This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Solubility behaviour of random and gradient copolymers of di- and oligo(ethylene oxide) methacrylate in water: Effect of various additives

Maryam Bozorg a, Birgit Hankiewicz a and Volker Abetz ab

Poly[oligo(ethylene oxide)] based gradient and random copolymers with different compositions are synthesized via Cu-based atom transfer radical polymerization. The solubility behavior of these copolymers in pure water and in the presence of different salts, surfactants and ethanol is investigated. According to dynamic light scattering results, the lower critical solution temperature (LCST) depends on the structure of the copolymer and changes slightly by the additives. Good cosolvents like ethanol can increase the LCST through dissolving the collapsed copolymer chains to some extent. The same effect is observed for surfactants that make the copolymer solution more stable by preventing aggregation. Above a certain concentration of surfactant, depending on the copolymer structure, the solution is stable at all temperatures (no LCST). The effect of salts on the solubility of the copolymers is following the Hofmeister series and it is related linearly to the salt concentration. According to their affinity to the copolymer, the salts can increase or decrease the LCST. There is a considerable difference in phase transition changes for gradient or random copolymer after salt addition. While both copolymers show two-step phase transition in the presence of different salts, the changes of hydrodynamic radius and normalized scattering intensity is rather broad for random compared to gradient copolymer. Contrary as expected, varying the cations has no distinguishable effect on the LCST for both copolymers. All chlorides decrease the LCST. This decrease is considerable difference in phase transition changes for gradient or random copolymer after salt addition. While both copolymers show two-step phase transition in the presence of different salts, the changes of hydrodynamic radius and normalized scattering intensity is rather broad for random compared to gradient copolymer. Contrary as expected, varying the cations has no distinguishable effect on the LCST for both copolymers. All chlorides decrease the LCST. This decrease is almost the same for gradient copolymer and fluctuates for random copolymers.

Introduction

POEOMAs are nonlinear poly(ethylene oxide) (PEO) or poly(ethylene glycol) (PEG) analogues. They can be up to 85 wt.% composed of ethylene oxide units (EO), and therefore are water soluble and biocompatible in most cases. POEOMAs combine both the properties of PEG and poly(N-isopropylacrylamide) (PNIPAM) in a single macromolecule and therefore are considered as ideal structures to use in biomedicine. The LCST of these polymers does not depend much on the solution concentration in water (above approx. 1 g/L), which is an important factor for application in biotechnology. Moreover, being produced from a commercially available monomer, POEOMA containing polymers are favorable to be used as smart biomaterials in biosensors, artificial tissues, smart gels for chromatography and hyperthermia-induced drug delivery. For POEOMAs, the phase transition temperature depends slightly on molecular weight, main-chain end-groups, tacticity and ionic strength. Though, the changes in LCST are generally rather small (1–5).

For a long time, PNIPAM has had the title of “gold standard” for thermoresponsive polymers in bio-application due to its LCST of 32 °C, which is close to the physiological temperature. However, POEOMAs can exhibit an adjustable LCST between 26 and 90 °C by simple copolymerization of OEMAs with different amounts of EO in the side chain. The LCST can be precisely adjusted by the copolymer composition considering that the comonomers have similar structure containing a methacrylate moiety and ethylene oxide units (1)(6). Moreover, the phase transition of these copolymers is reversible, in comparison to PNIPAM which shows an irreversible phase transition (4)(7). The solubility behavior of PNIPAM also shows a significant dependency to its end group (8). Furthermore, The presence of the amide group at the side chain of PNIPAM might cause hydrogen bonding with other polymides like proteins and result in bio-adhesion (9). PNIPAM also produces low molecular weight amines during hydrolysis which complicates its usage in biotechnological applications (10).

The outstanding solubility behavior of POEOMA is due to its hydrogen bonding with water and lack of strong polymer-polymer interactions in the collapsed state. Like PNIPAM, the phase transition of POEOMA is attributed to the competition between hydrophilic polymer-water interactions and hydrophobic polymer-polymer interactions. At temperatures
below the LCST, polymer-water interactions are thermodynamically favorable which makes the polymer soluble in water. Above LCST, the polymer-polymer interactions become more favorable which result in polymer’s self-aggregation and phase transition in the form of globules or micelles depending on the structure of POEOMA copolymer (11). Maeda et al. discovered that both C=O and C-H groups are hydrated in poly d(ethylene oxide) methyl ether methacrylate (PMEO2MA) aqueous solution, but due to the crowded position of carbonyl groups near the backbone, only about half of these moieties are hydrated. By increasing temperature above LCST, the fraction of hydrogen-bonded carbonyl groups decreases. Moreover, the complete breakage of the H-bond between the ether oxygens with water are reported. The fraction of hydrogen-bonding methoxy oxygens changes from one below LCST to zero after LCST. These results indicate that the hydrogen bond breakage is the main reason for phase separation of POEOMAs (12)(13).

A similar behavior is observed for the solubility of P(MEO2MA-stat-EOEGMA_{75}) copolymers in D_{2}O which shows a sharp change of the hydrodynamic radius at the LCST and a gradual change above LCST. The phase transition of P(MEO2MA-stat-EOEGMA_{75}) is happening due to multiple chain aggregation without preconnection of individual polymer chains. Self-aggregation of P(MEO2MA-stat-EOEGMA_{75}) is mainly based on the conformation change of ethylene oxide side chains. They first collapse to be near the hydrophobic backbone and then distort to bring hydrophilic ether oxygen groups to the “outer shell” of polymer chains as far as possible. As a result of disturbing the balance between hydrophobic and hydrophilic interactions, the single dehydrated polymer chains aggregate into more stable micelles and cause a sharp change below LCST (3).

P(MEO2MA-stat-PEGMA_{2080}) copolymer in aqueous dilute solution undergoes a similar phase separation mechanism. However, compared to other random copolymers of MEO2MA and OEOMA, this copolymer shows a weird two stage thermally induced phase separation. Instead of loose aggregates formed at the phase transition, the copolymer chains associate at the first thermal transition, followed by a rearrangement process at the second thermal transition to form stable micellar structures consisting of a methacrylate core stabilized by the longer ethylene oxide chains at the shell (14). A more detailed study shows four conformation changes of: “unimers−clusters−micelles−aggregates” during the two-step phase transition. Like other POEOMAs, the dehydration of the long hydrophilic ethylene oxide side chains take place before the dehydration of carbonyl groups and backbones during the whole phase transition process. Therefore, the driving force of the phase transition of P(MEO2MA-stat-PEGMA_{2080}) should be the hydration changes of the side chains. However, detailed FT-IR analysis explain that the peculiar behavior in the phase transition process could be attributed to the complex transition between hydrated C=O, semi-dehydrated C=O and dehydrated C=O (15).

A two-step phase transition is also observed by Yao and Tam for the behavior of PMEO2MA-block-(PMEO2MA-stat-POEGMA_{300}) block copolymers. They observed that by changing the ratio of MEO2MA/OEOMA from 80/20 to 70/30 the copolymer’s solution behavior changes from showing one thermal transition to two (16). A similar phenomenon has also been observed by Gibson et al. for the mixture of two POEGMA chains with different molecular weights. The independent phase transitions are described by the weak molecular weight dependence of the polymers’ cloud point (17).

By increasing the number of blocks, Kudo et al. observed a multi-step phase separation for the hexa-block copolymer of P(MEO2MA-block-{MEO2MA-stat-OEOMA}). They synthesized this hexa-block by semi-batch RAFT polymerization with addition of more OEOMA every two hours and therefore, the concentration of OEOMA in the reaction flask increased in batches, not gradually. Upon sequential dehydration, each block of the hexa-block copolymer showed its own temperature responsive behavior. The thermoresponsivity in each step was reversible with 2 °C hysteresis. Overall, the behavior of the block copolymer showed slight similarity to gradient copolymers (18). To the best of our knowledge, this is the only study mentioning the solubility behavior of gradient POEOMA copolymers with similar comonomer structure.

Studies on the solubility behavior of gradient copolymers consisting of a hydrophilic and a hydrophobic monomer show that the solubility behavior changes drastically, depending on the interaction of the comonomers with water and their hydrophobicity rather than their sequential order in the copolymer structure (19)(20). On the other hand, the thermal phase transition of other temperature responsive gradient copolymers consisting of monomers with similar chemical structure and therefore, similar hydrophobicity has shown considerable differences from the respective random and block copolymers and dependent on their sequential order in the gradient structure. This characteristic makes such gradient copolymers a great potential in biomimetic applications (21–29).

Various studies are done on the effect of additives on the solubility behavior of thermoresponsive polymers (30–39) including POEOMAs (40–44), but so far there is no comprehensive study to compare the effect of different additives on the phase transition of thermoresponsive copolymers with different structure.

In this study, the synthesis of gradient copolymers of MEO2MA and OEOMA via semi-batch ATRP is investigated. The injection procedure of the second monomer (OEOMA) is optimized to reach the best sequence control and gradient structure. Moreover, the solubility behavior of gradient and random copolymers with different compositions as well as their behavior in the presence of various additives are compared. As additives, different anions and cations, as well as ethanol as an organic polar solvent and Sodium dodecyl sulfate SDS (anionic surfactant) and cetyltrimethylammonium bromide CTAB (cationic surfactant) are used and the effect on the solubility behavior of random and gradient copolymers of MEO2MA and OEOMA are investigated. The knowledge of the copolymer’s responsivity towards different additives is essential for in vivo applications, as well as separation and membrane technology (37)(45). While adjusting the phase transition behavior of
POEOMAs by changing the structure seems to be expensive, time consuming and complicated, an easier way to alter the dynamic thermal transition is an addition of slight amounts of additives to the system.

