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Lower Critical Solution Temperature Behavior of Comb and Graft Shaped Poly[oligo(2-ethyl-2-oxazoline)methacrylate]s

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ABSTRACT: Comb and graft polymers with lower critical solution temperature (LCST) behavior based on hydrophilic oligo(2-ethyl-2-oxazoline) side chains and a hydrophobic methacrylate backbone were synthesized using the macromonomer method. Well-defined oligo(2-ethyl-2-oxazoline) methacrylate (OEtOxMA) macromonomers were obtained by direct end-capping of living oligo(2-ethyl-2-oxazoline) chains with in situ formed triethylammonium methacrylate. The macromonomers were subsequently polymerized in a controlled manner using the reversible addition−fragmentation chain transfer (RAFT) polymerization technique yielding a series of comb polymers with varying side chain length and backbone length. In addition, a series of graft copolymers were prepared by copolymerizing OEtOxMA with methyl methacrylate (MMA, 40−80 mol %). The copolymers were characterized by ^{1}H NMR spectroscopy, size exclusion chromatography (SEC), and, partially, by matrix-assisted laser desorption ionization (MALDI-TOF) mass spectrometry. The LCST behavior of aqueous polymer solutions was investigated by turbidity measurements revealing cloud points that can be tuned from 35 to 80 °C by variation of the MMA content.

Introduction

Nonionic water-soluble polymers are known to exhibit a phase transition in aqueous solution at their lower critical solution temperature (LCST), which depends on the hydrophilic−hydrophobic balance as well as the capability to form hydrogen bonds with water.[1] Such polymers are soluble below their LCST due to hydrogen bonds that are formed with the solvent resulting in a hydration shell around the polymer. These hydrogen bonds become weaker with increasing temperature, and when the cloud point of the solution is reached, the water molecules from the hydration shell are expelled into the water. Hence, the polymer becomes hydrophobic and precipitates. This behavior facilitates the "switching" between a hydrophilic−hydrophobic behavior and, thus, the solubility by changing the temperature, resulting in a wide range of potential applications, e.g., in drug delivery systems,[2] for solvatization or precipitation of nanoparticles[3,4] and in selective membranes.[1] Because of its LCST close to body temperature, the properties and potential applications of poly(*N*-isopropylacrylamide) (PNIPAm) have been extensively studied.[5,6] However, there are other classes of polymers that deserve attention with respect to their biocompatibility. Polyoxazolines are a class of synthetic polymers that can be synthesized in a very well-defined way due to the livingness of the polymerization of 2-oxazolines.[7−12] This enables the "fine tuning" of polymer properties like the LCST behavior by copolymerization of different monomers or by control of the polymer end group and broadens the range of potential applications.[13−22]

Graft and comb polymers are particularly interesting due to the fact that the properties of different polymeric units can be combined within one molecule offering the possibility of a variety of potential applications in, e.g., breathable membranes or permanent antistatic additives.[23] Moreover, graft copolymers that contain special labile moieties can be used for improved biodegradable drug delivery systems although they have high molar masses.[24] As some graft copolymers show LCST behavior or micelle formation in water, there is a general interest in the biomedical application of these amphiphilic substances.[1,25−27] The macromonomer method is a promising technique that makes it possible to synthesize comb shaped polymers where the side chains are connected to the backbone regularly and closely to each other.[28] The side chains of the comb are first polymerized and subsequently functionalized with a polymerizable end group, which is used to form the backbone in a second polymerization process. A further advantage of this method is the possibility to copolymerize the synthesized macromonomers with comonomers that have different properties and allows the introduction of other functionalities into the final polymer architecture. The living cationic ring-opening polymerization (CROP) of 2-oxazolines enables the synthesis of oligomers with well-defined radically polymerizable end groups applicable for this synthetic method as we and others have demonstrated in previous work.[29,30]

The copolymerization of oligo(2-methyl-2-oxazoline)acrylate and -methacrylate macromonomers with styrene or methyl methacrylate (MMA) is described in the literature[31,32] as well as the copolymerization of oligo(2-phenyl-2-oxazoline)acrylate macromonomers with MMA.[33] However, the synthesis of well-defined homopolymers of such oligooxazoline macromonomers by controlled radical polymerization has, to the best of our knowledge, not been reported until now. In addition, there are only few examples of well-defined copolymers with grafted polyoxazolines described in the literature up to now. David et al. used the macromonomer method in the copolymerization of NIPAm with poly(2-methyl-2-oxazoline) macromonomers derived from cinnamic and maleic acid.[34] The obtained polymers revealed a LCST behavior at 34−36 °C. In addition, copolymers of NIPAm and chloromethylstyrene have been used for the initiation of the CROP of 2-ethyl-2-oxazoline (EtOx) and 2-methyl-2-oxazoline (MeOx) in a "grafting-from" approach.[35]

