160 *Macromolecules* **2010**, *43*, 160–167
DOI: 10.1021/ma902014q

Macromolecules
ARTICLE

Dual Responsive Methacrylic Acid and Oligo(2-ethyl-2-oxazoline) Containing Graft Copolymers

Christine Weber,[†,‡] C. Remzi Becer,[†,‡,§] Wolfgang Guenther,[†] Richard Hoogenboom,*[,‡,§] and Ulrich S. Schubert*[,†,‡,§]

[†]*Laboratory of Organic and Macromolecular Chemistry, Friedrich-Schiller-University Jena, Humboldtstrasse 10, 07743 Germany,* [‡]*Dutch Polymer Institute (DPI), John F. Kennedylaan 2, 5612 AB Eindhoven, The Netherlands, and* [§]*Laboratory of Macromolecular Chemistry and Nanoscience, Eindhoven University of Technology, Den Dolech 2, 5612 AZ Eindhoven, The Netherlands*

Received September 9, 2009; Revised Manuscript Received October 29, 2009

ABSTRACT: Graft copolymers containing a poly(methacrylic acid) (PMAA) backbone and oligo(2-ethyl-2-oxazoline) (OEtOx) side chains were synthesized by reversible addition−fragmentation chain transfer (RAFT) polymerization of methacrylic acid (MAA) and OEtOx methacrylate macromonomers. In order to achieve a constant backbone length of the graft copolymer, living OEtOx chains were also directly grafted onto a deprotonated PMAA backbone in grafting densities from 7% to 92%. Both methods resulted in graft copolymers which were characterized by SEC (M_n = 5500 to 18 700 g mol^{-1}, PDI < 1.3), ^{1}H NMR spectroscopy, and acid/base titrations. The solubility behavior of the graft copolymers in aqueous media was investigated using turbidity measurements revealing a lower critical solution temperature (LCST) behavior of the polymers with a grafting density above 35%. The cloud points of the solutions can be varied in a temperature range from 8 to 90 °C by adjustment of the polymer composition and the pH of the solution. ^{1}H NMR measurements in D_2O at varying temperatures revealed a collapse of the polymer backbone above the cloud point of the solution whereas parts of the OEtOx side chains remained mobile.

Introduction

Polymers exhibiting lower critical solution temperature (LCST) behavior in aqueous media have attracted a significant interest during the past decade because this behavior enables the reversible switching of polymer properties by temperature variation.[1,2] Below the so-called cloud point of the polymer solution, the hydrogen bonds that are formed between the binding sites within the polymer and the surrounding water molecules enable a hydrophilic behavior of the polymer, and thus, the polymer is dissolved. The solubility transition is driven by water: At the cloud point the hydrogen bonds between the polymer and the water molecules are weakened, and it is entropically more favorable to release the water to form hydrogen bonds with other water molecules resulting in a precipitation of the polymer. In particular, poly(meth)acrylamides have been investigated in detail because the phase separation of aqueous solutions of poly(*N*-isopropylacrylamide) (PNiPAm) occurs in the range of the body temperature.[3−6] Another polymer class that is known to exhibit an LCST behavior are poly(2-oxazoline)s, which possess hydrogen bond accepting amide moieties in the polymer main chain.[7−17] The living cationic ring-opening polymerization (CROP) mechanism allows excellent control of the polymer chain length and the end groups but does not tolerate the direct incorporation of hydrogen bond donators.[18−23] Since the LCST behavior is based on hydrogen bonding, it is interesting to study the influence of hydrogen bond donating moieties on the phase transitions of aqueous solutions of poly(2-oxazoline)s if their behavior is to be fully elucidated. Because of the fact that such moieties would cause tremendous side reactions during a cationic polymerization process, they have to be introduced to the polymer via postpolymerization modification procedures.[21,22,24]

The advantage of radical polymerization techniques such as the reversible addition−fragmentation chain transfer (RAFT) polymerization over ionic polymerizations is their tolerance against many functional groups.[25] Consequently, the combination of the CROP and the RAFT polymerization techniques should provide a powerful tool to obtain poly(2-oxazoline)s with a hydrogen bond donating unit such as a carboxylic acid.

