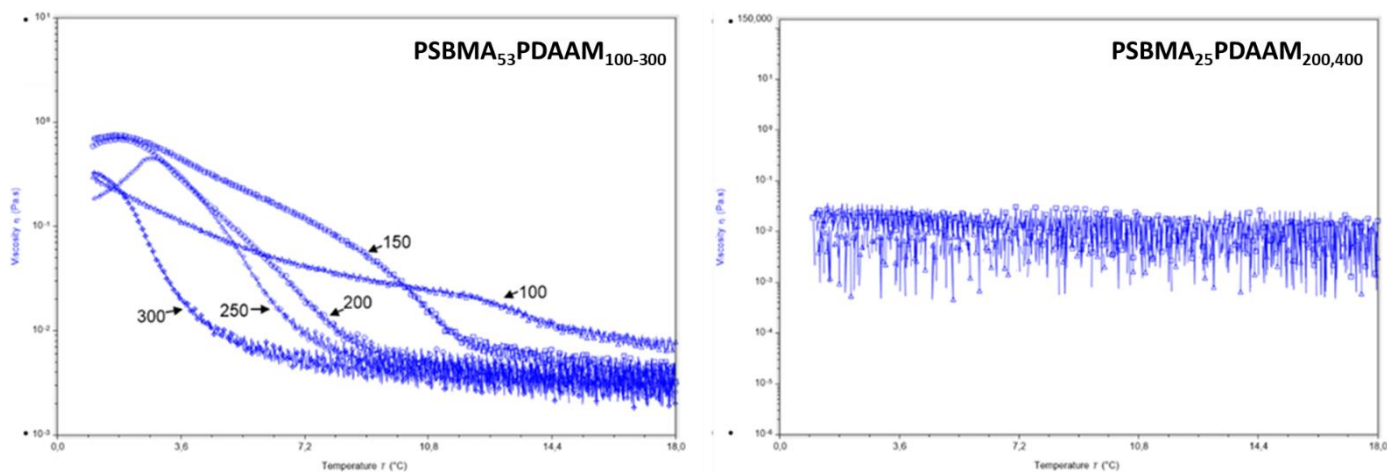
Figure 25. Temperature sweep of different copolymers measured at 10 1/s measured as cooling sweep.

PSBMA$_{25}$ doesn't give any difference in viscosity in the temperature area but CTA chain length 53 on the other hand gives change in viscosity when temperature is changed. There is a transition stage with each of the PSBMA$_{53}$PDAAM$_y$ chain lengths. There is a pattern of transition stage moving to colder temperatures when the PDAAM chain is getting longer, which is a bit unexpected but could be explained by the strength of longer chains. When cooling the polymer it needs lower temperatures to get to the transition stage when shorter chain lengths of PDAAM reacts more easily. Overall, we can say that PSBMA$_{53}$ shows more temperature dependence than shorted chain lengths at given temperatures.
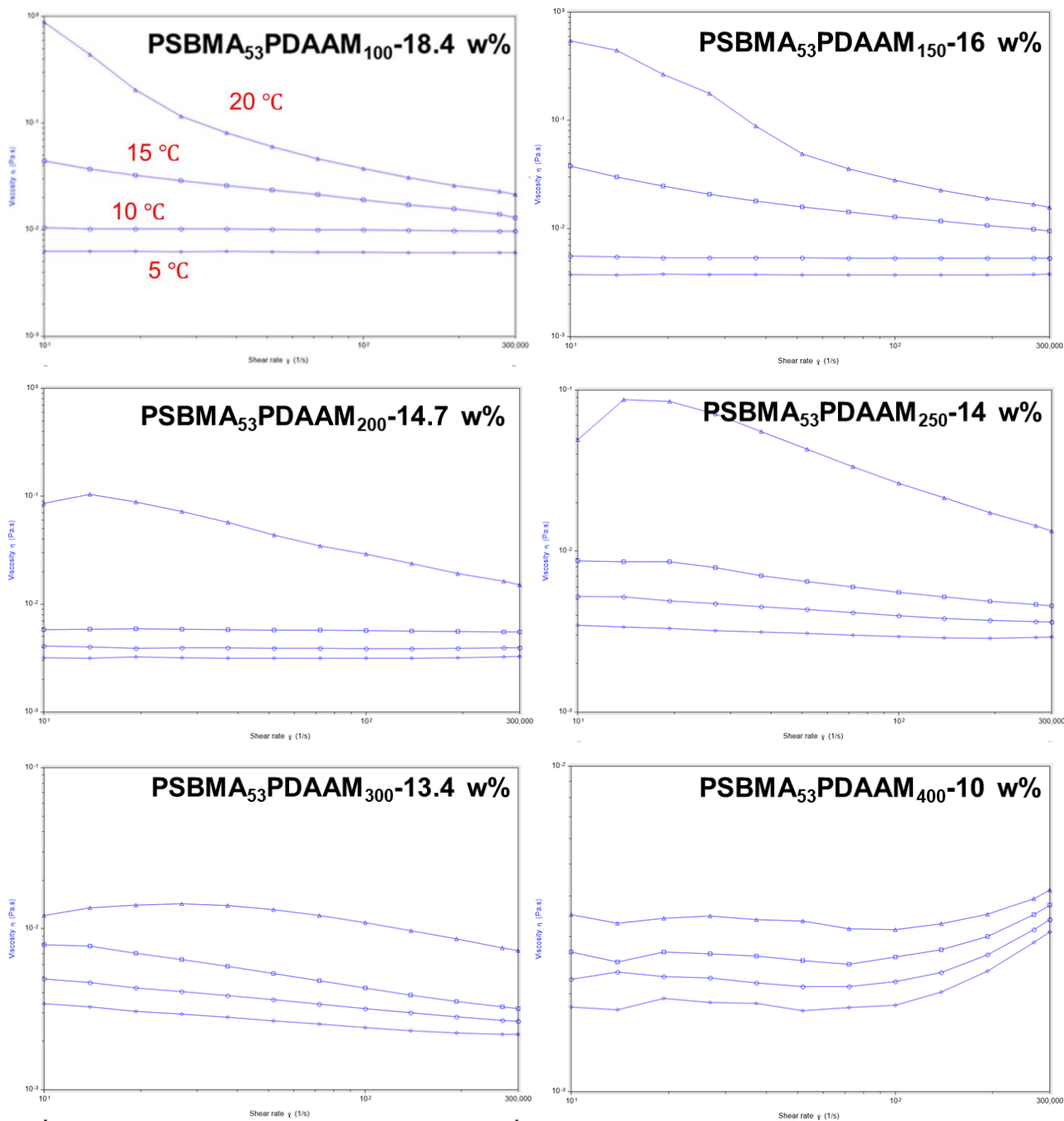
Figure 26. Four flow measurements for each polymer, at temperature 20, 15, 10 and 5 ℃.Each of the plots are shown at same scales so the differences can be seen easily. with every copolymer the topmost plot is at 20 ℃ and each of them decrease in viscosity when lowering the temperature.