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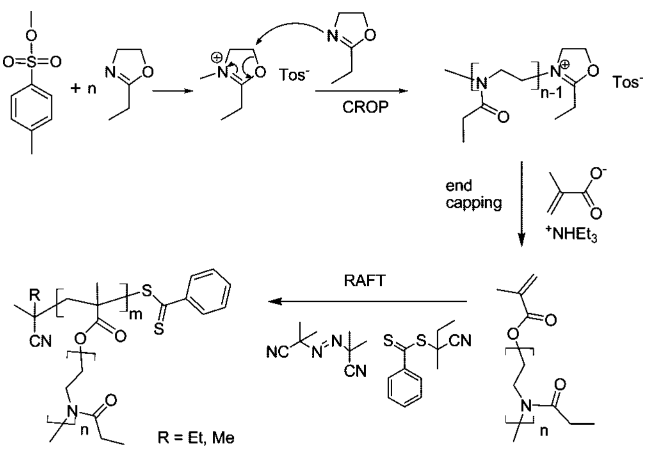
Scheme 1. Schematic Representation of the Synthesis Route toward Poly[oligo(2-ethyl-2-oxazoline)methacrylate]s[a]



[a] CROP: cationic ring-opening polymerization. RAFT: reversible addition−fragmentation chain transfer polymerization.

The obtained copolymers revealed LCST transitions in the range of 30−40 °C. Nevertheless, to the best of our knowledge, these are the only well-defined grafted Poly(2-oxazoline) structures described in the literature up to now.

Here, we report the synthesis of comb and graft copolymers containing hydrophilic oligo(2-ethyl-2-oxazoline) (OEtOx) side chains and a hydrophobic methacrylate backbone using the macromonomer method. This is achieved by the combination of the living CROP of 2-oxazolines with a controlled radical polymerization technique, namely the reversible addition−fragmentation chain transfer (RAFT) polymerization.[36] The synthetic approach is depicted in Scheme 1. The OEtOx macromonomers with different chain lengths have been homopolymerized as well as copolymerized with MMA. In addition, the LCST behavior of the thus-synthesized comb and graft copolymers was investigated in aqueous solution by turbidity measurements.

Experimental Section

Materials. 2-Ethyl-2-oxazoline (99%, Acros, EtOx) was dried over barium oxide and distilled under argon prior to use. Methyl tosylate (98%, Aldrich, MeTos) was distilled under reduced pressure and stored under argon. Acetonitrile (extra dry, Acros) was stored under argon. Methacrylic acid (99%, Aldrich, MAA) was used as received. Triethylamine (NEt_3) was dried over potassium hydroxide and distilled under Argon. 2,2′-Azobis(2-methylpropionitrile) (98%, Acros, AIBN) was recrystallized from hexane, and the chain transfer agent 2-cyanobutan-2-yl dithiobenzoate (CBDB) was kindly provided by AGFA. Methyl methacrylate (99%, Fluka, MMA) was passed over a short neutral aluminum oxide column directly before use to remove the stabilizer. For the purification of the comb polymers a column filled with BioBeads S-X1 (exclusion limit 14 000 Da) with THF (Biosolve, HPLC grade) as eluent was used. For the cloud point measurements phosphate buffered saline 10× concentrate (Aldrich), citrate buffered solution (pH = 4, Roth), and borate buffered solution (pH = 10, Roth) were used. All other chemicals and solvents were obtained from common commercial sources and used without further purification, unless otherwise noted.

Instrumentation. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker AC 250 MHz using the residual solvent resonance as an internal standard. Size exclusion chromatography (SEC) was measured on a Shimadzu system equipped with a SCL-10A system controller, a LC-10AD pump, and a RID-10A refractive index detector using a solvent mixture containing chloroform, triethylamine, and isopropanol (94:4:2) at a flow rate of 1 mL min^{-1} on a PSS-SDV-linear M 5 μm column at room temperature. The system was calibrated with polystyrene (370−67 500 Da) and PMMA (2000−88 000 Da) standards. For polymers with higher molar masses, a second Shimadzu system was equipped with a SCL-10A system controller, a LC-10AD pump, a RID-10A refractive index detector, and both a PSS Gram30 and a PSS Gram1000 column in series, whereby *N,N*-dimethylacetamide with 5 mmol of LiCl was used as an eluent at 1 mL min^{-1} flow rate and the column oven was set to 60 °C. For the measurement of the matrix-assisted laser desorption/ionization (MALDI) spectra an Ultraflex III TOF/TOF (Bruker Daltonics, Bremen, Germany) was used. The instrument was equipped with a Nd:YAG laser and a collision cell. All spectra were measured in the positive reflector or linear mode. The instrument was calibrated prior to each measurement with an external PMMA standard from PSS Polymer Standards Services GmbH (Mainz, Germany). The polymerization of EtOx was performed in a Biotage Initiator Sixty microwave synthesizer (for polymerizations in the microwave, see refs 37−40). Cloud points were determined either on a UV/vis spectrometer Specord 250 equipped with a control unit PTC 800 and a thermostat WC601 from Analytik Jena or on a Crystal 16 from Avantium Technologies being connected to a chiller (Julabo FP 40) using a wavelenghth of 500 nm and a heating ramp of 1 K min^{-1}. The concentration of the polymer was 5 mg mL^{-1}, unless otherwise stated.