We have recently demonstrated the feasibility of the macromonomer method for the combination of the living cationic ring-opening polymerization of 2-ethyl-2-oxazoline (EtOx) with the RAFT polymerization technique.[26,27] A straightforward approach to synthesize the targeted polymers would therefore be the copolymerization of oligo(2-ethyl-2-oxazoline) methacrylate (OEtOxMA) macromonomers with methacrylic acid, resulting in graft copolymers with a poly(methacrylic acid) (PMAA) backbone and OEtOx side chains. However, it should be noted that the resulting copolymers will not have a constant backbone length, a feature that can influence the solubility behavior as well as the distribution of grafted chains.

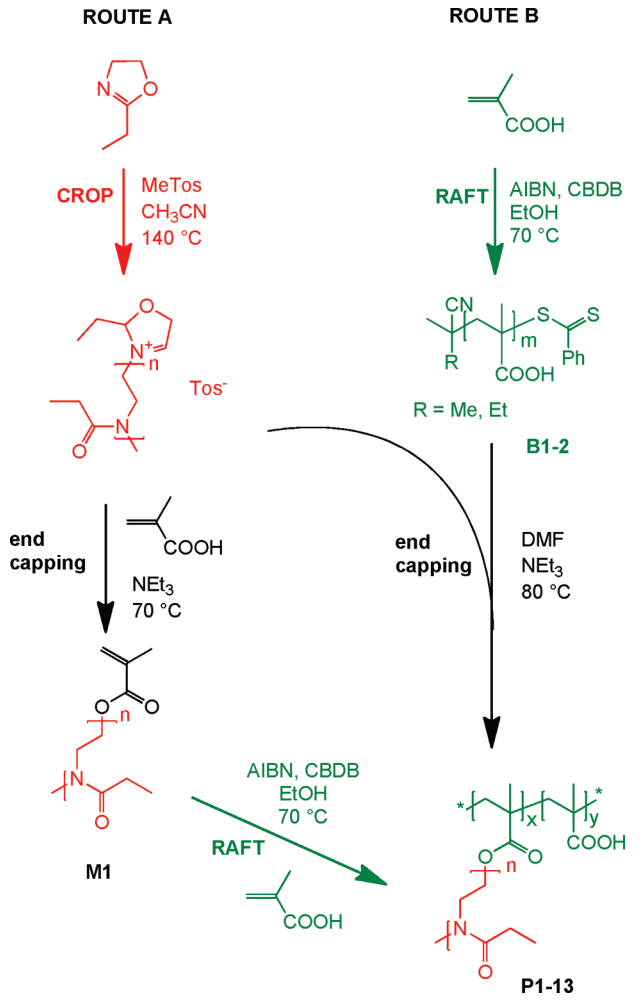
Alternatively, the grafting-onto method can be used for the preparation of PMAA with OEtOx grafts. The synthesis of a well-defined PMAA using the RAFT polymerization will provide a universal backbone onto which OEtOx side chains can be grafted in varying grafting densities, resulting in polymers with the same backbone length. A similar approach has been applied by McCormick et al.[28] using conventional free radical polymerization and CROP in order to investigate the properties of PMAA-*g*-PEtOx with relatively broad molar mass distributions at different pH values.

Here, we report the synthesis of graft copolymers composed of a PMAA backbone and OEtOx side chains using the macromonomer method on the one hand and a grafting-onto approach

*Corresponding authors: e-mail r.hoogenboom@tue.nl (R.H.) or ulrich.schubert@uni-jena.de (U.S.S.); Tel +49-(0)3641-948200, Fax +49-(0)3641-948202.

 Published on Web 11/17/2009

Scheme 1. Schematic Representation of the Synthesis Routes toward Poly[oligo(2-ethyl-2-oxazoline)methacrylate-*stat*-methacrylic acid] Graft Copolymers: Macromonomer Method (Route A); Grafting-Onto Method (Route B)[a]



[a] CROP: cationic ring-opening polymerization; RAFT: reversible addition−fragmentation chain transfer polymerization; CBDB: 2-cyanobutan-2-yl dithiobenzoate.

on the other hand. In both methods CROP, RAFT polymerization, and end-capping the CROP are combined but carried out in a different order (Scheme 1). In addition, we report detailed analysis of the solubility behavior and the cloud points of the graft copolymers in aqueous media using turbidity measurements as well as ^{1}H NMR spectroscopy at different measurement temperatures.