In the figures the topmost plot is at 20 ℃ and the plots are at lower viscosity when temperature is going down. At 20 ℃ PDAAM chain lengths 100-300 has shear thinning behavior and they start to behave like Newtonian liquid at lower temperatures. $PDAAM_{400}$ on the other hand behaves completely differently and has shear thickening behavior at every temperature measured.
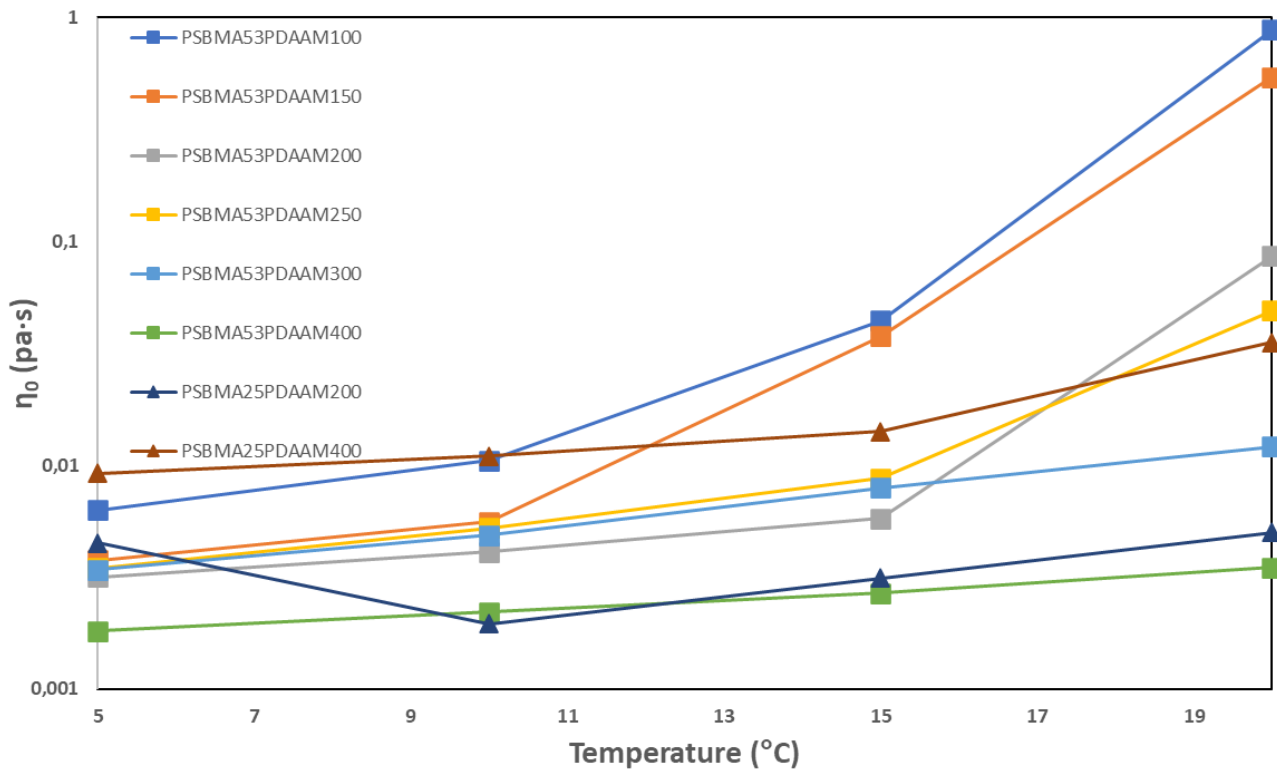
Figure 27. Zero shear viscosities of some copolymers at 10 1/s.