Synthesis. *Macromonomer Synthesis.* In a representative example, MeTos (2.490 g, 13.4 mmol), EtOx (3.971 g, 40.1 mmol), and acetonitrile (6.0 mL) were transferred to a predried microwave vial under inert conditions. In all cases the concentration of EtOx was kept at 4 mol L^{-1}. The amounts of MeTos and EtOx were calculated according to the desired [monomer] to [initiator] ratio for each oligomer. The vial was capped and placed in the autosampler of the microwave. After 10 s of prestirring, the reaction solution was heated to 140 °C, and the temperature was held for the desired reaction time (1−5 min, depending on the [monomer] to [initiator] ratio, absorption level very high). Subsequently, the vial was automatically cooled to room temperature by applying a nitrogen flow. MAA (1.7 mL, 20 mmol) was added in 1.5-fold excess via a syringe through the septum of the capped microwave vial containing the solution of living OEtOx (13 mmol). Thereafter, NEt_3 (3.7 mL, 26.7 mmol) was added similarly in 2-fold excess. The given amounts were varied according to the used [monomer] to [initiator] ratios. The reaction solution was heated to 80 °C for 15 h. The acetonitrile was evaporated, and the polymer was redissolved in chloroform. This solution was washed with saturated aqueous sodium hydrogen carbonate and saturated brine, dried with sodium sulfate, and filtered. The solvent was evaporated under reduced pressure, and the resulting white sticky polymer was dried under reduced pressure and stored at −18 °C under argon. ^{1}H NMR (250 MHz, $CDCl_3$): δ 6.07 (=CH_2), 5.58 (=CH_2), 4.27 (m, CH_2−COO), 3.44 (m, N−CH_2); 3.02 (m, N−CH_3), 2.35 (m, C−CH_2−C), 1.92 (m, CH_2=C−CH_3), 1.12 (m, C−CH_3) ppm.

Homopolymerization of the Macromonomers. In a representative example, 1.00 g (0.435 mmol) of macromonomer was transferred into the reaction vial (either 5 mL microwave vial or a small Schlenk tube) and dissolved in 0.832 mL of ethanol. Subsequently, 1.706 mg (7.25×10^{-3} mmol) of CBDB in 0.111 mL of ethanol and 0.297 mg (1.811×10^{-3} mmol) of AIBN in 0.143 mL of ethanol were added. The exact amounts of the used chemicals were calculated according to the molar mass of the macromonomer, keeping the ratio of [CBDB] to [AIBN] as 4/1 and the ratio of [monomer] to [CBDB] as 60/1, unless otherwise specified. Before closing the vial, the reaction solution was bubbled through with a flow of argon for 30 min. The reaction was carried out in an oil bath at 70 °C for 22 h. The obtained polymers were purified by precipitation into cold diethyl ether, column chromatography on a BioBeads S-X1 column (solvent THF, exclusion limit 14 000 Da), and a second precipitation into cold diethyl ether. After decantation of the solvent the slightly pink polymers were dried in a vacuum oven at 40 °C. M_n and PDI values were determined from SEC measurements using PMMA calibration. The conversion was calculated from the integrated peak areas of macromonomomer and comb polymer in the SEC elugram. The degree of polymerization

Table 1. Overview of Characterization Results of the Oligo(2-ethyl-2-oxazoline) Methacrylate Macromonomers[a]

code	[M]/[I] theo[b]	reaction time [min]	DP (^{1}H NMR)[c]	F (^{1}H NMR)[d] [%]	M_n[e] [g mol^{-1}]	PDI[e]
M1	3.0	1	2.7	86	860	1.10
M2	5.1	2	5.1	87	1120	1.08
M3	5.2	2	5.4	73	1170	1.07
M4	7.0	2	7.8	86	1420	1.08
M5	9.7	3	10.0	84	1730	1.08
M6	14.9	4	14.9	89	2360	1.08
M7	21.7	5	19.8	83	3270	1.08

[a] Polymerization conditions: $T = 140$ °C, [EtOx] = 4 mol L^{-1}. [b] Calculated from the amount of monomer and initiator used. [c] Calculated from the aromatic signals of the initiator and the methylene signals of the polymer backbone. [d] Degree of functionalization calculated from the vinylic protons of the end group and the methylene signals of the polymer backbone. [e] Obtained from SEC ($CHCl_3$, *i*PrOH, NEt_3) using polystyrene calibration.

was calculated from conversion and the used ratio of monomer to chain transfer agent. ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.01 (m, $CH_2{-}COO$), 3.44 (m, $N{-}CH_2$); 3.02 (m, $N{-}CH_3$), 2.35 (m, $C{-}CH_2{-}C$), 1.12 (m, $C{-}CH_3$) ppm.

Copolymerization of **M2** *with MMA.* The RAFT copolymerization was carried out in a similar manner to the homopolymerization of the macromonomers. The obtained polymers were purified by precipitating them three times into diethyl ether at room temperature and, after decantation of the solvent, dried in a vacuum oven at 40 °C. The monomer conversions were calculated from the integrals of monomer and polymer signals in the ^{1}H NMR spectrum ($CDCl_3$) of the reaction solution. The degree of polymerization was calculated from conversion and the used ratio of monomer to chain transfer agent. The final composition of the copolymer was determined from integration of the signals in the ^{1}H NMR spectrum of the purified product. ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.01 (m, $CH_2{-}COO$), 3.59 (m, $O{-}CH_3$), 3.44 (m, $N{-}CH_2$); 3.02 (m, $N{-}CH_3$), 2.35 (m, $C{-}CH_2{-}C$), 1.3−2.1 (CH_2 backbone), 1.12 (m, $C{-}CH_3$), 1.00 (m, CH_3 backbone), 0.83 (m, CH_3 backbone) ppm.