**Experimental**

**Materials**

The monomers, di(ethylene oxide) methyl ether methacrylate (MEO₂MA) (Aldrich, 95%) and oligo(ethylene oxide) methyl ether methacrylate (OEOMA) (Aldrich, Mₙ=500) were purified by passing through a basic alumina column to remove the inhibitors. The catalyst, Copper (I) bromide (CuBr) (Sigma-Aldrich > 98%) was purified by mixing with glacial acetic acid, followed by filtration. It was further washed three times with absolute ethanol and two times with diethyl ether. The colorless solid product was dried overnight under vacuum at room temperature and was stored under nitrogen. The initiator, ethyl α-bromoisobutyrate (EBiB) (Sigma-Aldrich 98%), the ligands, 4,4′-dinonyl-2,2′-dipyridyl (dNbpy) (Alfa Aesor 97%) and N,N,N′,N′′,N′′-pentamethyl diethylene triamine 99% (PMDETA) (TCI 98%), the reaction solvents, anisole (Merck 99%) and absolute ethanol and two times with diethyl ether. The colorless solid product was dried overnight under vacuum at room temperature and was stored under nitrogen. The initiator, ethyl α-bromoisobutyrate (EBiB) (Sigma-Aldrich 98%), the ligands, 4,4′-dinonyl-2,2′-dipyridyl (dNbpy) (Alfa Aesor 97%) and N,N,N′,N′′,N′′-pentamethyl diethylene triamine 99% (PMDETA) (TCI 98%), the reaction solvents, anisole (Merck 99%) and ethanol (Acros 99.5%) as well as other chemicals were used as received without further purification.

**Homopolymerization and random copolymerization of MEO₂MA and OEOMA via ATRP**

The polymerization was performed with [M][₀] : [I][₀] : [Cu][₀] : [L] ([Monomer][₀] : [Initiator][₀] : [CuBr][₀] : [Ligand]) of 100 : 1 : 0.5 : 1. For a general ATRP reaction, CuBr and a magnetic stirring bar were added to a 50 ml two necked flask connected to a condenser and Schlenk line. The reaction flask was then evacuated and backfilled with nitrogen at least three times and kept for each repetition at least 15 min under vacuum. The monomer and the initiator were added to a 50 ml Schlenk flask. The solvent and the ligand were added to another 50 ml Schlenk flask. The liquids were degassed via three freeze-pump-though cycles and kept under nitrogen. The solvent and the ligand were afterwards added to the reaction flask containing CuBr via a degassed airtight syringe and mixed for half an hour until the CuBr/ligand complex was formed. The monomer and the initiator were added afterwards with a degassed airtight syringe and the reaction started by placing the reaction flask in a preheated oil bath at 60°C. The reaction carried on under nitrogen flow. Samples were withdrawn with a degassed syringe at defined time intervals for kinetic study. The reaction was stopped by opening the flask to the air and cooling it in an ice bath. The reaction solution was diluted with tetrahydrofuran and passed through a neutral aluminum oxide column to remove the catalyst. The extra amount of solvent was removed via a rotary evaporator. The polymer was purified by precipitating the concentrated polymer solution in cyclohexane and dried under vacuum overnight.

The procedure for random copolymerization was similar to homo-polymerization; instead of a single monomer, two different monomers (MEO₂MA and OEOMA) with a defined molar ratio were added depending on the planned copolymer composition.

**Gradient copolymerization of MEO₂MA and OEOMA via ATRP**

Gradient copolymerization was performed with the ratio of [M][₀] : [I][₀] : [Cu][₀] : [L] : 100 : 1 : 0.5 : 1 (where [M][₀] is a mixture of MEO₂MA and OEOMA with a defined molar ratio) using PMDETA as ligand and anisole as solvent. In the procedure of optimization of the injection program, the aspired ratio of two monomers was chosen to be [MEO₂MA] : [OEOMA] : 80 : 20. It was subsequently changed according to the desired composition. To achieve the gradual composition of the product, a programmable single syringe pump was used. The injection procedures differed in the overall injection time as well as the number of steps and the injection volume of each step of gradual addition of OEOMA to the reaction solution. The procedures differed in the overall injection time as well as the number of steps and the injection volume of each step of gradual addition of OEOMA to the reaction solution. The procedures differed in the overall injection time as well as the number of steps and the injection volume of each step of gradual addition of OEOMA to the reaction solution. The procedures differed in the overall injection time as well as the number of steps and the injection volume of each step of gradual addition of OEOMA to the reaction solution. The procedures differed in the overall injection time as well as the number of steps and the injection volume of each step of gradual addition of OEOMA to the reaction solution.
injection programs are shown in Figure S1. They were calculated for the given injection volume and injection time with an increasing injection speed in order to compensate the increasing viscosity of the reaction solution and the decreasing reaction rate. Based on the results of the first syntheses, the injection program was adjusted to achieve the gradient copolymer with desired composition and good gradient quality. The effect of the injection procedure on the copolymer structure will be explained later. In order to avoid the very fast reaction at the beginning due to the high concentration of catalyst, the CuBr/L complex and the reaction solvent are divided into two different monomer flasks with the same equivalent ([M]₀ : [Cu]₀ : [L] of 100 : 0.5 : 1). This also helps to keep the ratio between catalyst/ligand and monomer constant during the reaction. To prevent a reaction in the syringe, the whole amount of initiator is added at the beginning to the reaction flask. **Error! Reference source not found.** also shows the addition procedure of the different reagents. In general, the reaction procedure is as it was mentioned before for homopolymerization, with an extra step of monomer solution preparation. The reaction flask was prepared and afterwards charged with MEO₂MA, while another flask of OEMA was prepared in a similar way (without initiator). MEO₂MA and the initiator were not added to the CuBr solution before the reaction started but degassed separately. Both flasks containing CuBr were degassed via evacuation and backfilling with nitrogen as mentioned before. The mixtures of liquids were degassed via three freeze-pump-thaw cycles. To dissolve the solids and build up the copper complex, solution C (anisole and PMDETA, the equivalent amount for MEO₂MA) and E (anisole and PMDETA, the equivalent amount for OEMA and the monomer OEOA) were added to flask A (containing CuBr, the equivalent amount for MEO₂MA) and D (containing CuBr, the equivalent amount for OEOA) respectively and mixed for 30 minutes. The reaction was started by adding the MEO₂MA and the EBriB from flask B to the reaction solution A and immersing the flask into an oil bath (T = 60 °C). The injection program of the OEOA solution was started simultaneously. The reaction was quenched as described for homopolymerization before and the final product was purified by column chromatography and precipitation in cyclohexane.

**Analytics**

**Size exclusion chromatography (SEC)**

SEC was used to obtain the products’ molecular weight and size distribution. The measurement was performed on a system PSS Agilent Technologies 1260 Infinity including a pre-column (8 mm × 50 mm) and three analytical columns (mesh size 1 × 30 Å and 2 × 1000 Å) with a polyester copolymer network (GRAM) as stationary phase, SECurity autoinjector and an isocratic SECurity pump. The elugrammes were recorded by a refractive index and a UV-Vis detector, working at a wavelength of 280 nm. The system was operated by WinGPC Unichrom software. A 0.1 M solution of Lithium Bromide (LiBr) (Acros Organics) in N,N-dimethylacetamide (DMAc) (HPLC Optiprep, Promochim) with a flow rate of 1.0 mL min⁻¹ at a temperature of 50 °C was utilized as eluent. Methyl benzoate was added as internal standard to the analyzed polymer solutions which had a polymer concentration of 2 mg mL⁻¹. The injection volume was 100 µL. For determination of relative MWs and D-values, the system was calibrated with narrowly distributed PMMA standards with the molecular weights between 2.2 and 1190 kDa.

**¹H NMR**

For the determination of the monomer conversion in the ATRP polymerization as well as the composition of copolymers, ¹H NMR was used. The NMR experiments were conducted on a Bruker Avance II 400 MHz spectrometer at 300 K with Bruker TopSpin Software. For a typical ¹H NMR spectrum, 16 scans were recorded, and a relaxation delay of 5 s was applied. Solutions were measured in CDCl₃ at concentrations of ~ 20 g L⁻¹. The internal solvent signal of CDCl₃ was used as reference (δ = 7.260 ppm). The spectra were analyzed with the software MestReNova 7.1.

**Dynamic Light Scattering (DLS)**

The thermoresponsivity of the polymers was studied via DLS. DLS measurements were performed using an ALV/CSG-3 Compact Goniometer System with an ALV/LS-7004 multiple tau digital correlator working with pseudo cross correlation and the ALV Digital Correlator Software 3.0 (ALV-GmbH, Langen, Germany). All of the measurements were performed at an angle of 90 ° and the measurement duration was 120 s with a post wait time of 10 s. A Nd:YAG laser emitting at 532 nm was used as a light source. The viscosity and refractive index of the solvent were automatically corrected by the DLS software according to the temperature. For the measurements with ethanol as additive, extra correction on the viscosity and refractive index was done based on the solvent mixture (46–49). In case of salt solutions, since, the change of viscosity and refractive index after salt addition was very small, no further correction has been done.

The polymer solutions were prepared with the concentration of 1 mg/mL and were shaken overnight to ensure complete dissolution. Each solution was filled in a dust-free glass tube through a microporous regenerated cellulose filter with the average pore diameter of 200 nm. The DLS samples were let to rest at least 1 h prior to measurement in order to assure that the possible dust particles present in the system settle and not interfere with the measurement. To investigate the effect of different additives, the solution of the certain additive with an exact concentration was prepared beforehand and added to the polymer instead of pure solvent.