PSBMA$_{25}$PDAAM$_Y$ doesn't show any remarkable difference in zero shear viscosity at measured temperatures. PSBMA$_{53}$PDAAM$_Y$, Y=100 and 150 shows 100 times smaller zero shear viscosities at 5 ℃ than at 20 ℃. This means that the copolymers are stiffer at high temperatures than at low temperature. When comparing these three figures from rheology studies, we can notice that they argue with each other's. This can be explained by the experimental set up, only temperature sweep was cooling down and other experiments were heating. Therefore, it seems that the longer PDAAM chain lengths reacts slower to temperature changes than shorter chains lengths.
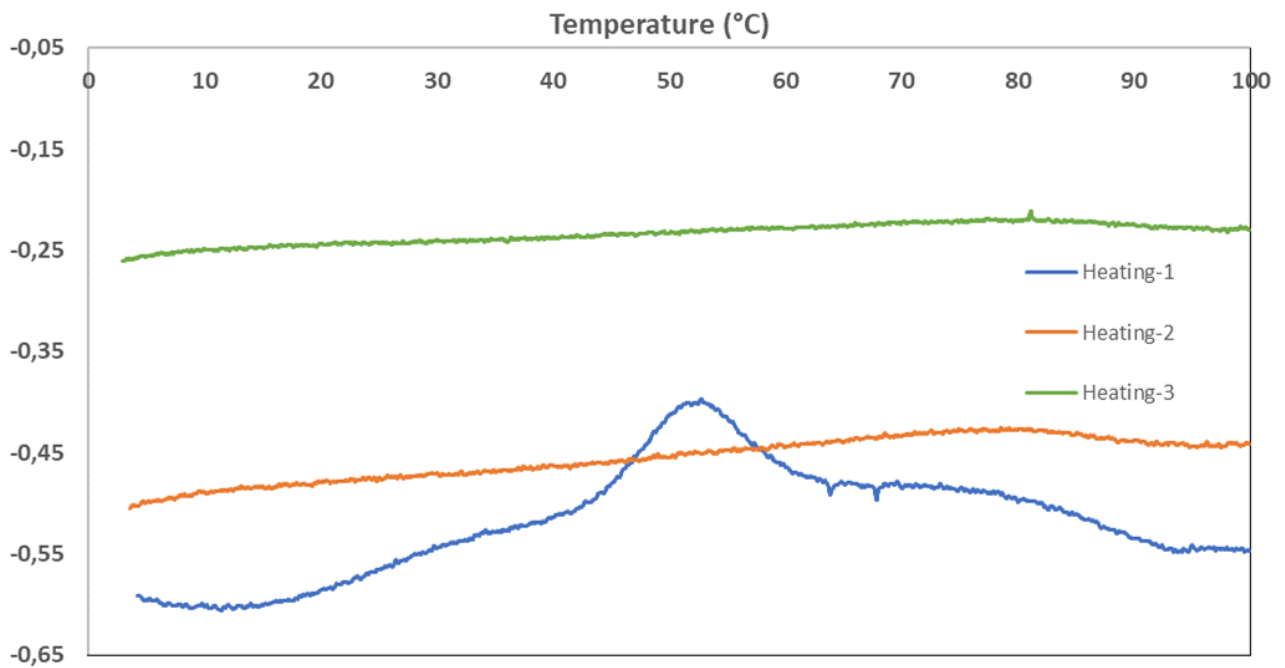
Figure 28. Micro-calorimetry heating curves of PSBMA$_{53}$PDAAM$_{100}$.

In the first heating polymer has an endotherm stage at around 50 ℃ but in the second heating we can barely see small transition at 80 ℃. The Y-axis is only the relative enthalpy change and the values seen in the axis doesn't really tell anything. It seems that there is a conformational transition only at the first heating. Polymer reorganizes and does not recover anymore. This could be thought to be a behavior of the core block.
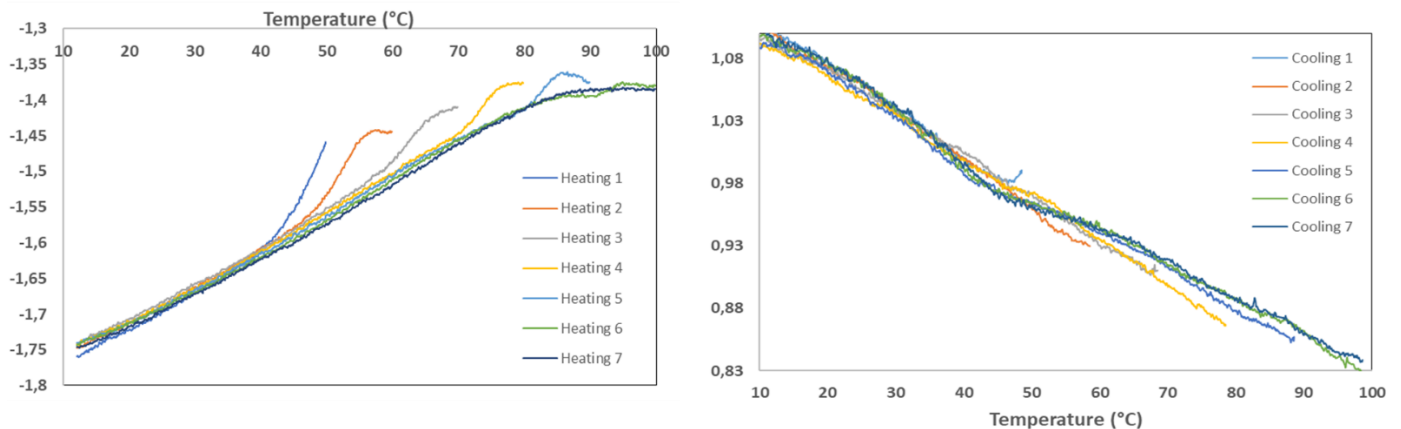