Kinetic Studies of the RAFT Polymerization. The preparation of the reaction solution was carried out in a similar manner as described above. The initial sample was taken before the reaction solution was heated to 70 °C. After the predefined time intervals, samples were taken (under a slight argon flow) with a syringe directly through the septum of the microwave vial or the valve of the Schlenk tube. The samples were analyzed by SEC in order to determine the conversion of the macromonomer and the molar mass as well as the PDI value of the formed comb polymer.

Results and Discussion

Synthesis and Characterization of Macromonomers. In order to obtain hydrophilic OEtOx macromonomers, a direct end-capping method was used for the functionalization of the OEtOx with a methacrylate unit.[29] The living cationic ring-opening polymerization of EtOx was performed with MeTos as initiator and acetonitrile as solvent under microwave irradiation.[29,41−44] The thus-obtained oligomers with living oxazolinium species at the chain end were attacked with nucleophilic methacrylate anions that were formed by in situ deprotonation of methacrylic acid with triethylamine.[30] Using this method, a series of well-defined oligo(2-ethyl-2-oxazoline)methacrylates (OEtOxMA) with varying degrees of polymerization were synthesized and characterized by SEC, ^{1}H NMR spectroscopy, and MALDI-TOF mass spectrometry as reported previously.[29] An overview of the analytical data of the OEtOxMA macromonomers is given in Table 1.

The macromonomers show narrow molar mass distributions with polydispersity indices below 1.2 and a single molar mass distribution in their MALDI-TOF mass spectra. The molar masses of the corresponding peaks fit to a OEtOx structure with both methyl and methacrylate end groups and have a distance of 99 Da, which is the molar mass of one monomeric unit. It should be noted that each species is ionized by a sodium cation. The functionality was determined by comparing the integrals of the signals derived from the vinylic protons with the polymer backbone signal. The large amount of protons that are present in the polymer backbone was compared to the end group leading to a comparison of peaks with very different intensities. Therefore, the error of this kind of determination is supposed to be larger than the 5%, which NMR studies show in general. Nevertheless, the degree of functionalization (F) was higher than 70% for all macromonomers, and unfunctionalized oligomers will not react during the RAFT polymerization due to the fact that they do not bear any radically polymerizable unit. Such oligomers were separated together with the unreacted macromonomer from the final comb polymer.

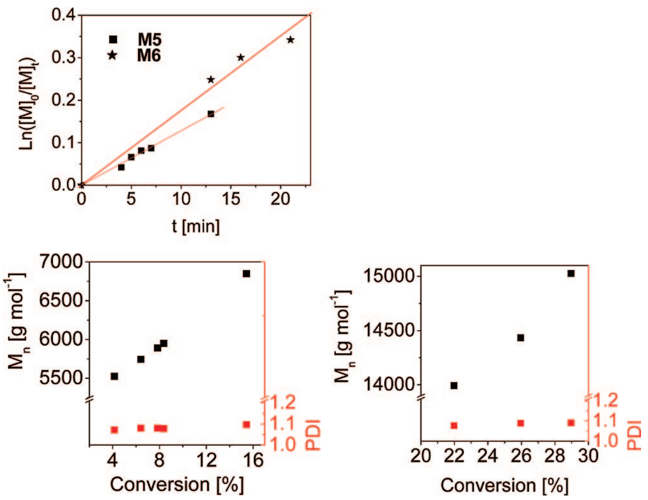


Figure 1. Kinetic plots for the RAFT polymerization of oligo(2-ethyl-2-oxazoline) methacrylates **M5** and **M6**. Top: semilogarithmic kinetic plot. Bottom left: molar mass and PDI values against conversion for **M5**. Bottom right: molar mass and PDI values against conversion for **M6**.

Homopolymerization of OEtOxMA. The RAFT polymerizations were carried out at 70 °C in ethanol as solvent, using CBDB as chain transfer agent and AIBN as initiator. The concentration (unless otherwise noted: 0.4 mol L^{-1}) of the macromonomer was kept low due to the high molar mass of the macromonomers, leading to relatively long reaction times. The optimum reaction conditions for a comparable RAFT polymerization (MMA, AIBN, CBDB) with a 2 M monomer concentration resulted in 80% conversion after 10 h.[45] In order to determine the required optimal polymerization time, kinetic studies were performed for the polymerization of two macromonomers with different molar masses, namely **M5** and **M6** with DP 10 and 15, respectively. The conversion was calculated from the integrals of the peaks of macromonomer and comb polymer in the SEC traces ($CHCl_3$:*i*PrOH:NEt_3, PMMA calibration) whereby it is assumed that they have equal response in the RI detector. The obtained PDI values are below 1.3, the molar mass increases linearly with conversion, and the semilogarithmic kinetic plot indicates a reaction of pseudo first order (see Figure 1). Therefore, it can be concluded that the polymerizations proceed in a controlled manner in the investigated range of conversions. The polymerizations of both macromonomers proceed with a comparable rate although the macromonomers **M5** and **M6** have a different chain length, indicating that the difference in molar mass is too small to affect the sterical hindrance and, thus, the polymerization rate.