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. The used solvents (extra dry) acetonitrile and dimethylformamide were purchased from Acros and stored over molecular sieves and 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 synthesized according to a literature procedure.[29] For the cloud point measurements, demineralized water, phosphate buffered saline 10× concentrate (Aldrich), and citrate buffered solution (pH = 4, Roth) or 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 DMSO-d_6 on a Bruker Avance 250 MHz using the residual solvent resonance as an internal standard. Temperature-dependent ^{1}H NMR spectroscopy measurements were preformed in deuterated water utilizing a Bruker Avance 400 MHz using 3-(trimethylsilyl)-propionic acid-2,2,3,3-d_4 sodium salt (TSP) as a temperature inert standard. Size exclusion chromatography (SEC) was measured on a Agilent 1200 Series system equipped with an G1310A isocratic pump, a G1329A autosampler, a G1362A refractive index detector, and both a PSS Gram30 and a PSS Gram1000 column in series, whereby *N,N*-dimethylacetamide with 2.1 g L^{-1} of LiCl was used as an eluent at 1 mL min^{-1} flow rate and the column oven was set to 40 °C. The system was calibrated with poly(methyl methacrylate) (2000−88 000 g mol^{-1}) standards. Matrix-assisted laser desorption/ionization (MALDI) spectra were recorded on an Ultraflex III TOF/TOF (Bruker Daltonics, Bremen, Germany) 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 microwave-assisted polymerizations, see refs 30−33). 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 kept constant at 5 mg mL^{-1}. For the acid/base titration the polymer was dissolved in deionized water, and 1 mL of 0.1 M hydrochloric acid solution was added. The titration was performed against 0.1 M aqueous sodium hydroxide solution using a 765 Dosimat from Metrohm, a digital pH/mV-thermometer GMH 3530 from Greisinger electronic, and the EBS9 M Recorder software. Polymer solutions with cloud points below room temperature were cooled with an ice bath during the titration process.

Synthesis. *RAFT Polymerization.* Methacrylic acid (4.0 g, 46.5 mmol) and 2 mL of anisole were dissolved in 1.5 mL of ethanol and a solution of 31.8 mg of AIBN in ethanol as well as a solution of 182 mg of CBDB in ethanol were added. The concentration of monomer was 2.0 mol L^{-1} and the ratio of [monomer]:[CBDB]:[AIBN] was 60:1:0.25. Subsequently, the mixture was bubbled with a gentle flow of argon for 30 min in order to remove the oxygen before the vial was capped and heated to 70 °C in an oil bath for 8 h. The poly(methacrylic acid) was obtained as pink powder by precipitation into chloroform. The conversion was determined by comparison of the integral ratios of the anisole and methacrylic acid peaks in the ^{1}H NMR (MeOH-d_4) spectrum before and after the reaction. **B1**: conversion = 63%; DP = 38; M_n(SEC) = 7700 g mol^{-1}; PDI(SEC) = 1.27. **B2**: conversion = 45%; DP = 28; M_n(SEC) = 7000 g mol^{-1}; PDI(SEC) = 1.28.

CROP of EtOx. A stock solution of EtOx (4.651 g, 46.9 mmol), MeTos (1.771 g, 9.5 mmol), and acetonitrile (6.86 mL) was prepared under inert conditions and transferred into predried microwave vials that were capped subsequently. The monomer concentration was 4.0 mol L^{-1}, and the ratio of [monomer] to [intitator] was set to 5. After performing the polymerization at 140 °C for 1 min using microwave irradiation as heating source, a sample was taken for analysis of the oligomeric side chains by SEC, ^{1}H NMR spectroscopy, and MALDI-TOF mass spectrometry.

Grafting-Onto Procedure. 1.6 g of PMAA **B1** or **B2** and 3.9 mL of NEt_3 were dissolved in 7 mL of DMF. The desired amount of that stock solution was added via a syringe into the