Figure 29. Heating and cooling were done for PSBMA$_{53}$PDAAM$_{100}$ 7 times by using the same parameters than in the earlier experiment. Heating was performed to 50, 60, 70, 80, 90 degrees and two times to 100℃.

We can see that the behavior changes when we start heating the polymer to higher temperatures. Transition starts to move to higher temperature when we heat more the polymer and when heating first time to 100 ℃ only small transition can be seen and second time there is not any transition to be seen. From the figure it can be predicted that 90 ℃ is the critical temperature since when heating to 100 ℃ we can´t see proper transition like in the other ramps. It is possible that the core breaks down and can´t reform again after heating high enough. Also, in the previous figure this can be seen, in the first cycle to 100 ℃ clear transition can be seen but already in the second heating there is small transition in higher temperatures.
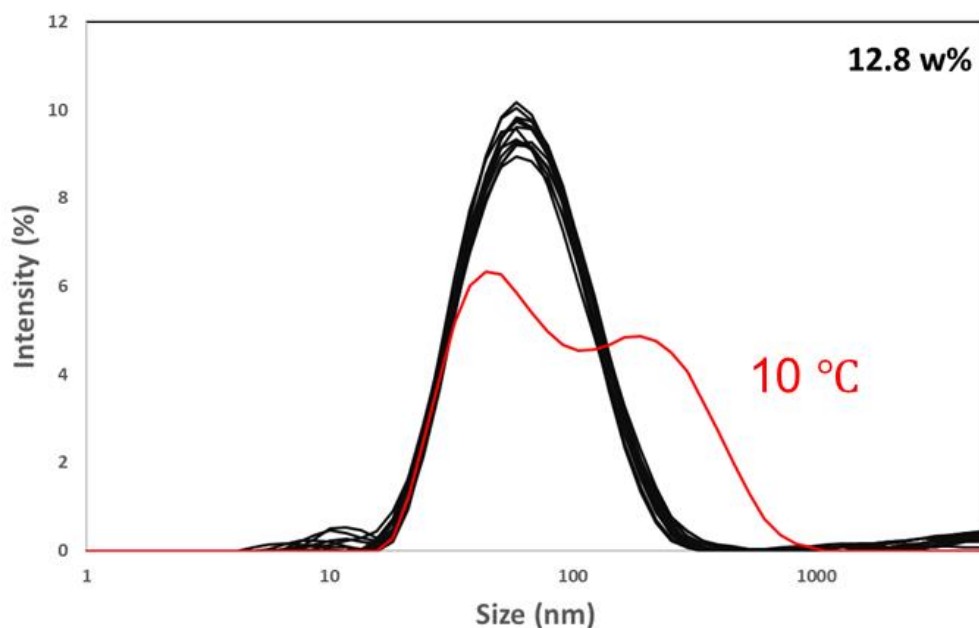

Figure 30. PSBMA$_{53}$PDAAM$_{400}$ sample measured at different temperatures with Malvern instruments ZetaSizer. Cooling is proceeded from 70 to 10 ℃.
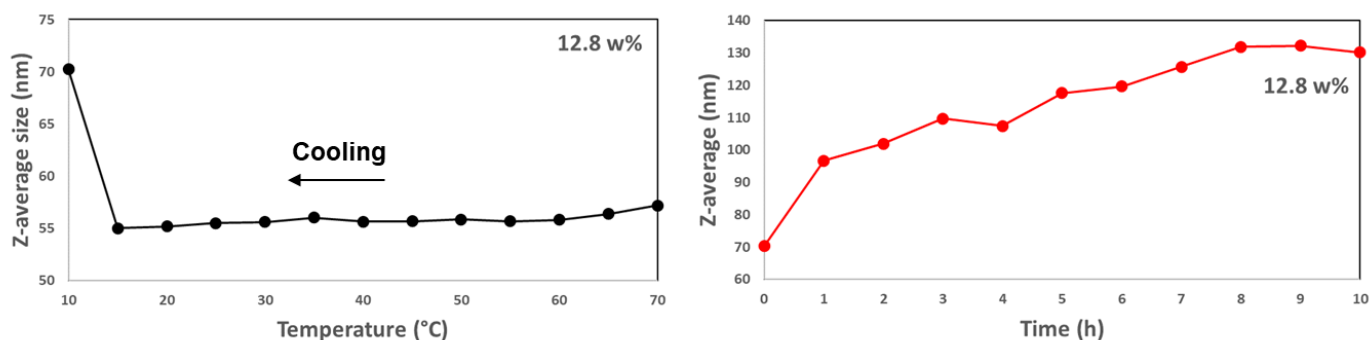
Figure 31. Temperature measurement of PSBMA$_{53}$PDAAM$_{400}$. First figure in the left is cooling the sample from 70 to 10 ℃ and in the right is time figure of time against the Z-average size at 10 ℃.