A series of comb shaped polymers with varying side chain length could be synthesized using the OEtOxMA macromono-

Table 2. Overview of Characterization Results of the Poly[oligo(2-ethyl-2-oxazoline)methacrylate] Comb Polymers Synthesized via RAFT[a]

comb polymer	macromonomer	DP (EtOx)	DP[b] (backbone)	conv[c] [%]	PDI[d]	M_n[d] [g mol^{-1}]	M_n[e] [g mol^{-1}]
P1	**M1**	3	50	84	1.17	12 170	20 000
P2[f]	**M1**	3	86	43	1.29	10 570[g]	34 400
P3[h]	**M3**	5	11	18	1.10	5 700	6 600
P4	**M3**	5	38	64	1.31	7 970	22 800
P5	**M2**	5	20	33	1.18	7 110	12 000
P6	**M4**	7	30	50	1.27	9 190	24 000
P7	**M5**	10	26	69	1.20	14 540	28 600
P8	**M6**	15	34	57	1.17	22 520	54 400
P9[h]	**M7**	22	9	15	1.18	14 500	20 700
P10	**M7**	22	25	42	1.11	23 170	57 500

[a] Polymerization conditions: [OEtOxMA] = 0.4 mol L^{-1}, [M]:[CBDB]:[AIBN] = 60:1:0.25, T = 70 °C, t = 22 h. [b] Calculated from conversion. [c] Conversion calculated from peak areas of macromonomer and comb polymer in the SEC ($CHCl_3$:*i*PrOH:NEt_3). [d] Obtained from SEC ($CHCl_3$:*i*PrOH:NEt_3) using PMMA calibration. [e] Calculated from conversion. [f] [**M1**]:[CBDB] = 200, t = 45 h. [g] Obtained from SEC (DMA) using PMMA calibration. [h] Polymerization time 7 h.

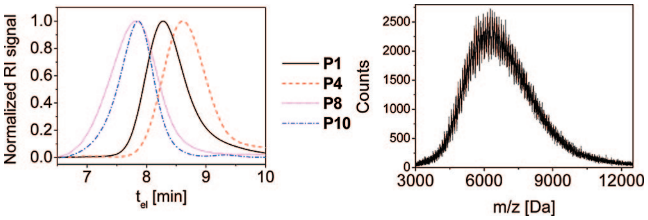


Figure 2. Left: SEC traces ($CHCl_3$, PMMA calibration) of selected poly[oligo(2-ethyl-2-oxazoline)methacrylate]s. Right: MALDI-TOF mass spectrum (matrix: DCTB; salt: NaI) of **P3**.

mers **M1**−**M7**. The RAFT polymerizations were aimed at a conversion of ~50% in order to avoid undesired side reactions such as chain coupling. Because of the fact that the conversion of the macromonomers was not complete, the residual macromonomers had to be removed from the final products. As the comb polymer and the longer macromonomers show similar solubility, it was in some cases not possible to separate the residual macromonomer by common precipitation techniques. Instead, a BioBeads S-X1 preparative size exclusion column was used, and the samples were separated according to their hydrodynamic volume. It was possible to obtain homopolymers of OEtOxMA with a wide range of backbone length (12−86 units) with narrow molar mass distributions and PDI values below 1.3. The PDI values obtained for the RAFT polymerization with dithiobenzoates generally range from 1.1 to 1.3 due to often observed hybrid behavior during initiation.[46] As such, the reported PDI values are as expected for RAFT polymerizations. The differences observed for the different polymerizations might be due to a number of factors such as RAFT agent concentration, residual oxygen levels, and purification procedure. The results of the characterization of the poly[oligo(2-ethyl-2-oxazoline)methacrylate] comb polymers are summarized in Table 2.

The molar masses that are obtained from SEC measurements (see Figure 2 for representative SEC traces) are much lower than the calculated molar masses for all of the polymers due to the polymer architecture which influences the hydrodynamic volume of the polymers. Graft or comb polymers have much smaller hydrodynamic volumes in solution compared to linear polymers because the chains are forced to stay close to each other. As the calibration of the SEC is carried out with linear standard polymers, the comb polymer appears at a later elution volume during the SEC measurement.

Because of the presence of repeating units in the comb polymer whose mass differs only by 1 Da (M(EtOx) = 99 Da, M(methyl + methacrylate) = 100 Da), the MALDI-TOF mass spectrum (Figure 2) shows a broad distribution consisting of a large number of overlapping peaks. Nevertheless, the maximum of the molar mass of the distribution corresponds to the M_n value for **P3** that was calculated from conversion.

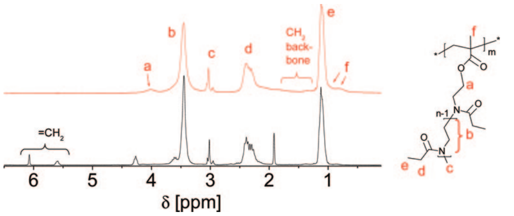


Figure 3. ^{1}H NMR spectra ($CHCl_3$) of poly[oligo(2-ethyl-2-oxazoline)methacrylate] comb polymer **P6** and the corresponding macromonomer **M4**. Top: macromonomer; bottom: comb polymer.