Temperature-dependent DLS measurements were performed at temperature steps of 2 °C with 3 runs per temperature, except for more detailed measurements which were done with 1 °C temperature steps. A Julabo F25 thermostat functioning with a mixture of water and ethylene glycol with a temperature accuracy of 0.01 °C was used as a heating system. There was a 3 min time interval for the stabilization of the temperature prior to each measurement. A MATLAB program was used to analyze the electric field autocorrelation functions g²(q,t) by means of a cumulant fit up to the second order for a monomodal distribution, \( \langle \ln g_1(q,t) \rangle = \ln A - \tau + \tau \left[ \frac{1}{2} + \tau \right] \) (1).
\[ \ln(g^2(q,t)) = \ln A - \Gamma \times t + \frac{\mu_2}{2} \times t^2 \] (1)

A is the amplitude, \( \Gamma \) is the average decay rate, \( t \) is the time, \( \mu_2 \) is the second moment, and \( \Gamma \) presents a measure of the relative width of the size distribution (particle size dispersity, PSD):

\[ PSD = \frac{\mu_2}{\Gamma} \] (2)

The translational diffusion coefficient \( D \) was determined from \( \Gamma \) with

\[ q = \frac{4\pi\sin(\theta)}{\lambda_0} \] (3)

where \( q \) is the absolute value of the scattering vector, \( n \) is the refractive index of the solvent, \( \vartheta \) is the scattering angle, and \( \lambda_0 \) is the vacuum wavelength of the laser. The hydrodynamic radius was calculated from the Stokes–Einstein equation.

\[ R_h = \frac{k_B T}{6\pi\eta D} \] (4)

with \( k_B \) being the Boltzmann constant, \( T \) is the temperature, \( \eta \) is the solvent viscosity and \( D \) is the apparent diffusion coefficient, respectively (50).

**Results and discussion**

Controlled radical polymerization (CRP) techniques like ATRP provide versatile routes for macromolecular engineering such as synthesis of tailor-made polymers with controlled chain-length, dispersity, functionality, composition and architecture. Moreover, the polymers synthesized via CRP, have a reactive end group which could be used in further end functionalization or even as a macroinitiator for new polymerization (1).

The first polymerization of an OEOA via ATRP was carried out by Armes et al. in aqueous medium. But the high polarity of water makes it very complicated to control the reaction (51). In early researches, Ishizone et al. also synthesized various poly(oligo(ethylene glycol) methacrylate)s using living anionic polymerization. They studied the effect of methyl, ethyl, vinyl and hydroxyl end groups as well as the number of ethylene oxide moieties in the side chain, on the polymers’ solubility in water and organic solvents (52). ATRP as a synthesis route is advantageous compared to anionic polymerization due to its flexibility and simpler reaction conditions. Moreover, the prospects of obtaining new structures like gradient, random or star copolymers are higher via ATRP. It is worth mentioning that the precise dependency of LCST on the copolymer composition could only be observed for copolymers prepared by controlled polymerization techniques. In a conventional free radical polymerization, the chains can present a strong chain-to-chain transitions (1)(37).

The synthesis route of this research is an optimized work based on the research of Lutz et al. (55) and Matyjaszewski et al. (6). Lutz et al. reported a well-controlled ATRP of OEOAs in pure ethanol. The polarity of ethanol establishes fast polymerization kinetics (55), but since OEOMA monomers have a slight polarity, their polymerization can also be successfully done in apolar solvents like anisole as reported by Matyjaszewski’s group (6).

For selecting the best reaction conditions for ATRP gradient polymerization of OEOMAs, first the homopolymerization of MEO₂MA was optimized. Several test reactions with different solvents, ligands and reaction times were conducted. Samples were taken at defined time intervals and analyzed via NMR and SEC. The NMR spectra of MEO₂MA monomer and its changes during polymerization are plotted in Figure S2 where the calculation of monomer conversion is explained as well. To analyze and compare the control over different reaction systems, in Fig. 2 (a) the semi-logarithmic plot of monomer conversion i.e. \( \ln([M]_0/[M]) \) is plotted versus reaction time. The reaction time is 100 min for all reactions, except for the solvent ethanol and the ligand PMDETA, which is stopped after 80 min due to its
higher rate. The semi-logarithmic plots are linear for all of the polymerizations indicating that the polymerization rate is proportional to the monomer concentration (first order polymerization with respect to monomer) and moreover, the radical concentration is constant during the polymerization, according to (5)(51)(56).

\[
\frac{R}{[M]} = \frac{1}{\tau} \ln \left( \frac{[M]_0}{[M]} \right) = k_p K_{eq} \frac{[I][Cu]}{[Cu]_{eq}} = k_{app}
\]

The highest slope in the semi-logarithmic plots is observed for the system of ethanol and PMDETA showing the highest apparent reaction rate with lower control over polymerization compared to the other systems.

The best control over polymerization is observed when using anisole as a solvent and PMDETA as a ligand. In comparison to anisole, the polymerization in ethanol is faster and less controlled. This could be explained by the relative polarity of ethanol (0.654) which is higher than anisole (0.198); (relative polarity, normalized from measurements of solvent shifts in absorption spectra) (57).

Among different solvents used to conduct ATRP, good control mostly resulted from bulk polymerization or using nonpolar solvents, while using polar solvents especially water, often leads to limited control over polymerization and poor livingness and the reaction is incredibly fast (58)(59). Wang and Armes suggested that, by increasing the solvent polarity, a higher concentration of mononuclear copper catalyst is produced which increases the radical concentration and the polymerization rate. As a result, the molecular weight distribution is broadened distinctly (51). According to other studies, increasing the solvents’ polarity increases the k_{act} while decreasing k_{deact} with approximately the same ratio: Δlog k_{act} ≈ Δlog k_{deact}. Therefore, changing to a more polar solvent increases K_{ATRP} since K_{ATRP} = k_{act}/k_{deact} and results in overall faster and less controlled polymerization (59–63).

Furthermore, the solvent effect on the Cu ions’ redox potential appears to be higher for complexes of copper with ligands having high degrees of freedom, compared to more rigid structures (59). Moreover, by increasing the number of coordination sites of a ligand, the redox potential of its copper complex increases (62). Comparing the ligands that are used in this research, PMDETA has the highest redox potential considering the fastest polymerization in both ethanol and anisole. Incidentally, the reaction with PMDETA in anisole is still well controlled based on its first order kinetics and SEC results. The SEC results of different reaction conditions (Error! Reference source not found.) and summarized information (in Table 1) prove the good control over polymerization and low D for all systems excluding the polymerization using ethanol as a solvent and PMDETA as a ligand. The abovementioned system shows a small shoulder at higher molecular weights which is related to faster activation than deactivation reaction in ATRP equilibrium and therefore a small portion of bimolecular termination close to the end of reaction. The addition of Cu atr or decreasing the CuBr concentration does not help to decrease the propagating radicals’ concentration and improving the control over polymerization noticeably.

Table 1 shows that the apparent molecular weight of the polymers synthesized in anisole are in good agreement with the theoretical calculation, while for the polymers synthesized in ethanol the molecular weight achieved is slightly lower than from theoretical calculation which is in agreement with previous studies (6). A possible explanation for such behavior is the chain transfer reaction to solvent due to higher reactivity of ethanol than anisole. Such a chain transfer reaction to solvent was also indicated in previous ATRP studies. (64–66). Among the test polymerizations conducted, the one in anisole using PMDETA as a ligand shows a very good control over reaction while being fast. Considering the fact that less molar amount of PMDETA is needed to provide a good control compared to dNbyp and the easier way of handling such small amounts (because PMDETA is liquid while dNbyp is solid) the final choice was to carry out ATRP polymerization of MEO2MA and further copolymerizations in anisole using PMDETA as a ligand.

| Solvent | Ligand | [M]_0 [I]_0 [Cu]_0 [L]_0 | Conv. | Time [min] | M_n[theory] [kDa] | M_n[SEC] [kDa] | M_w/M_n |
|---------|--------|---------------------------|-------|-----------|-----------------|----------------|-----------|
| ethanol | dNbyp | 100:1:0.5:2               | 0.85  | 100       | 15.99           | 12.03          | 1.36      |
| 2       | ethanol| PMDETA                    | 100:1:0.5:1 | 0.92  | 80        | 17.32          | 14.95          | 1.43      |
| 3       | anisole| dNbyp                     | 100:1:0.5:1 | 0.65  | 100       | 12.23          | 12.86          | 1.22      |
| 4       | anisole| PMDETA                    | 100:1:0.5:1 | 0.75  | 100       | 14.12          | 13.66          | 1.23      |

a [M]_0: [I]_0: [Cu]_0: [L]_0: [MEO2MA]_0: [EBiB]_0: [CuBr]_0: [Ligand]: the ratio of the ingredients at the start of reaction.

b Monomer conversion is determined by ^H NMR

\[M_n{\text{theory}} = \text{Conv.} \times \frac{[M]}{[I]}\]

Conv.: conversion

M_n: monomer’s molecular weight

[M]: molar amount of monomer

Reference source not found.
Random and gradient copolymerization of MEO₂MA and OEOMA

Since MEO₂MA and OEOMA differ only in the side chain, therefore, their reactivity ratios are very similar. This is also proven by very similar ATRP kinetics of these monomers at similar reaction conditions as can be seen in the Figure S4 and S5 in the supporting information. Therefore, forced gradient copolymerization could be an optimal synthesis route to prepare gradient copolymers of MEO₂MA and OEOMA. This technique involves a semi-batch reaction, in which one of the monomers is added continuously from an external reservoir, via a syringe pump into the polymerization mixture. The forced gradient method with living polymerization allows precise synthesis of gradient compositions with high sequence control (21).