Change in the size distribution can be seen at 10 ℃ and there are no changes in the size before 10 ℃. This is the critical temperature where the properties changes. From the figures can be seen that critical size change is happening between 15 and 10 ℃. Also, the size is increasing when time is passing so the reaction mixture is deforming over the time and aggregating.
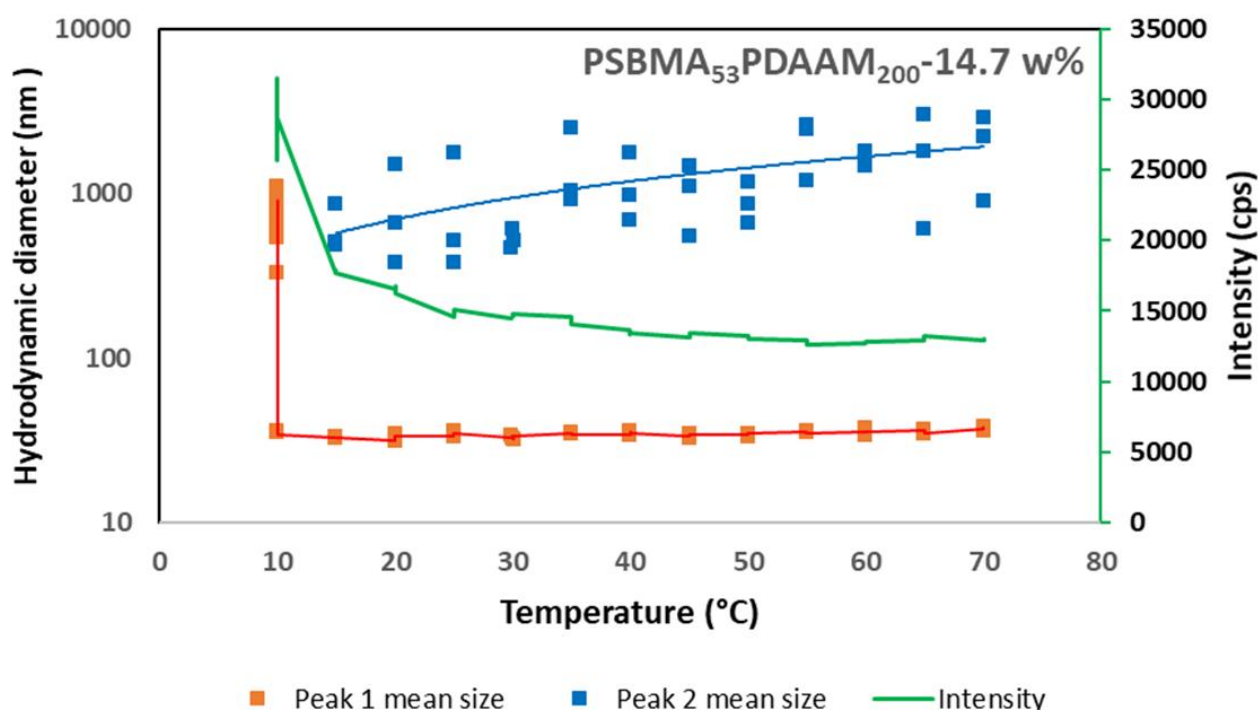

Figure 32. Figure shows the size of the two peaks when cooling down the copolymer as peak 1 and peak 2 mean size. These two peaks are the ones always showing in earlier DLS figures having different intensities. Also, the intensity of the measurement is shown in this figure.

Intensity stays the same until 15 ℃ and between 15-10 ℃ it changes dramatically, and this is the cloud point of the polymer. Below this temperature the polymer forms cloudy appearance phase separates from the solution. Above this the polymer mixture can be seen as a clear transparent solution.

9.2.3 Reproducibility of the temperature measurements

With PSBMA$_{53}$PDAAM$_{400}$ reproducibility and the effect of concentration was studied with Malvern instruments ZetaSizer Nano-ZS equipment. Measurement was done two times following exactly same procedure and the size was followed during the measurement.
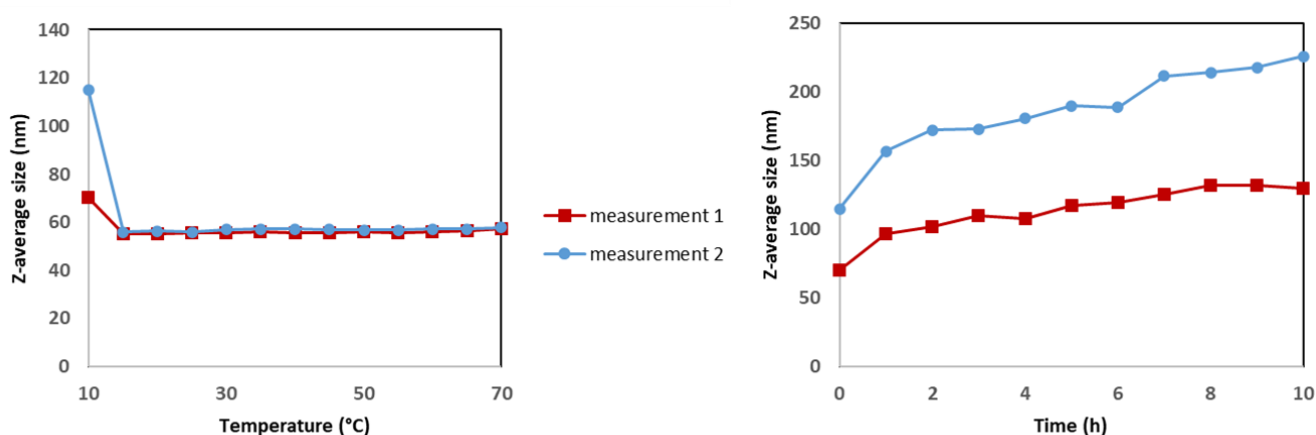