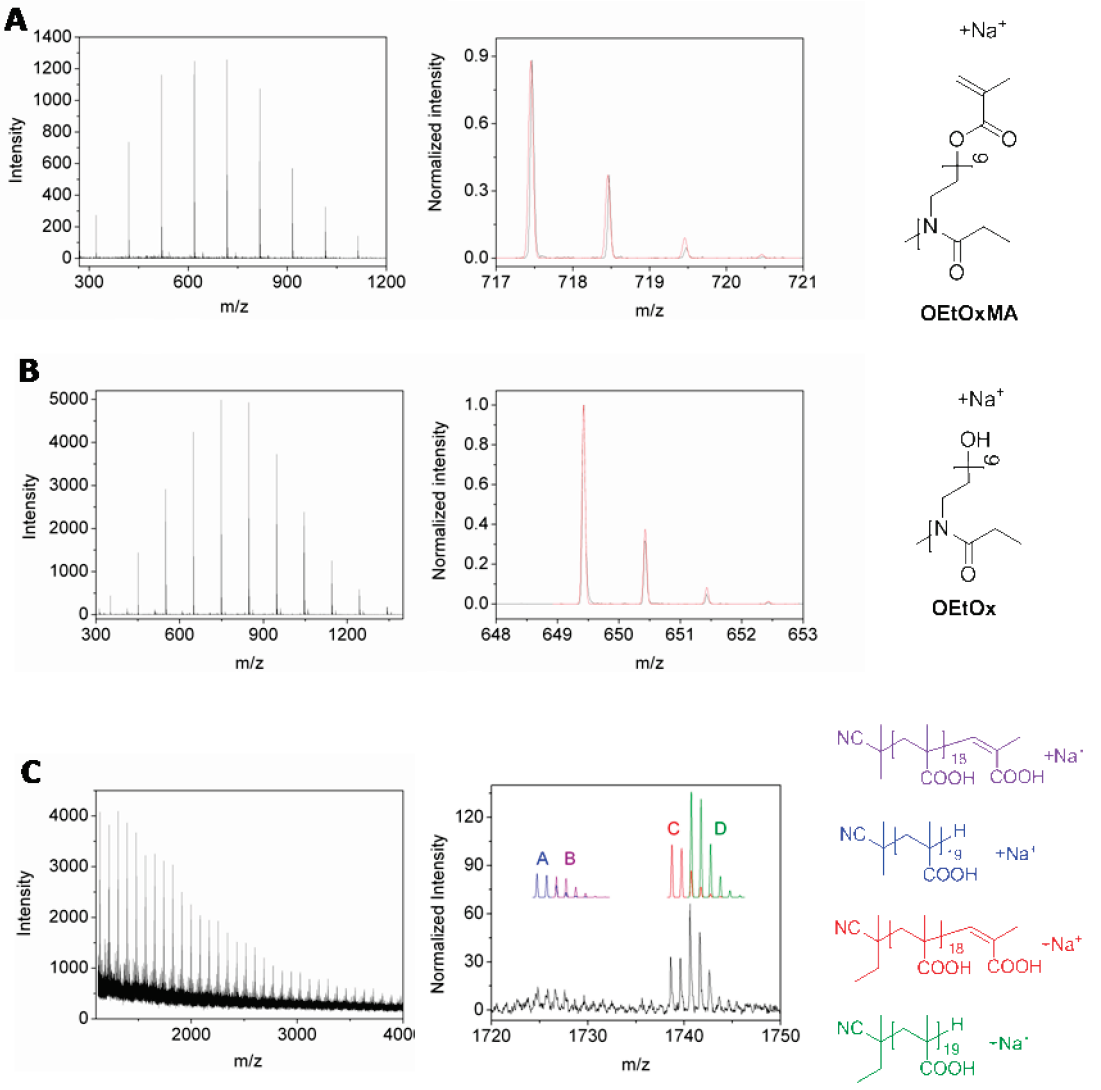


Figure 1. Representative MALDI-TOF mass spectra (left) and expanded regions showing a comparison of experimental (black) and calculated (colored) isotopic patterns (center) of the assigned structures (right). (A) OEtOxMA macromonomer **M1**; (B) OEtOx side chains of **P9** after addition of a drop of water; (C) PMAA backbone **B2**.

capped microwave vial containing the living oligomeric oxazolinium species obtained as described in the CROP of EtOx section. This mixture was heated to 80 °C for 15 h. The resulting grafting products were purified by precipitation into diethyl ether or into a mixture of diethyl ether and chloroform (2:1) and a second precipitation from DMF at pH = 4 into diethyl ether.