To follow the change of copolymer structure during reaction, copolymers’ compositions were studied by NMR spectroscopy. The NMR spectra as well as the calculation of the copolymers’ compositions is explained in the Supplementary Information (Figure S6 and S7). The calculated copolymer composition for a random copolymer of MEO₂MA and OEOMA with a designed composition of E₀₂MA : OEOMA is plotted in Error! Reference source not found.. The composition is staying constant during reaction which shows a perfect random copolymerization of the two monomers (38).

Since the reactivity ratios of the two monomers are very similar, a gradient copolymerization in a batch system is impossible (67), as it is also proven by the perfect random copolymerization of the two monomers in a batch system (Error! Reference source not found.). Therefore, gradient copolymerization was done via ATRP semi-batch polymerization. Three different injection programs (P1-P3) were used in this work to inject the OEOMA solution to the reaction system. In all programs, the injection speed was increased stepwise during the reaction leading to the parabolic curve of the injected volume against time. The reason for injecting OEOMA to the system is its lower ratio in copolymer’s desired composition compared to MEO₂MA and its higher viscosity. Since the two monomers have almost the same structure and they differ only at the side chain, their reactivity ratios are the same and almost equal to one. The similar reactivity ratios of MEO₂MA and OEOMA make it easier to adjust the injection program according to the desired composition.

In the first synthesis (P1), the injection was carried out for the first 80 min of the total reaction time of 100 min. In the second synthesis (P2), the starting injection speed was decreased 30% while the injection time was reduced to 70 min of the overall reaction time of 100 min leading to a steeper injection volume curve. The third reaction (P3) was done like the first one (injecting for 80 min of 100 min reaction time) but with 30% higher starting speed and less injection steps which changes the injection volume curve toward a linear graph.

The composition development during the three polymerizations is shown in Error! Reference source not found.. The plots of all syntheses show a gradual, almost linear increase of OEOMA and decrease of MEO₂MA in the copolymer composition which indicates a gradient structure for all systems. For syntheses P1 and P2, the change of composition at the beginning of reaction is very small which led us to increase the starting injection speed for the last reaction. It is also to be noted that for both P1 and P2, the aspired copolymer composition (OEOMA : MEO₂MA of 80:20) was not achieved. The final composition is 87 : 13 for P1 and 85 : 15 for P2. In the last reaction P3, a perfect linear graph of composition vs. time is observed and the copolymer reached the desired composition.

The developments of the composition qualitatively follow the injection program. Since the chain growth is directional with only one active end, the increasing incorporation of OEOMA during the reaction indicates an increasing OEOMA...
concentration from one chain end to the other. Hence, the synthesized polymers exhibit the aspired gradient structure.

A direct conclusion about the kinetics or the control over the reaction is difficult because of the continuous injection of monomer. A kinetic study of polymerization control via conversion calculation is hence not possible. However, the implied high control over the reaction is reflected in the SEC analysis and the molecular weight development (see Error! Reference source not found. SI). Furthermore, the molecular weight change for gradient and random copolymerization versus time is linear showing a good controlled polymerization. The lower molecular weight of the gradient copolymer compared to the random copolymer is also a proof for the gradient structure because less OEOMA was added to the polymer chain compared to the random copolymer.

**Solubility study of PMEO<sub>2</sub>MA homopolymer and random and gradient copolymer of MEO<sub>2</sub>MA and OEOMA in water**

To investigate the solubility behavior of a temperature responsive polymer, mostly turbidimetry is used, which gives good information about the cloud point; but it cannot explain the transition procedure well. By using DLS, we are able to follow the changes in particles’ size, size distribution and scattering intensity, and therefore, study the solution behavior in more detail. Error! Reference source not found. shows the solubility behavior of PMEO<sub>2</sub>Ma as well as the solubility behavior of a random and a gradient copolymer of MEO<sub>2</sub>MA and OEOMA with approximately 20 mol% OEOMA in a dilute water solution. As seen in the graph, both normalized scattering intensity (I) and hydrodynamic radius (R<sub>h</sub>) increase sharply above LCST, then they increase gradually to reach a maximum and then decrease very slowly (the red graph). The amphiphilic structure of PMEO<sub>2</sub>MA is the reason for its thermoresponsive behavior. Below LCST, the ether oxygens of the side chains form hydrogen bonds with water. On the other hand, the apolar carbon-carbon backbone causes a competitive hydrophobic effect. This results in a balance between favorable polymer-water interactions and unfavorable polymer–polymer interactions which grants solubilization. By increasing temperature above LCST, this balance is disrupted as the hydrogen bonds break and hydrophobic polymer–polymer interactions become thermodynamically favored as compared to polymer-water interactions (1). Therefore, the hydrophobicity of the polymer increases as temperature gets higher and this results in aggregation of polymer chains and phase separation (6). This also explains why the LCST increases by increasing the amount of ethylene oxide in the side chain of POEOMA, as more EO unities result in increasing the amount of hydrogen bonds and therefore stronger polymer-water interaction.

At temperatures lower than LCST, the polymer is dissolved in the form of unimers with the R<sub>h</sub> of around 20 nm. When the temperature reaches LCST, as was explained before, the polymer chains form aggregates and R<sub>h</sub> suddenly increases to 1300 nm at 32 °C. The size growth continues until R<sub>h</sub> reaches 1500 nm, due to binding more PMEO<sub>2</sub>MA chains to the aggregate. The aggregates size then start to decrease while more and more water molecules are expelled and PMEO<sub>2</sub>MA chains get dehydrated and as a result, the aggregates slightly

![Fig. 4](image_url)
shrink. It should also be noted that there is no size change observed for PMEO$_2$MA before LCST, proving that the coil of PMEO$_2$MA does not collapse (mainly by the distortion of backbones) into a crumpled structure at low temperatures. This is totally different from PNIPAM which shows a precontraction of individual polymer chains before the phase transition (3)(68).

It is also obvious from Error! Reference source not found. that there is a hysteresis in the heating and cooling cycle of the solubility behavior of PMEO$_2$MA, which is due to the small delay in the dissolution of large precipitated globular particles. This is attributed to the procedure of DLS measurements which excludes any type of stirring while measuring and makes it more difficult for the large globules to dissolve fast. However, the hysteresis according to literature is still much less than for PNIPAM that is assigned as golden standard of temperature responsive polymers (1)(3).

Wu and coworkers explain the large hysteresis in the solution behavior of PNIPAM as a result of the intramolecular and intermolecular $\text{NH} \cdots \text{O} = \text{C}$ hydrogen bonding interactions that are formed in the collapsed state. These strong hydrogen bonds hinder the rehydration of PNIPAM during the cooling process and result in a pronounced hysteresis (69). In contrast, a reversible dehydration is observed for POEOMAs due to the lack of strong H-bond donor in the molecular structure of these polymers and as a result, there is no possibility of stabilizing H-bonds formation in the collapsed state (1).

For comparing the solubility behavior of random and gradient copolymer of ME0$_2$MA and OEOMA both copolymers are chosen to have similar molecular weight and dispersity index, as the LCST shows a slight decrease by increasing the molecular weight (6). According to Error! Reference source not found. (b), the LCST of random copolymer (50.7 °C) is almost 15 °C higher than the one for the gradient copolymer (36.6 °C). Around 8 °C hysteresis is observable for gradient copolymer like in case of PMEO$_2$MA homopolymer. But it is less (5 °C) for the random copolymer. The behaviors of gradient and random copolymers also differ distinguishably above LCST. Despite the random copolymer which shows a large broad transition in normalized scattering intensity ($4.7 \times 10^{-5} - 1.6 \times 10^{-3}$ a.u.) and hydrodynamic radius (20 - 1280 nm), the change in gradient copolymers ($I: 2.7 \times 10^{-5} - 5.2 \times 10^{-4}$ a.u., $R_h: 24 - 292$ nm) is rather sharp but with less increase in the amount. This data shows the formation of micelles rather than globular aggregates in terms of gradient copolymers which are held in solution by the hydrophilic OEOMA segments that are not yet aggregated (6).

As mentioned before, there are no strong intermolecular hydrogen bonding interactions between polymer chains. Therefore, the phase transition occurs mainly because of the multiple chain aggregation without a precontraction process of individual polymer chains. Moreover, the self-aggregation process of P(ME0$_2$MA-co-OEGMA) is mainly dominated or driven by the conformation changes of oxyethylene side chains, which collapse first to get close to the hydrophobic backbones and then distort to expose hydrophilic ether oxygen groups to the “outer shell” of polymer chains as much as...
possible(2)(3)(11)(12). According to Sun and Wu (3), P(MEO₂MA-co-OEGMA) random copolymers exhibit “hydrated chains, dehydrated chains, loosely aggregated agglomerates and finally densely aggregated agglomerates” conformations during the phase transition. As there is no precontraction process before phase transition, the conformation of hydrophobic backbones with the slowest response does not change much and the micelle size stays constant. It should be noted that the cores in the micelles are only physically or loosely cross-linked by hydrogen bond bridges between ether oxygen groups and water molecules. By increasing the temperature above the LCST, due to the increased molecular motion and decreased density, the amount of water molecules which participate in hydrogen bonding decrease or in another word, more water molecules expel from micelles. Therefore, the micelles get more densely aggregated which results in a gradual change as seen in DLS (3).