Figure 33. Reproducibility of the measurement with PSBMA$_{53}$PDAAM$_{400}$. Figures follow the same theme so the measurements should be reproducible to some extent.

9.2.4 Effect of the concentration

Different solution concentrations from the same solids concentration PISA particles are measured and shown in the figure below. The original sample formed with PISA is 12.8 w % and it is diluted to see if the diluted, lower concentrations gives same results. Results show that the concentrations of the samples is an important factor and lower concentrations give no change in sizes of the particles. Measurements were conducted cooling from 70 to 10 degrees and with 5 step program. After cooling down to 10 ℃, size is followed for 10 hours. The sample is PSBMA$_{53}$PDAAM$_{400}$ and different concentrations are 1 w%, 5 w%, 10 w% and the original dispersion 12.8 w%. Different concentrations were formed by diluting the sample to ionized water. Only the original dispersion showed results

with changing size of the particles. Particle size is changing at 10 °C and after that the size distribution is changing when time is passing.
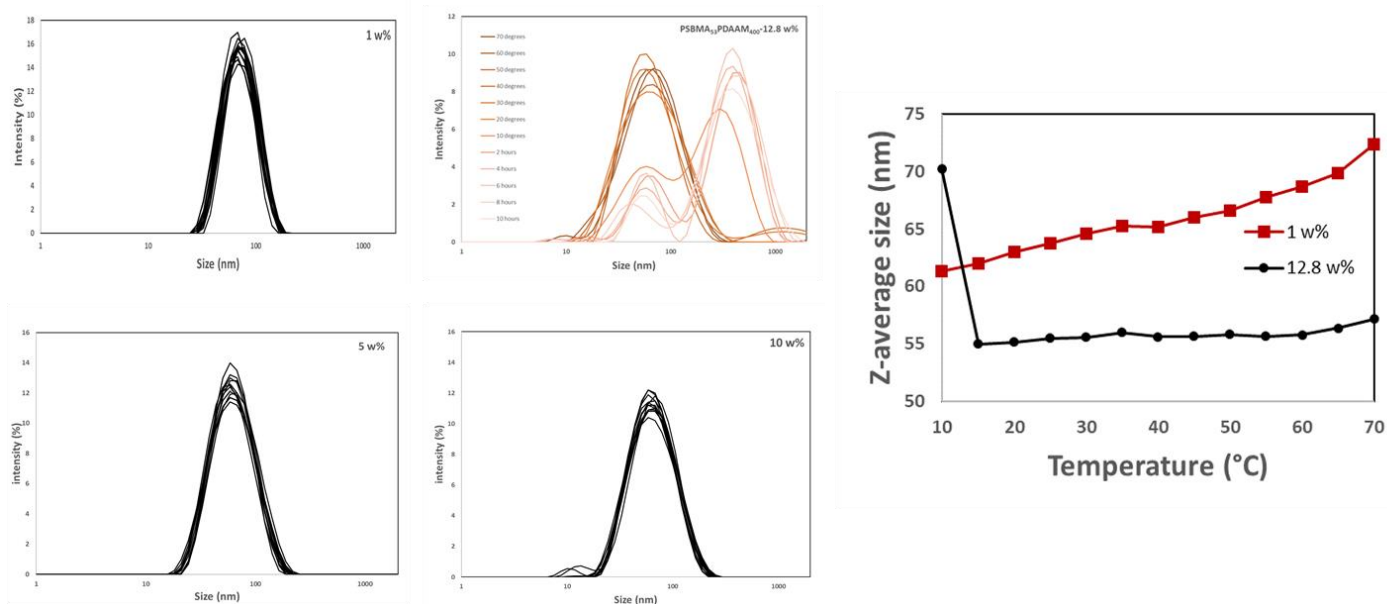

Figure 34. Different concentrations of PSBMA$_{53}$PDAAM$_{400}$ are recorded cooling from 70 to 10 degrees with 5 step program and after cooling down, size of the particles is followed for 10 hours.