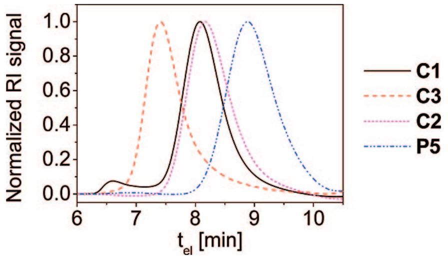


Figure 4. SEC traces ($CHCl_3$, *i*PrOH, NEt_3, PMMA calibration) of poly[oligo(2-ethyl-2-oxazoline)methacrylate-*r*-methyl methacrylate] copolymers **C1**−**C3** and the poly[oligo(2-ethyl-2-oxazoline) methacrylate] homopolymer **P5** (synthesized from the same macromonomer batch).

The ^{1}H NMR spectrum of **P6** shows the disappearance of the vinylic protons and a shift of the methylene signal next to the ester function when compared to the macromonomer, as shown in Figure 3. Furthermore, the signals are broadened after polymerization due to the lower chain mobility in solution.

Copolymerization of the OEtOxMA Macromonomers with Methyl Methacrylate. OEtOxMA macromonomer **M2** was copolymerized with varying amounts of MMA. A series of graft copolymers with a varying number of hydrophilic side chains were synthesized. Since only one batch of macromonomer was used, it can be assured that the average side chain length is kept constant (five repeating units of EtOx) for the series of statistical copolymers. It is depicted in Figure 4 that the SEC traces of these well-defined polymers show narrow molar mass distributions; the obtained PDI and molar mass values are summarized in Table 3. The high molar mass impurity in the SEC trace of **C1** is a product of autopolymerization of **M2** that was unfortunately not noticed before the synthesis of **C1** was carried out.

The MMA content in the purified copolymers was calculated from the corresponding ^{1}H NMR spectra (see Figure 5). With increasing content of MMA, the O−CH_3 peak at 3.7 ppm and the signals belonging to the methacrylate backbone gain intensity

Table 3. Overview of Characterization Results of the Poly[oligo(2-ethyl-2-oxazoline)methacrylate-*stat*-methyl methacrylate] Copolymers Synthesized via RAFT of M2[a]

					M2/MMA				
							polymer composition		
polymer	[**M2**]:[MMA]:[CBDB]	M_n[b] [g mol^{-1}]	M_n[c] [g mol^{-1}]	PDI[b]	conv [%]	DP[d]	conv [%]	NMR [%]	EA [%]
C1	60:15:1	15 780	25 400	1.29	67/96	40/14	74/26	56/44	52/48
C2	60:60:1	12 800	18 900	1.23	41/66	25/39	39/61	38/62	47/53
C3	60:240:1	25 190	18 500	1.25	61/71	36/170	17/83	21/79	19/81

[a] Polymerization conditions: [OEtOxMA] = 0.4 mol L^{-1}, [CBDB]:[AIBN] = 4:1, T = 70 °C, t = 22 h. [b] Calculated from SEC (CHCl$_3$, *i*PrOH, NEt$_3$) using PMMA calibration. [c] Calculated from conversion and the used ratio of [**M2**]:[MMA]:[CBDB]. [d] Degree of polymerization calculated from conversion and the used ratio of [**M2**]:[MMA]:[CBDB].

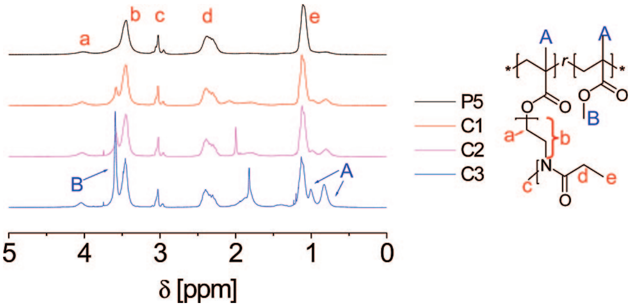


Figure 5. ^{1}H NMR spectra (250 MHz, CDCl$_3$) of the poly[oligo(2-ethyl-2-oxazoline)methacrylate-*stat*-(methyl methacrylate)] copolymers **C1**−**C3** and the poly[oligo(2-ethyl-2-oxazoline)methacrylate] homopolymer **P5** (synthesized from the same macromonomer batch).

as the ratio of backbone units to the side chain units is increasing.