The case is significantly different for the gradient copolymer based on its phase behavior in water (Error! Reference source not found. (c)). The change of $R_h$ and $I$ are rather sharp than broad and, in the amount, less pronounced than the random copolymer. Above LCST, by further temperature increase, first $R_h$ decreases and then stays constant. This could be attributed to the formation of micelles rather than big agglomerates. Peng et al. reported a similar behavior in case of poly(MEO₂MA-co-PEGMA₂₀₈₀) due to the association of the polymer chains as a result of the dehydration while increasing the temperature, followed by a rearrangement process and micelle formation (14). In the system of P(MEO₂MA-grad-OEOMA), there are no long PEGMA₂₀₈₀ side chains to stabilize the micelles in the system. Whereas the dense OEOMA at one end of the polymer chain can help the micelle stabilization to some extent. The decrease in $R_h$ is explained by further dehydration and water expelling from the system that results in shrinking of the micelles and decreasing the particle size while the scattering intensity stays constant. This is also to a certain degree similar to the behavior of block copolymers (16)(18), but without showing multiple transition temperatures, while micelles cannot stay in the system long enough to reach a second LCST. The solubility behavior of several gradient and random copolymers with various comonomer compositions were studied via DLS and the change of hydrodynamic radii and normalized scattering intensities are plotted in Error! Reference source not found.. For simplicity, just the heating cycles are shown. The solubility behavior depends strongly on the copolymer structure and therefore is similar for all gradient copolymers or all random copolymers, in a similar manner shown in Error! Reference source not found.. For both gradient and random structures, the LCST increases at higher portion of OEOMA in the comonomer composition. This change is plotted in Error! Reference source not found.. By increasing the mol% of OEOMA, the LCSTs of gradient and random copolymers get closer to each other. The higher slope of LCST versus temperature for random copolymers shows the higher dependency of LCST to OEOMA’s ratio for random copolymers rather than gradient copolymers.

Effect of additives

Effect of anions and cations

Hofmeister discovered and explained the effect of salts on the denaturation of proteins in aqueous solution for the first time (70)(71). The results of his work are still used as a guide to study the thermodynamics of the effect of salts on macromolecules’ solubility (34)(72–75). Salts are well-known to have a high impact on the behavior of thermoresponsive polymers in solution (37). Both the cation and anion in the salt are considered to influence the solubility behavior of the...
macromolecules (71). To study the effect of different anions on the solubility behavior of gradient and random copolymer, sodium salts of these anions are used. The order of anions in the Hofmeister series is as follows, with decreasing denaturation ability from left to right. The bold anions are studied in this research.

\[
\text{CO}_3^{2-} > \text{SO}_4^{2-} > \text{H}_2\text{PO}_4^- > F^- > Cl^- > Br^- \approx N\text{O}_3^- > I^- > ClO_4^- > SCN^- 
\]

Fig. 7 The change of LCST versus the copolymer composition for gradient and random copolymer. As the amount of OEOMA in the polymer increases, the LCSTs of gradient and random copolymer get closer to each other.

**Error! Reference source not found.** shows the change of LCST for gradient and random copolymers of MEOA MA and OEOMA with 20 % OEOMA in their chains in the presence of different salts. The results of DLS measurements (normalized scattering intensity and hydrodynamic radius versus temperature) in the presence of various salts, are plotted in Figure S8 - S24. To make the comparison easier, the concentration of all the salts was set to 0.5 M. Although, generally less amount of salt is present in most of biological systems such as blood plasma, this higher concentration was used as for some of the salts at concentrations lower than 0.5 M, no change was observed in the LCST of the copolymers in water. Surprisingly, there is no distinguishable trend in the behavior of different cations on the LCST for both random and gradient copolymers. All chlorides studied in this work decrease the LCST. The decrease of LCST is almost similar for the various chlorides to the solution of gradient polymer and is fluctuating for random copolymer. The effect of anions on the solubility behavior of gradient and random copolymers is in agreement with the Hofmeister series and similar to their effect on the solubility of PNIPAM (33). F\(^-\), Cl\(^-\) and Br\(^-\) decrease the LCST and therefore, show salting out effect while I\(^-\) and SCN\(^-\) increase the LCST and show salting in effect. The biggest change in solubility is observed for SO\(_4^{2-}\) which makes the copolymer precipitate immediately after it is added to the solution and therefore, it is not plotted in Error! Reference source not found.. The next maximum of change is observed for F\(^-\) with the most salting out effect that decreases the LCST around 17.5 °C for random copolymer and gradient copolymer. The biggest salting in effect is observed for SCN\(^-\) which causes 8.5 °C increase in LCST for the random copolymer and 10 °C increase for the gradient copolymer.

The salting-out effect is assumptively related to the high surface charge density of the kosmotropic anions; this increases the surface tension in the inner hydration shell of the polymer, leading also to a relatively rigid and well-ordered anion hydration shell. As a result, the kosmotropic salts show a highly negative hydration entropy (76). Therefore, in the presence of kosmotropic anions, less water molecules are available to hydrate the polymer and the LCST decreases. Furthermore, in the presence of salt, the solvent polarity increases which enforces the hydrophobic–hydrophobic interactions. On the other hand, the salting-in effect is related to the high polarizability of the chaotropic salts which results in less negative hydration entropy. This can partially distribute the rigid cage-like water structure and as a result, more water molecules can hydrate the polymer. Moreover, chaotropic anions can bind directly to the polymer and increase its surface charge and thus its solubility (37).

According to Zhang et al. (33), the change of LCST after adding salt to a polymeric solution could be modeled based on three facts:

- If the concentration of an inorganic salt is not too high, the surface tension of water at the hydrophobic/aqueous interface changes linearly with salt concentration.
At the first hydration shell of a macromolecular solute the polarization of water molecules is also dependent linearly to the salt concentration. Each of these two effects can be the cause of the polymer precipitation depending on whether the anion is a kosmotrope or a chaotrope. For the most weakly hydrated anions, enthalpically favorable anion-polymer interactions can result in salting-in effect. As a result, the change of LCST by addition of salt could be described by:

\[ T = T_0 + \kappa \left[ A^- \right] + \frac{B_{\text{max}} K_A \left[ A^- \right]}{1 + K_A \left[ A^- \right]} \]

\( T_0 \) is the LCST with no salt, \( \kappa \) is a constant with the unit of temperature/molarity which is proportional to the surface tension or hydration entropy of the anion. \( B_{\text{max}} \) is the increase in LCST related to anion binding at saturation and \( K_A \) is the binding constant of the anion to the polymer.

The binding isotherm \( \frac{1}{T} + K_A \left[ A^- \right] \) is attributed to the direct ion binding to the polymer (third fact) which is considered to be a saturation phenomenon and is relevant just for the chaotrope salts. For kosmotrope salts, the LCST is related linearly to the salt concentration as:

\[ T = T_0 + \kappa \left[ A^- \right] \]

**Error! Reference source not found.** shows the effect of salt concentration of different salts on the LCST of gradient and random copolymers. From these graphs it is visible that the effect of salt concentration on the LCST of gradient copolymers is sharper than for random copolymers in NaSCN’s salting in effect. The calculated amount of \( \kappa \) as well as \( B_{\text{max}} \) and \( K_A \) are presented in **Error! Reference source not found..** As it is also obvious in **Error! Reference source not found..** the increase of \( \kappa \) for both gradient and random copolymer is in agreement with the order in Hofmeister series. Moreover, except for \( \text{SO}_4^{2-} \) which shows a considerably lower value of \( \kappa \) for the random copolymer as compared to the gradient copolymer, for the rest of anions, \( \kappa \) and as a result the effect of anion on the LCST is similar for gradient and random copolymer.

**Effect of surfactant**

Fig. shows the change of LCST for gradient and random copolymer after addition of SDS (anionic surfactant) and CTAB (cationic surfactant). The surfactant concentration is chosen to be lower than or close to the critical micelle concentration (CMC) (~8.1 mM for SDS (77) and for ~1 mM for CTAB (78)(79)). In general, addition of a surfactant increases the LCST, due to its effect on stabilizing the formed polymeric micelles or globules in the solution. The surfactant molecules interact with the polymer and anchor on the surface of the formed micelles or globules in solution and increase the repulsion between adjacent polymer-bound micelles (32). This can also explain the lesser increase of particle size above the LCST when increasing the surfactant concentration (Figure S25 – S28). Based on Fig. , LCST increases linearly with increasing surfactant concentration until it reaches the boiling point. This result is completely different from PNIPAM, which shows the abnormal behavior of not precipitating in the presence of SDS until the concentration of SDS reaches the critical aggregation concentration (CAC) (80). The increase in LCST is larger for CTAB than for SDS, which is different from the general trend observed for PNIPAM (81–83). For PNIPAM in general, the LCST increases in the order of nonionic < cationic < anionic which is the general surfactant adsorption on the polymer (32). The reason could be the longer alkyl chain for CTAB which according to the literature can also influence the increase of LCST after addition of surfactant (82)(84). Although it was mentioned that the effect of ionic structure is more relevant than of the length of alkyl chain, this is not in agreement with our observation for POEOMAs.

**Effect of ethanol**

The change of LCST of the gradient and random copolymers of MEOMA and OEOMA with 20 mol% OEOMA in presence of ethanol and a cosolvent is plotted in **Error! Reference source not found..** The results of DLS measurements (normalized scattering intensity and hydrodynamic radius versus temperature) for gradient and random copolymer in the presence of various amount of ethanol in aqueous solution, are plotted in Figure S29 and S30. The LCST increases with increasing amount of ethanol until the polymer is completely soluble and does not show any LCST below the solvents’ boiling point. The change of LCST is exponential for the gradient copolymer and linear for the gradient copolymer. This behavior is in agreement with copolymers of similar structure in the literature (85). Addition of ethanol as a good solvent for POEOMAs increases their solubility. Moreover, the competitive interaction with polymer between water and alcohol enhances
the solubility, thus increases the LCST. No cononsolvency effect (i.e. lower compatibility of the polymer with the solvent at a certain range of solvent composition) is observed for these copolymers in the system of water-ethanol in contrary to other temperature responsive polymers like PNIPAM \(32\)(36) (86)(87) or other thermo-responsive polymers with nitrogen atom as the source of hydrogen bonding (38). This can be a result of no preference to form water-ethanol interactions rather than water-polymer or polymer-polymer interactions in this system.