Macromonomer Synthesis. The synthesis of the OEtOxMA macromonomer **M1** was carried out according to our previously reported method.[27] **M1**: $DP_{(1H\text{-}NMR)}$ = 5.0; $M_{n(SEC)}$ = 660 g mol^{-1}; PDI(SEC) = 1.15.

*Copolymerization of **M1** with Methacrylic Acid*. The RAFT polymerization was carried out at 70 °C for 18 h in a similar manner as described for the homopolymerization of MAA. The overall concentration of the monomer was set to 0.5 mol L^{-1}, and the ratio of [monomer]:[CBDB]:[AIBN] was 60:1:0.25. The used amounts of MAA and **M1** were varied according to the ratios given in Table 2. The resulting pink graft copolymers were precipitated into a 2:1 mixture of diethyl ether with chloroform.

Results and Discussion

Macromonomer Method (Route A, Scheme 1). We have recently demonstrated the possibility to obtain well-defined OEtOxMA macromonomers by microwave-assisted polymerization of EtOx using MeTos as initiator and subsequent end-capping of the living oligomeric oxazolinium species with in situ formed triethylammonium methacrylate.[27] Macromonomer **M1** was synthesized according to this procedure and has a low PDI value of 1.15 and reveals a single distribution in the MALDI-TOF mass spectrum (see Figure 1A). Each polymer chain is cationized by a sodium ion and bears the desired methyl and methacrylate end groups. The masses that were experimentally found are compared to the calculated ones in Table 1 and are within the mass accuracy of the used TOF analyzer.

The degree of functionalization with a methacrylate unit was determined to be 97% by comparison of the integral ratios of the appropriate peaks in the ^{1}H NMR spectrum, and the degree of polymerization was found to be 5.0.

This OEtOxMA macromonomer was copolymerized with varying amounts of methacrylic acid using the RAFT polymerization technique yielding graft copolymers **P1**−**P3** with a poly(methacrylic acid) backbone and OEtOx side chains. The RAFT polymerization was carried out in a 0.5 M solution in ethanol at 70 °C using CBDB as chain transfer agent, AIBN as initiator, and anisole as internal standard. For all radical polymerizations, the ratio of [CBDB] to

Table 1. Assignment of the Observed Peaks in the MALDI-TOF Mass Spectra of the Used Building Blocks

	structure	*m/z* calc	*m/z* found
OEtOxMA **M1**	$CH_3(C_5H_9NO)_6C_4H_5O_2 + Na^+$	717.45	717.47
		718.46	718.47
		719.46	719.48
OEtOx side chains **P9**	$CH_3(C_5H_9NO)_6C_4H_5O_2 + Na^+$	649.43	649.42
		650.43	650.42
		651.43	651.43
PMAA **B2**	$C_4H_6N(C_4H_6O_2)_{18}C_4H_5O_2 + Na^+$	1724.73	1724.82
		1725.73	1725.84
		1726.74	1726.64
		1727.74	1727.60
	$C_4H_6N(C_4H_6O_2)_{19}H + Na^+$	1726.75	1726.64
		1727.75	1727.60
		1728.75	1728.75
		1729.76	
	$C_5H_8N(C_4H_6O_2)_{18}C_4H_5O_2 + Na^+$	1738.75	1738.64
		1739.75	1739.63
		1740.75	1740.65
		1741.76	1741.65
		1742.76	
	$C_5H_8N(C_4H_6O_2)_{19}H + Na^+$	1740.76	1740.65
		1741.77	1741.65
		1742.77	1742.66
		1743.77	1743.66
		1744.77	

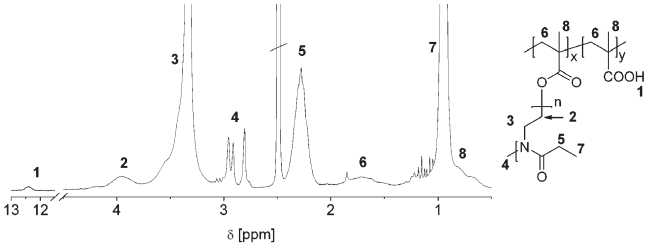


Figure 2. ^{1}H NMR spectrum (250 MHz, DMSO-d_6) of **P2** and assignment of the proton signals to the schematic representation of the polymeric structure.