From the figure in right, we can see that the 12.8 w% sample particle size is increasing at 10 °C strongly and the 1 w% sample is not reacting at all to the temperature change from 15 to 10 °C. This can be also explained by the aggregation since the mixture is diluted the particles have more space and don't aggregate.
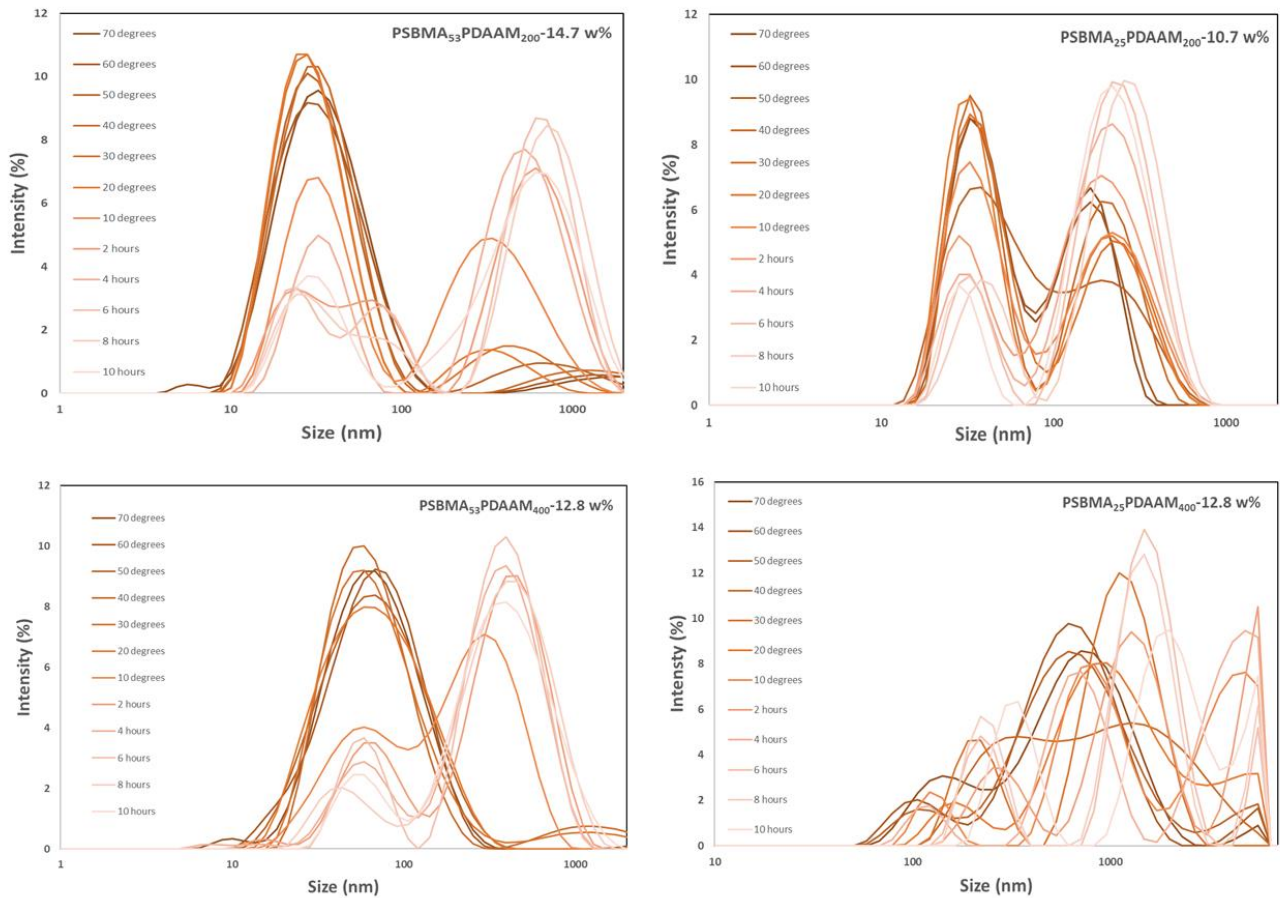
9.2.5 Effect of the solids content

Figure 35. The effect of different chain lengths to the size distribution.