By increasing the ethanol fraction, there are no more sufficient water molecules to hydrate all ethanol molecules. As a result, the mobility of ethanol molecules increases and destroys the water network built by hydrogen bonds. At very high concentrations of ethanol, the water molecules form clusters which are surrounded by ethanol molecules \(88\)(89). Cononsolvency is also a reason for lower polymer solubility of this type of copolymer at high alcohol concentration and the appearance of a UCST as reported by Roth et al. \(40\).

| anion   | Random copolymer | Gradient copolymer | Random copolymer | Gradient copolymer | Random copolymer | Gradient copolymer |
|---------|------------------|--------------------|------------------|--------------------|------------------|--------------------|
| SO\(^-\) | -69              | -46                | 34.5             | 69.2               | 0.65             | 0.68               |
| F\(^-\)  | -27              | -30                | 34.5             | 69.2               | 0.65             | 0.68               |
| Cl\(^-\) | -19              | -16                | 34.5             | 69.2               | 0.65             | 0.68               |
| Br\(^-\) | -8               | -7                 | 34.5             | 69.2               | 0.65             | 0.68               |
| SCN\(^-\) | -2.5             | -12                | 34.5             | 69.2               | 0.65             | 0.68               |

**Conclusion**

Poly(oligo(ethylene glycol)) (POEMA) based gradient and random copolymers with various compositions were synthesized via semi-batch Cu-based ATRP. The continuous injection of OEOMA to the system is a very straightforward method of force gradient copolymerization to provide optimized sequence control. Solubility behavior of gradient and random copolymers of ME0\(_2\)MA and OEOMA with various amounts of OEOMA were investigated by DLS. Both copolymers show reversible thermoresponsivity with a small hysteresis that is slightly higher for gradient copolymers compared to random.
copolymers. Both copolymers undergo the phase transition in one step, but with different mechanisms. While the random copolymer shows a simple coil to globule transition, the gradient copolymer undergoes micelle formation followed by micelle shrinkage as the temperature increases. By increasing the amount of OEOMA in the copolymer composition, the LCST of both gradient and random copolymers increases linearly, and their values tend to converge. The effect of different additives including various salts, ethanol and surfactants on the solubility behavior of a gradient and a random copolymer was investigated by DLS. The copolymers show different phase transition behavior in the presence of various additives. While the transition of the random copolymer shows a broad transition with a vast change in hydrodynamic radius and normalized scattering intensity, the gradient copolymer displays a rather sharp transition but with less changes in hydrodynamic radius and normalized scattering intensity. The effect of anions on the solubility of both copolymers follows the Hofmeister series. Among the anions studied in this work, SO42−, Cl− and Br− show kosmotropic while I− and SCN− show chaotropic effect on the solubility of copolymers in pure water. Whereas, there is no distinguishable trend observed for the decrease of LCST in presence of various cations. The phase transition behavior of both gradient and random copolymer changes from one-step to two-step phase transition in the presence of salts.

Addition of a good solvent as well as an anionic or cationic surfactant increases the LCST of both gradient and random copolymers. While the gradient copolymer shows a linear increase in LCST vs the amount of ethanol added to the solution, the LCST of the random copolymer changes exponentially. The increase in LCST in the presence of ethanol is attributed to the aggregation of polymer’s solubility in presence of ethanol as a good solvent for POEMAs which itself is a result of the competitive interaction with polymer between water and ethanol. There is no cononsolvency observed despite the cononsolvency behavior found for other temperature responsive polymers in presence on ethanol (38). On the other hand, the addition of surfactants to the aqueous solution of gradient and random copolymers makes the solution more stable by stabilizing the formed micelles and prevention the aggregation. The latter results in a stable solution regardless of temperature over a certain concentration (depending on the copolymer’s structural architecture) of surfactant in the system.

Conflict of interest

There are no conflicts to declare.

References

1. Lutz JF. Polymerization of oligo(ethylene glycol) (meth)acrylates: Toward new generations of smart biocompatible materials. J Polym Sci Part A Polym Chem. 2008;46(11):3459–70.
2. Lutz JF. Thermo-switchable materials prepared using the OEGMA-platform. Adv Mater. 2011;23(19):2237–43.
3. Sun S, Wu P. On the thermally reversible dynamic hydration behavior of oligo(ethylene glycol) methacrylate-based polymers in water. Macromolecules. 2013;46(1):236–46.
4. Lutz JF, Akdemir Ö, Hoth A. Point by point comparison of two thermosensitive polymers exhibiting a similar LCST: Is the age of poly(NIPAM) over? J Am Chem Soc. 2006;128(40):13046–7.
5. Han S, Hagiwara M, Ishizone T. Synthesis of Thermally Sensitive Water-Soluble Polymeric Acrylates by Living Anionic Polymerizations of Oligo(ethylene glycol) Methyl Ether Methacrylates. Macromolecules. 2003;36(6):8312–9.
6. Yamamoto S-I, Pietraski J, Matyjaszewski K. The Effect of Structure on the Thermoresponsive Nature of Well-Defined Poly(oligo(ethylene oxide) methacrylate) synthesized by ATRP. J Polym Sci Part A Polym Chem. 2008;46:194–202.
7. Wang X, Qiu X, Wu C. Comparison of the Coil-to-Globule and the Globule-to-Coil Transitions of a Single Poly(N-isopropylacrylamide) Homopolymer Chain in Water. Macromolecules [Internet]. 1998;31(9):2972–6. Available from: http://pubs.acs.org/doi/abs/10.1021/ma971873p
8. Kujawa P, Segui F, Shaban S, Diab C, Okada Y, Tanaka F, et al. Impact of End-Group Association and Main-Chain Hydration on the Thermosensitive Properties of Hydrophobically Modified Telechelic Poly(N-isopropylacrylamides) in Water. Macromolecules. 2006;39:341–8.
9. Havazelet B-P, Gryc S. Binding of Amino Acids to “Smart” Sorbents: Where Does Hydrophobicity Come into Play? Langmuir. 2004;20(1):169–74.
10. Wu J-Y, Liu S-Q, Heng PW-S, Yang Y-Y. Evaluating proteins release from, and their interactions with, thermosensitive poly(N-isopropylacrylamide) hydrogels. J Control Release. 2005;10(2):361–72.
11. Lutz J-F, Weichenhan K, Akdemir Ö, Hoth A. About the phase transitions in aqueous solutions of thermoresponsive copolymers and hydrogels based on 2-(2-methoxyethoxy) ethyl methacrylate and oligo (ethylene glycol) methacrylate. Macromolecules. 2007;40:2503–8.
12. Maeda Y, Kubota T, Yamauchi H, Nakaji T, Kitano H. Hydration changes of poly(2-(2-methoxyethoxy)ethyl methacrylate) during thermosensitive phase separation in water. Langmuir. 2007;23(22):11259–65.
13. Lutz J-F, Weichenhan K, Akdemir Ö, Hoth A. About the phase transitions in aqueous solutions of thermoresponsive copolymers and hydrogels based on 2-(2-methoxyethoxy) ethyl methacrylate and oligo (ethylene glycol) methacrylate. Macromolecules. 2007;40:2503–8.
14. Peng B, Grishkewich N, Yao Z, Han X, Liu H, Tam KC. Self-assembly behavior of thermoresponsive oligo(ethylene glycol) methacrylates random copolymer. ACS Macro Lett. 2012;1(5):632–5.
15. Zhang B, Tang H, Wu P. In depth analysis on the unusual multistep aggregation process of oligo(ethylene glycol) methacrylate-based polymers in water. Macromolecules. 2014;47(14):4728–37.
16. Yao ZL, Tam KC. Temperature induced micellization and
aggregation of biocompatible poly (oligo(ethylene glycol)methyl ether methacrylate) block copolymer analogs in aqueous solutions. Polymer (Guildf). 2012;53(16):3446–53.

17. Ieong NS, Hasan M, Phillips DJ, Saaka Y, O’Reilly RK, Gibson MI. Polymers with molecular weight dependent LCSTs are essential for cooperative behaviour. Polym Chem. 2012;3:794–9.

18. Kudo Y, Mori H, Kotsuchibashi Y. Preparation of an ethylene glycol-based block copolymer consisting of six different temperature-responsive blocks. Polym J [Internet]. 2018;50:1013–20. Available from: http://www.nature.com/articles/s41428-018-0091-1

19. Ogura Y, Terashima T, Sawamoto M. Amphiphilic PEG-Functionalized Gradient Copolymers via Tandem Catalysis of Living Radical Polymerization and Transesterification. Macromolecules. 2017;50(3):822–31.

20. Medel S, Garcia JM, Garrido L, Quijada-Garrido I, Paris R. Thermo- and pH-responsive gradient and block copolymers based on 2-(2-methoxyethoxy)ethyl methacrylate synthesized via atom transfer radical polymerization and the formation of thermoresponsive surfaces. J Polym Sci Part A Polym Chem. 2011;49:690–700.

21. Seno K-I, Tsujimoto I, Kanaoka S, Aoshima S. Synthesis of Various Stimuli-Responsive Gradient Copolymers by Living Cationic Polymerization and Their Thermally or Solvent Induced Association Behavior. J Polym Sci Part A Polym Chem. 2008;46:6444–6454.

22. Seno K-I, Tsujimoto I, Kikuchi T, Kanaoka S, Aoshima S. Thermosensitive Gradient Copolymers by Living Cationic Polymerization: Semibatch Precision Synthesis and Stepwise Dehydration-Induced Micellization and Physical Gelation. J Polym Sci Part A Polym Chem. 2008;46:6151–6164.