[AIBN] was 4 to 1 based on our previous optimization studies,[34] and the overall [monomer] to [CBDB] ratio was 60 to 1 whereas the ratio of the two monomers [OEtOxMA] to [MAA] was varied (2:1; 1:2; 1:3). The monomer conversions were determined by comparison of the integral ratios of the vinylic proton signals of the two monomers with the integral of the anisole peaks in the ^{1}H NMR spectra of the reaction solution before and after the polymerization. The conversions of both monomers were in the range of 30%−40% in all cases, hinting at the fact that MAA and OEtOxMA are incorporated into the polymer in an equal rate. This observation is supported by the ^{1}H NMR spectra (see Figure 2) of the purified polymers that indeed revealed a ratio of OEtOxMA to MAA units in the graft copolymer that is close to the used feed ratio.

The ^{1}H NMR spectra (DMSO-d_6, see Figure 2) of the graft copolymers are dominated by the signals that belong to the large amount of protons in the OEtOx side chains. Nevertheless, the grafting density can be estimated by comparison of the integrals of the −COOH signal belonging to the methacrylic acid segments at 12.5 ppm with the integral of the OEtOx side chain signals.

The SEC traces of the graft copolymers (see Figure 3) reveal narrow molar mass distributions with PDI values below 1.3 as commonly observed for controlled radical polymerizations as well as a quantitative removal of the

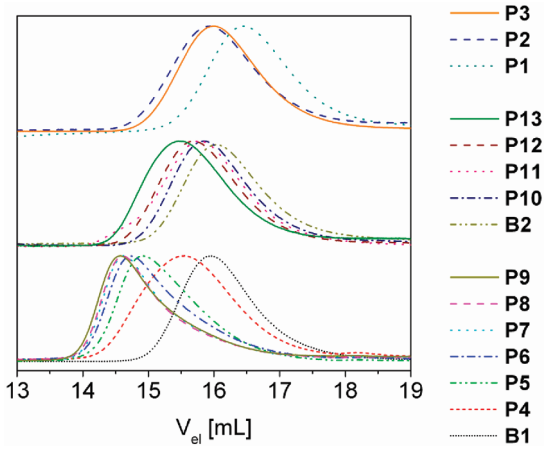


Figure 3. Normalized SEC traces (DMA/LiCl, RI detection) of the synthesized grafting products. Top: graft copolymers **P1**−**P3** synthesized via the macromomomer method. Center: graft copolymers **P10**−**P13** synthesized by grafting-onto **B2**. Bottom: graft copolymers **P4**−**P9** synthesized by grafting-onto **B1**.

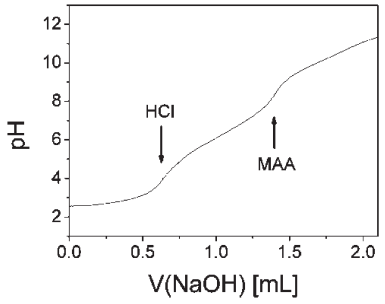


Figure 4. Determination of the acid content of **P3** by acid/base titration (0.1 M sodium hydroxide solution, flow rate 0.05 mL min^{-1}).

residual macromonomer. The low molar mass tailing in some samples might indicate nonuniform grafting. Unfortunately, an accurate determination of the molar mass of the polymers using a MALLS detector failed due to the relatively low molar mass of the polymers. Therefore, the molar masses are currently investigated in detail by analytical ultracentrifugation (AUC).

A method that is very sensitive toward the presence of acidic functions is an acid/base titration. For this purpose a known amount of polymer was dissolved in water and a 0.1 M HCl solution was added in order to ensure a complete protonation of all MAA groups of the polymer. This solution was titrated against a 0.1 M NaOH solution. The titration curve of **P3** (see Figure 4) reveals a first inflection point upon neutralization of the excess of HCl and a second one upon neutralization of MAA. The amount of NaOH that was added between these two inflection points corresponds to the amount of MAA groups that are present in the graft copolymer. The resulting grafting densities of **P1**−**P3** are in good agreement with the data obtained from conversion of the two monomers as well as the data obtained from ^{1}H NMR spectroscopy (see Table 2).

The characterization data obtained by SEC (DMA, LiCl), ^{1}H NMR spectroscopy (DMSO-d_6), and acid/base titration of the synthesized copolymers are summarized in Table 2.