In addition, it is possible to determine the nitrogen and carbon content of the purified product by elemental analysis. The composition of the copolymer can be calculated because nitrogen is only present in the oxazoline side chain and carbon is present in both monomers (macromonomer and MMA). On the basis of the integrals of monomer and polymer peaks in the ^{1}H NMR spectra of the reaction solution before and after the polymerization, the conversion of MMA and OEtOxMA was calculated. Using these conversions as well as the used ratio of monomers and chain transfer agent, the copolymer composition was calculated. The values for the polymer composition obtained by ^{1}H NMR spectroscopy of the purified product and by elemental analysis are in good agreement for **C1** and **C3**. The deviation in the value that is obtained from elemental analysis for **C2** is most likely caused by a contamination that could have still been present in the polymer and is not visible in the NMR spectrum. For the copolymers **C2** and **C3** the data calculated from the conversion of the two different monomers are consistent with the results from ^{1}H NMR spectroscopy, too. However, this is not the case for the copolymer **C1** with the highest macromonomer content. During the synthesis of this polymer the conversion of MMA was almost complete (96%). Consequently, the signals in the ^{1}H NMR spectrum of the t_{end} sample, which were used for integration and calculation of the conversion, were of very low intensity, and thus, inaccuracies in integration are supposed to cause the deviation of the value for the copolymer composition calculated from the conversion.

The amount of MMA which is actually incorporated in the polymer is higher than the used ratio of MMA to macromonomer. This indicates that MMA polymerizes faster than the more sterically hindered macromonomer as commonly observed for the copolymerization of macromonomers with small monomers.[47,48] On the basis of the lower reactivity of the macromonomer, it might be suggested that the statistical copolymers consist of a gradient monomer composition.

LCST Behavior of the Synthesized (Co)polymers. The temperature dependence of the solubility of the synthesized comb and graft (*co*)poly(OEtOxMA)s in aqueous solutions was investigated by turbidity measurements. Therefore, a defined amount of the synthesized polymer was dissolved in the desired solvent, either a buffered solution or in deionized water. This solution was heated and cooled at a rate of 1 °C min^{-1} while the transmittance of visible light (500 nm) was measured. The transmittance suddenly decreases upon heating as soon as the cloud point is reached and the polymer precipitates. The values for the cloud points are taken at a transmittance of 50% in the first heating ramp. When the solution is cooled, the polymer becomes soluble again, and therefore the transmittance of light increases. Such a heating−cooling cycle is repeated two times in order to investigate the reversibility of the phase transition. A typical curve for the determination of the cloud point is shown in Figure 6 for **P6**.

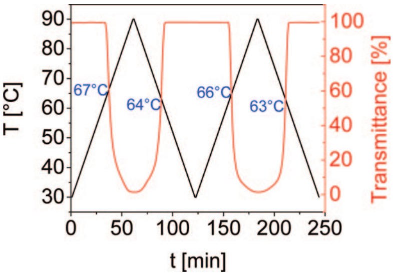


Figure 6. Transmittance of a solution of **P6** (5 g L^{-1} in H$_2$O) under temperature variation (heating rate 1 K min^{-1}). Blue: observed cloud point temperatures.

Table 4. Detected Cloud Points (cp) of Poly[oligo(2-ethyl-2-oxazoline)methacrylate]s in Aqueous Solutions (c = 5 g L^{-1}, Heating Rate 1 K min^{-1})

sample	cp[a] (H$_2$O) [°C]	cp[a] (pH = 4) [°C]	cp[a] (pH = 10) [°C]	cp[a] (PBS) [°C]
P1	69.3	63.5	63.1	63.4
P4	84.5	n.m.[b]	76.8	n.m.[b]
P5	84.4	77.1	77.2	77.1
P6	66.5	47.3	58.0	60.1
P8	73.3	67.4	69.5	70.4
P9	85.3	n.m.[b]	80.0	78.8
P10	73.7	70.7	71.3	71.0
C1	55.6	44.0	51.6	51.7
C2	55.1	49.9	50.1	50.2
C3	40.3	35.3	35.3	36.0

[a] cp = cloud point determined at 50% transmittance in the first heating run. [b] n.m. = not measured due to too little amount of polymer.

All of the synthesized comb and graft copolymers show LCST behavior. The obtained cloud points in water, buffered aqueous solution at pH 4 and 10, and phosphate buffered saline (PBS) are given in Table 4. In general, the cloud points decrease when going from water to the buffered solutions, indicating that the cloud point is somewhat affected by the presence of salts but not by the pH.

The salting-out effect of different additives to the solution is currently under detailed investigation.

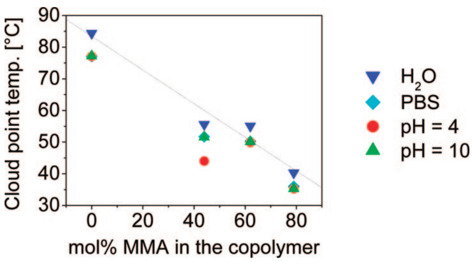


Figure 7. Detected cloud points for aqueous solutions of the copolymers of PEtOxMA with MMA ($c = 5$ mg mL^{-1}, heating rate 1 K min^{-1}). The line is added to guide the eye.

When the cloud points of comb polymers with identical length of the side chains are compared (**P9** and **P10**), an influence of the backbone length on the cloud point of the polymer solution is observed. The polymer becomes less soluble and the cloud point of the solution is decreased with increasing backbone chain length and increasing molar mass. According to the literature,[49,50] this effect is not observed for poly[oligo(ethylene glycol)methacrylate] (PEGMA) comb polymers with a rather similar molecular comb architecture, but it is clearly shown for linear PEtOx, a polymer which shows a classical type I Flory−Huggins demixing behavior in its aqueous solutions.[19,21,22] It might be speculated that it is more difficult to hydrate the polymer with higher molar mass due to decreased accessibility of the larger polymer coil similar to linear PEtOx.