23. Seno K-I, Shokoyoku K, Sadahito A. Thermosensitive Diblock Copolymers with Designed Molecular Weight Distribution: Synthesis by Continuous Living Cationic Polymerization and Micellization Behavior. J Polym Sci Part A Polym Chem. 2007;46:2212–21.

24. Konefal R, Svecavecz J, Cernoch P. Thermoresponsive poly(2-oxazoline) homopolymers and copolymers in aqueous solutions studied by NMR spectroscopy and dynamic light scattering. Eur Polym J [Internet]. 2018 Mar 1 [cited 2019 Jul 8];100:241–52. Available from: https://www.sciencedirect.com/science/article/pii/S0010077316322270

25. Matsumoto S, Kanazawa A, Kanaoka S, Aoshima S. Dual stimuli-responsive copolymers with precisely arranged degradable units: synthesis by controlled alternating copolymerization of oxethylene-containing vinyl ethers and conjugated aldehydes. Polym Chem [Internet]. 2019;10:4134–41. Available from: http://link.rsc.org/?DOI=C9PS00301A

26. Eggers S, Eckert T, Abetz V. Double thermoresponsive block-random copolymers with adjustable phase transition temperatures: From block-like to gradient-like behavior. J Polym Sci Part A Polym Chem. 2017;399–411.

27. Oleszko-Torbus N, Utrata-Wesolek A, Walach W, Dworak A. Solution behavior of thermoresponsive random and gradient copolymers of 2-n-propyl-2-oxazoline. Eur Polym J. 2017;88:613–22.

28. Jaksh S, Schulz A, Kyriakos K, Zhang J, Grillo I, Pipich V, et al. The collapse and aggregation of thermoresponsive poly(2-oxazoline) gradient copolymers: a time-resolved SANS study. Colloid Polym Sci. 2014;292(10):2413–3425. Steinhauser W, Hoogenboom R, Keul H, Moeller M. Block and gradient copolymers of 2-hydroxyethyl acrylate and 2-methoxyethyl acrylate via RAFT: Polymerization kinetics, thermoresponsive properties, and micellization. Macromolecules. 2013;46(4):1447–60.

29. Gandhi A, Paul A, Sen SO, Sen KK. Studies on thermoresponsive polymers: Phase behaviour, drug delivery and biomedical applications. Asian J Pharm Sci [Internet]. 2015;10(2):99–107. Available from: http://dx.doi.org/10.1016/j.ajps.2014.08.010

30. Costa MCM, Silva SMC, Antunes FE. Adjusting the low critical solution temperature of poly(N-isopropyl acrylamide) solutions by salts, ionic surfactants and solvents: A rheological study. J Mol Liq [Internet]. 2015;210:113–8. Available from: http://dx.doi.org/10.1016/j.molliq.2015.02.008

31. Dhara D, Chatterji P. Phase Transition in Linear and Cross-Linked Poly(N-isopropylacrylamide) in Water: Effect of Various Types of Additives. Polym Rev [Internet]. 2000;40(1):51–68. Available from: http://www.informaworld.com/openurl?genre=article&doi=10.1081/MC-100100578&magic=crossref%7C%7CD040A21C5BB053405B1A640AFFD44AE3

32. Zhang YJ, Furyk S, Bergbreiter DE, Cremer PS. Specific ion effects on the water solubility of acromolecules: PNIPAM and the Hofmeister series. J Am Chem Soc [Internet]. 2005;127(23):14505–10. Available from: http://pubs.acs.org/doi/abs/10.1021/ja0546424

33. Algaer EA, Van Der Vegt NFA. Hofmeister ion interactions with model amide compounds. J Phys Chem B. 2005;127(1):4372–81. Available from: http://doi.wiley.com/10.1002/jpcb.200500339

34. Michailova VI, Momkova DB, Velichkova HA, Ivanov EH, Kotsikova RK, Karashanova DB, et al. Self-Assembly of a Thermally Responsive Double-Hydrophilic Copolymer in Ethanol-Water Mixtures: The Effect of Preferential Adsorption and Co-Nonsolvency. J Phys Chem B. 2018;122(2):490–504. Available from: http://dx.doi.org/10.1021/acs.jpcb.7b08562

35. Eggerson J, Flickr S, Bergbreiter DE, Cremer PS. Effect of organic and inorganic ions on the lower critical solution transition and aggregation of PNIPAM. Soft Matter. 2018;14(38):7818–28.

36. Michailova VI, Momkova DB, Velichkova HA, Ivanov EH, Kotsikova RK, Karashanova DB, et al. Self-Assembly of a Thermally Responsive Double-Hydrophilic Copolymer in Ethanol-Water Mixtures: The Effect of Preferential Adsorption and Co-Nonsolvency. J Phys Chem B. 2018;122(2):490–504. Available from: http://dx.doi.org/10.1021/acs.jpcb.7b08562

37. Lucht N, Eggers S, Abetz V. Cononsolvency in the ‘drunken’ state: the thermoresponsiveness of a new acrylamide
copolymer in water–alcohol mixtures. Polym Chem [Internet]. 2017;8(7):1196–205. Available from: http://xlink.rsc.org/?DOI=C6PY01751G

39. Dilip M, Bridges NJ, Rodriguez H, Pereira JFB, Rodgers RD. Effect of Temperature on Salt-Salt Aqueous Biphasic Systems: Manifestations of Upper Critical Solution Temperature. J Solution Chem. 2014;44:454–68.

40. Roth PJ, Jochum FD, Theato P. UCST-type behavior of poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA) in aliphatic alcohols: solvent, co-solvent, molecular weight, and end group dependences. Soft Matter. 2011;7(6):2484.

41. Guang N, Shou-xin Liu, Li X, Tian L, Hongguang M. Micellization and Gelation of the Double Thermoresponsive ABC-type Triblock Copolymer by One-pot RAFT Synthesis. Chinese J Polym Sci. 2016;34(8):956–80.

42. Yuan W, Wang J. Oligo(ethylene glycol) and quaternary ammonium-based block copolymer micelles: from tunable thermoresponsive to dual salt response. RSC Adv [Internet]. 2014;4:38855. Available from: http://xlink.rsc.org/?DOI=CARA05096G

43. Fang Q, Chen T, Zhong Q, Wang J. Thermoresponsive polymers based on oligo(ethylene glycol) methyl ether methacrylate and modified substrates with thermosensitivity. Macromol Res. 2017;25(3):206–13.

44. Murdoch TJ, Humphreys BA, Willott JD, Prescott SW, Nelson A, Webber GB, et al. Enhanced specific ion effects in ethylene glycol-based thermoresponsive polymer brushes. J Colloid Interface Sci [Internet]. 2017;490:869–78. Available from: http://dx.doi.org/10.1016/j.jcis.2016.11.044

45. Badi N, Lutz JF. Sequence control in polymer synthesis. Chem Soc Rev. 2009;38(12):3383–90.

46. Troy A, Scott J. Refractive Index of Ethanol-Water Mixtures and Density and Refractive Index of Ethanol-Water- Ethyl ether Mixtures. J Phys Chem. 1946;50:406–12.

47. Herraez J V, Belda R. Viscous Synergy of Pure Monoalcohol Water-soluble Polymethacrylates by Living Anionic Polymerizations of Trialkylsilyl-Protected Oligo (ethylene glycol ) Methacrylates. Macromolecules. 2003;36:42–9.

48. Lutz JF, Hoth A. Preparation of Ideal Analogues with a Tunable Thermosensitivity by Controlled Radical Copolymerization of 2-(2-Methoxyethoxy)ethyl Methacrylate and Oligo(ethylene glycol) Methacrylate. Macromolecules. 2006;39:893–6.

49. Ishizone T, Han S, Okuyama S, Nakahama S. Synthesis of Water-soluble Polymethacrylates by Living Anionic Polymerizations of Trialkylsilyl-Protected Oligo(ethylene glycol) Methacrylates. Macromolecules. 2003;36:42–9.

50. Yamanaka J, Kayasuga T, Ito M, Yokoyama H, Ishizone T. Synthesis of water-soluble poly(oligo(ethylene glycol) methacrylate) by living anionic polymerization of oligo(ethylene glycol) vinyl ether methacrylates. Polym Chem [Internet]. 2011;2(8):1837. Available from: http://xlink.rsc.org/?DOI=C1PY00118c

51. Ahmed S, El-Sherif M. Effect of variation of PMDETA/[Cu(I)Br]0 ratio on atom transfer radical polymerization of N-vinylpyrrolidone. J Polym Mater. 2004;21(4):279–82.

52. Yamanaka J, Kayasuga T, Ito M, Yokoyama H, Ishizone T. Synthesis of water-soluble poly(oligo(ethylene glycol) methacrylate) by living anionic polymerization of oligo(ethylene glycol) vinyl ether methacrylates. Polym Chem [Internet]. 2011;2(8):1837. Available from: http://xlink.rsc.org/?DOI=C1PY00118c

53. Ishizone T, Han S, Hagiwara M, Yokoyama H. Synthesis and surface characterization of well-defined amphiphilic block copolymers containing poly [oligo(ethylene glycol) methacrylate] segments. Macromolecules. 2006;39(3):362–70.

54. Ishizone T, Han S, Okuyama S, Nakahama S. Synthesis of Water-soluble Polymethacrylates by Living Anionic Polymerizations of Trialkylsilyl-Protected Oligo (ethylene glycol) Methacrylates. Macromolecules. 2003;36:42–9.

55. Lutz JF, Hoth A. Preparation of Ideal Analogues with a Tunable Thermosensitivity by Controlled Radical Copolymerization of 2-(2-Methoxyethoxy)ethyl Methacrylate and Oligo(ethylene glycol) Methacrylate. Macromolecules. 2006;39:893–6.