Samples were put to capillary cuvettes for the measurements. Back scattering technology was used in the measurements, since the samples were so turbid. Procedure used in the measurements is cooling from 70 to 10 ℃ and then the size of the particles is followed for 10 hours. Measurement is 5 ℃ step measurement. Samples in the measurements are $PSBMA_{25}PDAAM_{200}$, $PSBMA_{25}PDAAM_{400}$, $PSBMA_{53}PDAAM_{200}$ $PSBMA_{53}PDAAM_{400}$ copolymers to understand how the different chain lengths affect to size distribution when temperature measurements are done. Both of the longer chain lengths of PSBMA shows temperature dependence and also $PSBMA_{25}PDAAM_{200}$ but with longer PDAAM chain length, the change are not certain because the distribution doesn't follow a clear pattern.
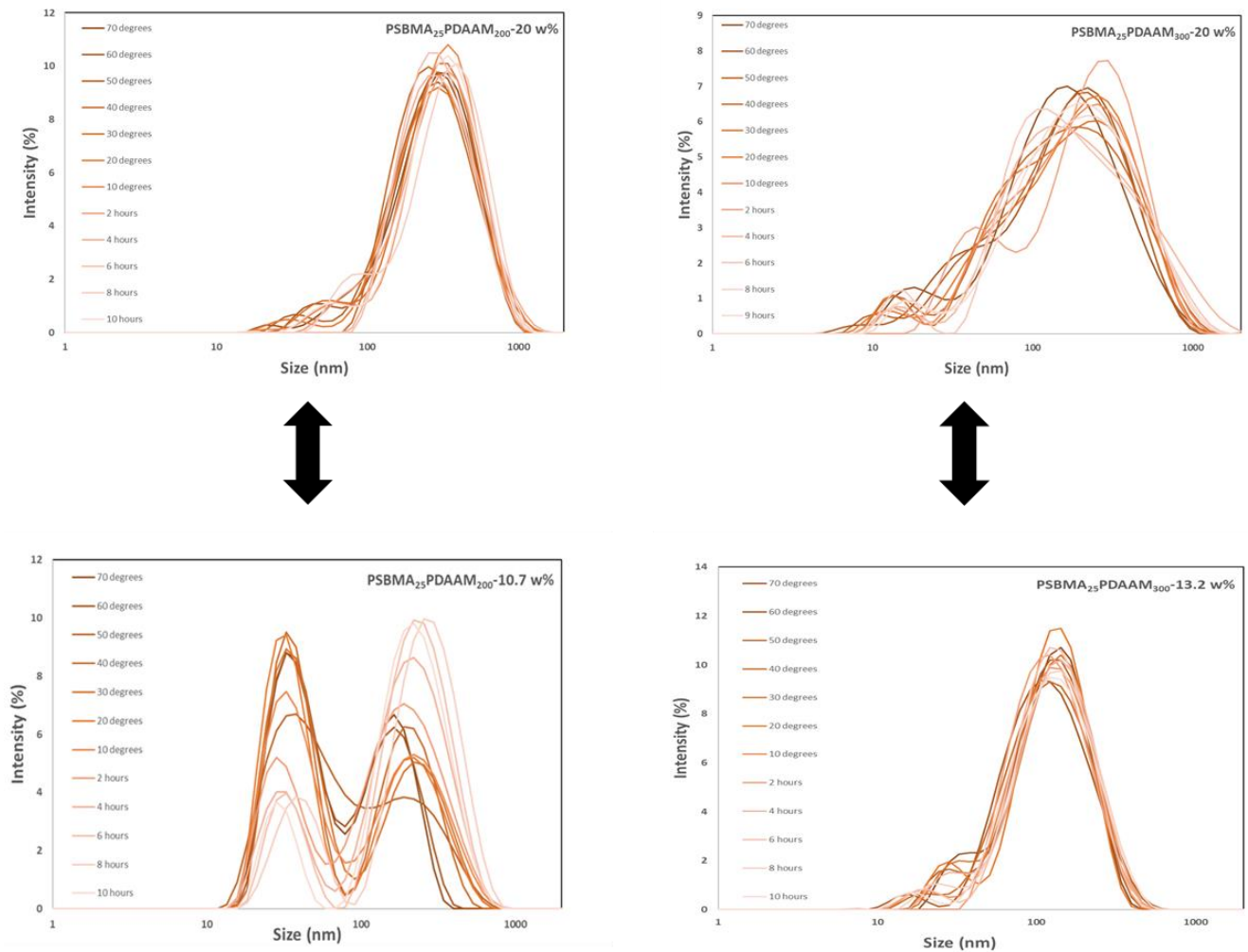
Figure 36. Effect of solids content to DLS results. Procedure used in the measurements is cooling from 70 to 10 ℃ and then the size of the particles is followed 10 hours. Measurement is 5 ℃ step measurement.

$PSBMA_{25}PDAAM_{200}$ 10.7 w% is the only one of these that shows clear change in the size distribution when temperature is changed. If we look the TEM pictures shown earlier, we can notice that other copolymers are vesicles apart from $PSBMA_{25}PDAAM_{200}$-10.7 w% that shows temperature dependance in this DLS experiment. Also, all the copolymers with long PSBMA chain length were spheres so this could be maybe a behavior that has to do something with each other.

10. Conclusions

The synthesis of PSBMA macro-CTA was a great success and conversions were quite predictable. PISA synthesis for $PSBMA_xPDAAM_y$ copolymer was successful and different particle sizes and morphologies were achieved, also the kinetic studies show that the monomer conversion is linear, so the conversion is easy to follow and predict. Nevertheless, GPC spectra showed low polydispersity and narrow distribution for PSBMA macro-CTAs which is the goal when polymerizing materials.

From DLS studies, more worm and sphere particles were expected but as we saw in TEM results, most of the copolymers that gave size bigger than 100 nm in DLS, were just extremely aggregated. Morphology of these formed particles is dependent on PSBMA and PDAAM chain lengths and solids content. Full morphologies were observed for short PSBMA chain lengths, where as long chain length only produced only spheres or fused spheres. Also some networking was witnessed, so these copolymers have possibilities to be modified. Particles with $PSBMA_{53}$ undergo UCST type phase transition where the large aggregates build up upon cooling the dispersion below 15 ℃ which is the cloud point of the particles. This was studied also with UV/Vis but the results from this are not reliable since the transmittance curves didn't show any pattern and samples were too cloudy in the beginning of the experiments to study with this method, so temperature measurements with DLS was chosen to show the transition more reliably. Morphology after cooling down was studied with TEM but no changes in morphology was witnessed, only strong aggregation so seems like the increase in size when cooling down the polymer was because aggregation was increasing. With Micro-cal disintegration of the particles was noticed when heating and cooling down the polymer. This needs further experiments for other copolymers and homopolymers to make clear conclusions.

Overall, the measurements were great success, and the study of this copolymer should be continued since it showed many interesting properties. Application areas could be wide because many different morphologies can be formed and also temperature dependance is a great advantage.

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