From theoretical considerations a decreasing cloud point with decreasing DP of the hydrophilic side chains would be expected due to the increasing fraction of hydrophobic backbone. However, this effect cannot be observed for the homopolymers with a constant backbone length around 30 units (**P4**, **P8**, **P10**). All of the cloud points obtained from measurements of these polymers can be found in a comparable region (60−74 °C). Aqueous solutions of linear PEtOx with $M_n > 20\,000$ g mol^{-1} show cloud points in the same temperature region.[21,22] Nevertheless, the region below 70 °C can only be reached with linear PEtOx of a much higher molar mass ($M_n > 10^5$ g mol^{-1}), whereas all of the synthesized comb shaped PEtOx have a $M_n < 6 \times 10^4$ g mol^{-1}. It has to be clearly stated that PEtOx with a degree of polymerization below 100 does not show any LCST behavior at all. By the polymerization of low molar mass PEtOx macromonomers without LCST behavior it is possible to obtain a PEtOx comb polymer, which shows the LCST behavior of high molar mass linear PEtOx. The length of the side chain of the comb polymer does not affect the cloud points of the solutions in a significant way. Thus, it can be concluded that the hydrophobic backbone is not accessible for the water molecules and shielded by the hydrophilic side chains. This results in polyoxazoline-like properties of the comb polymer in solution rather than methacrylate ones. The observed lowering of the cloud point of these comb polymers compared to linear PEtOx is similar to the lowering that was observed for PEtOx$_{90}$-*b*-NonOx$_{10}$, which was ascribed to the formation of a hydrophobic PNonOx microdomain at the chain end obstructing good hydration of this chain end.[51]

It is anticipated that increasing the hydrophobic content in the polymer by incorporation of MMA into the backbone and, thus, decreasing the amount of the hydrophilic side chains will allow tuning of the cloud point. As expected, the cloud points of the solutions of these copolymers (see Figure 7) are lower when the MMA content in the polymer is higher since the polymer becomes more hydrophobic. A similar effect is reported in the literature for copolymers of PEGMA with MMA,[52,53] which can be regarded as PEG analogues to the here reported OEtOxMA-based systems. When altering the composition from pure OEtOxMA to a copolymer with 80 mol % MMA the cloud points decrease approximately linear from 80 to 40 °C, making these polymers suitable for a large range of potential applications.

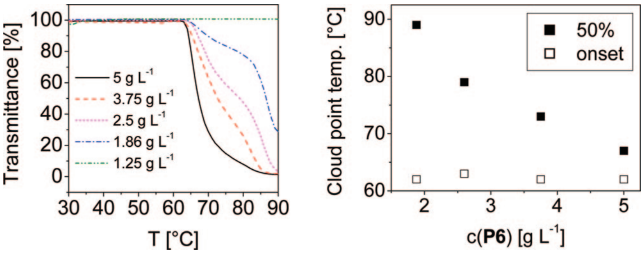


Figure 8. Turbidity curves and detected cloud points for **P6** in solutions with varying polymer concentration in deionized water (heating rate 1 K min^{-1}).

Furthermore, the cloud point of a PEtOxMA solution was found to be strongly dependent on the concentration of the polymer in aqueous solution (Figure 8). When the concentration of the solution is lowered, the cloud point (at 50% transmittance) is increased although the onset of the decrease in transmittance stays constant. A closer look at the shape of the turbidity curve reveals a two-step process for all concentrations except 1.25 g L^{-1}, which does not show a decrease in transmittance. A first decrease in transmittance occurs at 65 °C and a second decrease around 85 °C. The ratio of these two transitions shifts toward the 85 °C transition at lower concentration. A diminished aggregation of the precipitated polymer chains in lower concentrated solutions is supposed to cause this concentration effect and will be the focus of future studies.

Conclusion

Well-defined OEtOxMA macromonomers were obtained by direct end-capping of living OEtOx chains with in situ formed triethylammonium methacrylate. The obtained macromonomers could be polymerized in a controlled manner using the RAFT polymerization procedure. In that way a series of comb polymers with varying side chain length as well as a series of graft polymers with varying content of MMA were prepared.

The LCST behavior of aqueous polymer solutions was investigated by turbidity measurements revealing cloud points which are dependent on the polymer composition and can be tuned from 35 to 80 °C by varying the MMA content. The comb polymer architecture with very closely connected branched side chains leads to a significant decrease of the cloud points in aqueous solutions when compared to values from literature describing the LCST behavior of linear PEtOx. A remarkable concentration effect was found on the shape of the turbidity curve, which will be studied in future work to determine the underlying mechanism.

The further application of the developed general synthesis method offers the possibility to obtain a larger amount of copolymers by copolymerization of different monomers with the OEtOxMA macromonomers. By changing the nature of the comonomer, either hydrophobic, hydrophilic, or further functionalized, the properties of the grafted polymers can be varied and further studied. In addition, the macromonomer composition can be varied by (co)polymerizing different 2-oxazoline monomers.

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