56. Xue L, Agarwal US, Lemstra PJ. High molecular weight PMMA by ATRP. Macromolecules. 2002;35(22):8650–2.

57. Reichardt C, Welston T. Solvents and Solvent Effects in Organic Chemistry. 4th ed. wiley-VCH; 2011.

58. Coullerez G, Carilmark A, Malmström E, Jonsson M. Understanding copper-based Atom Transfer Radical Polymerization in aqueous media. J Phys Chem. 2004;108(35):1–4.

59. Burgenudd H, Coullerez G, Jonsson M, Malmström E. Solvent effects on ATRP of oligo(ethylene glycol) methacrylate. exploring the limits of control. Macromolecules. 2009;42(9):3302–8.

60. Horn M, Matyjaszewski K. Solvent effects on the activation rate constant in atom transfer radical polymerization. Macromolecules. 2013;46(9):3350–7.

61. Matyjaszewski K, Gobel B, Paik H, Horwitz CP. Tridentate Nitrogen-Based Ligands in Cu-Based ATRP: A Structure - Activity Study. Macromolecules. 2001;34:430–40.

62. Qi J, Matyjaszewski K, Thouin L, Amatore C. Cyclic voltammetric studies of copper complexes catalyzing atom transfer radical polymerization. Macromol Chem Phys. 2000;201(14):1625.

63. Matyjaszewski K. Structure-reactivity correlation in Atom Transfer Radical Polymerization. Macromol Symp. 2002;182:209–24.

64. Göbel B, Matyjaszewski K. Diimino- and diaminopyridine complexes of CuBr2 and FeBr2 as catalysts in atom transfer radical polymerization (ATRP). Macromol Chem Phys [Internet]. 2000;201(14):1619–24. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-0001082698&partnerID=40&md5=502f9465103b700c2b9602885b99b6

65. Huang J, Pintauer T, Matyjaszewski K. Effect of variation of [PMDETA]/[Cu(I)Br]0 ratio on atom transfer radical polymerization of butyl acrylate. J Polym Sci Part A Polym Chem [Internet]. 2004;42(13):3285–92. Available from: http://doi.wiley.com/10.1002/pola.20175

66. Matyjaszewski K, Wu T, Amatore C. Copper complex catalyzed atom transfer radical polymerization: poly(ethylene glycol) based amphiphilic block copolymers. Macromolecules. 2003;36:8936–45.

67. Matyjaszewski K, Wu T, Amatore C. Copper complex catalyzed atom transfer radical polymerization: poly(ethylene glycol) based amphiphilic block copolymers. Macromolecules. 2003;36:8936–45.

68. Matyjaszewski K, Wu T, Amatore C. Copper complex catalyzed atom transfer radical polymerization: poly(ethylene glycol) based amphiphilic block copolymers. Macromolecules. 2003;36:8936–45.

69. Matyjaszewski K, Wu T, Amatore C. Copper complex catalyzed atom transfer radical polymerization: poly(ethylene glycol) based amphiphilic block copolymers. Macromolecules. 2003;36:8936–45.

70. Matyjaszewski K, Wu T, Amatore C. Copper complex catalyzed atom transfer radical polymerization: poly(ethylene glycol) based amphiphilic block copolymers. Macromolecules. 2003;36:8936–45.

71. Matyjaszewski K, Wu T, Amatore C. Copper complex catalyzed atom transfer radical polymerization: poly(ethylene glycol) based amphiphilic block copolymers. Macromolecules. 2003;36:8936–45.

72. Matyjaszewski K, Wu T, Amatore C. Copper complex catalyzed atom transfer radical polymerization: poly(ethylene glycol) based amphiphilic block copolymers. Macromolecules. 2003;36:8936–45.

73. Matyjaszewski K, Wu T, Amatore C. Copper complex catalyzed atom transfer radical polymerization: poly(ethylene glycol) based amphiphilic block copolymers. Macromolecules. 2003;36:8936–45.

74. Matyjaszewski K, Wu T, Amatore C. Copper complex catalyzed atom transfer radical polymerization: poly(ethylene glycol) based amphiphilic block copolymers. Macromolecules. 2003;36:8936–45.

75. Matyjaszewski K, Wu T, Amatore C. Copper complex catalyzed atom transfer radical polymerization: poly(ethylene glycol) based amphiphilic block copolymers. Macromolecules. 2003;36:8936–45.

76. Matyjaszewski K, Wu T, Amatore C. Copper complex catalyzed atom transfer radical polymerization: poly(ethylene glycol) based amphiphilic block copolymers. Macromolecules. 2003;36:8936–45.
66. Bergenudd H, Coullerez G, Jonsson M, Malmstro M. Solvent Effects on ATRP of Oligo (ethylene glycol) Methacrylate. Exploring the Limits of Control-SI. Macromolecules. 2009;42:3302–8.

67. Matyjaszewski K, Ziegler MJ, Arehart S V, Greszta D, Pakula T. Gradient copolymers by atom transfer radical copolymerization. J Phys Org Chem [Internet]. 2000;13:775–86. Available from: http://doi.wiley.com/10.1002/1099-1395(200012)13:12%3C775::AID-POC314%3E3.0.CO;2-D

68. Wang X, Qiu X, Wu C. Comparison of the Coil-to-Globule and the Globule-to-Coil Transitions of a Single Poly(N-isopropylacrylamide) Homopolymer Chain in Water. Macromolecules [Internet]. 1998;31(9):2972–6. Available from: https://pubs.acs.org/doi/10.1021/ma971873p

69. Wang X, Qiu X, Wu C. Comparison of the Coil-to-Globule and the Globule-to-Coil Transitions of a Single Poly(N-isopropylacrylamide) Homopolymer Chain in Water. Macromolecules. 1998;31(9):2972–6.

70. Hofmeister F. Zur Lehre von der Wirkung der Salze - Dritte Mittheilung. Arch für Exp Pathol und Pharmakologie. 1889;25(1):1–30.

71. Kunz W, Henle J, Ninham BW. “Zur Lehre von der Wirkung der Salze” (about the science of the effect of salts): Franz Hofmeister’s historical papers. Curr Opin Colloid Interface Sci. 2004;9:19–37.

72. Heyda J, Dzubiella J. Thermodynamic Description of Hofmeister Effects on the LCST of Thermosensitive Polymers. J Phys Chem B. 2014;118:10979–88.

73. Zhang Y, Cremer PS. Chemistry of Hofmeister anions and osmotolites. Annu Rev Phys Chem [Internet]. 2010;61:63–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20055667

74. Peter H. von Hippel TS. Ion effects on the Solution Structure of Biological macromolecules. Acc Chem Res. 1969;2:257–65.

75. Gao YQ. Simple Theory for Salt Effects on the Solubility of Amide. J Phys Chem B. 2012;116:9934–9943.

76. Ciferri A, Perico A. Ionic interactions in Natural and Synthetic Macromolecules. Wiley; 2012.

77. Mysels PM and KJ. Mukerjee, P. and Mysels, K.J. (1971) Critical Micelle Concentration of Aqueous Surfactant Systems. Vol. 36, US Government Printing Office, Washington DC. Vol. 36. US Government Printing Office; 1971.

78. Neugebauer J.M. Detergents: An overview. Methods in Enzymology. 1990;182:239–53.

79. Roe S. Protein Purification Application: A Practical Approach. Oxford University Press; 1990.

80. R. Walter, J.Ricka, Ch. Quellet, Ch. Nyffenegger TB. Coil-Globule Transition of Poly(N-isopropylacrylamide), A Study of Polymer–Surfactant Association.pdf. Macromolecules. 1996;29:4019–28.

81. Wu C, Zhou S. Effects of surfactants on the phase transition of poly(N-isopropylacrylamide) in water. J Polym Sci Part B Polym Phys. 1996;34(9):1597–604.

82. Sakai M, Satoh N, Tsuji K, Zhang YQ, Tanaka T. Effects of Surfactants on the Phase Transition of a Hydrophobic Polymer Gel. Langmuir. 1995;11(7):2493–5.

83. Kokufuta E, Zhang Y-Q, Tanaka T, Mamada A. Effects of surfactants on the phase transition of poly(N-isopropylacrylamide) Gel. Macromolecules. 1996;26:1053–9.

84. Schild HG, Tirrell DA. Interaction of Poly(N-isopropylacrylamide) with Sodium n-Alkyl Sulfates in Aqueous Solution. Langmuir. 1991;7(4):665–71.

85. Fang Q, Chen T, Zhong Q, Wang J. Thermoresponsive polymers based on oligo(ethylene glycol) methyl ether methacrylate and modified substrates with thermostensitivity. Macromol Res [Internet]. 2017;25(3):206–13. Available from: http://link.springer.com/10.1007/s13233-017-5037-5

86. Kyriakos K, Philipp M, Lin CH, Dyakonova M, Vishnevetskaya N, Grillo I, et al. Quantifying the Interactions in the Aggregation of Thermoresponsive Polymers: The Effect of Cononsolvency. Macromol Rapid Commun. 2016;37(5):420–5.

87. Costa RO., Freitas RF. Phase behavior of poly(N-isopropylacrylamide) in binary aqueous solutions. Polymer (Guildf). 2002;43(22):5879–85.

88. Noskov SY, Lamoureux G, Roux B. Molecular dynamics study of hydration in ethanol-water mixtures using a polarizable force field. J Phys Chem B. 2005;109(14):6705–13.

89. Roth PJ, Collin M, Boyer C. Advancing the boundary of insolubility of non-linear PEG-analogues in alcohols: UCST transitions in ethanol-water mixtures. Soft Matter [Internet]. 2013;9(6):1825–34. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-84872587600&partnerID=40&md5=37d1918a61d04b2d63ba949a28f86c78
Gradient copolymers of oligo(ethylene oxide)methacrylates in water, show different solubility behaviour in presence of additives from their random analogue.