Grafting-Onto Method (Route B). In contrast to the macromonomer method, which starts with the synthesis of the side chains of the graft copolymer, the first step involved in the grafting-onto technique is the synthesis of the PMAA backbone via RAFT polymerization. The polymerization of

Table 2. Overview of the Characterization Results of the POEtOx-*g*-MAA Graft Copolymers P1−P3 Synthesized by Copolymerization of the OEtOxMA Macromonomer M1[a] (Route A)

	[M1]:[MAA] (feed)	conv[b] [M1]:[MAA] [%]	DP[c] [M1]:[MAA]	M_n[d] [g mol^{-1}]	PDI[d]	% grafting ^{1}H NMR	% grafting titration	[MAA]:[EtOx]
P1	20:40	21:11	4:4	5500	1.29	38	33	1:2.5
P2	40:20	34:38	14:8	8200	1.26	60	62	1:8.2
P3	15:45	32:30	5:14	8000	1.23	28	26	1:1.8

[a] $DP_{(1H\text{-}NMR)}$ = 5; $M_{n(SEC)}$ = 700 g mol^{-1}; $PDI_{(SEC)}$ = 1.15. SEC measured in $CHCl_3$, PS calibration. [b] Conversion calculated from ^{1}H NMR, addition of anisole as standard. [c] Degree of polymerization calculated from the used ratio of [CBDB] to [monomer] and the conversion. [d] Obtained from SEC (DMA/LiCl, PMMA calibration).

Table 3. Overview of the Characterization Results of the POEtOx-*g*-MAA Graft Copolymers P4−P9 Synthesized via Grafting onto B1[a] (Route B)

	M_n[b] [g mol^{-1}]	PDI[b]	% grafting theor	% grafting titration	[MAA]:[EtOx]
P4	10 500	1.32	20	22	1:1.4
P5	14 800	1.28	40	45	1:4.1
P6	16 300	1.29	60	66	1:9.7
P7	17 900	1.28	80	77	1:16.7
P8	18 000	1.29	100	88	1:36.7
P9	18 700	1.28	5 × excess	92	1:57.7

[a] [MAA]:[CBDB] = 60; $conv_{(HNMR)}$ = 63%; DP = 38; $M_{n(SEC)}$ = 7700 g mol^{-1}; $PDI_{(SEC)}$ = 1.27. [b] Obtained from SEC (DMA/LiCl, PMMA calibration).

Table 4. Overview of the Characterization Results of the POEtOx-*g*-MAA Graft Copolymers P10−P13 Synthesized via Grafting onto B2[a] (Route B)

	M_n[b] [g mol^{-1}]	PDI[b]	% grafting theor	% grafting titration	[MAA]:[EtOx]
P10	7800	1.32	20	7	1:0.4
P11	9300	1.31	30	22	1:1.4
P12	9000	1.29	40	12	1:0.7
P13	11100	1.24	50	25	1:1.7

[a] [MAA]:[CBDB] = 60; $conv_{(HNMR)}$ = 45%; DP = 28; $M_{n(SEC)}$ = 7000 g mol^{-1}; $PDI_{(SEC)}$ = 1.28. [b] Obtained from SEC (DMA/LiCl, PMMA calibration).

MAA was carried out using the same chemicals in equal ratios that were used during the RAFT via route A. The fact that no macromonomers are involved in the polymerization process enables the use of a higher monomer concentration (2.0 mol L^{-1}) leading to shorter reaction times (8 h) when compared to route A. Using these synthetic conditions, two PMAA backbones **B1** and **B2** were synthesized and characterized by means of SEC and ^{1}H NMR spectroscopy. The PDI values that were determined from SEC measurements are below 1.3, and the monomer conversions were determined to be 63% and 45%, respectively (^{1}H NMR, anisole standard). An advantage of route B is the possibility to characterize the backbone of the future graft copolymer in detail by MALDI-TOF mass spectrometry (DCTB, NaI, see Figure 1C). The mass spectrum shows in this case a mass discrimination effect but enables the analysis of the polymer end groups. The main distribution can be assigned to polymer chains that bear the initiating group of the RAFT agent CBDB and that are ionized by a sodium cation. During the MALDI process, the dithioester end group derived from the RAFT agent is cleaved, resulting in two kinds of end groups: a saturated and an unsaturated one.[35] Most likely, this is caused by fragmentation of the RAFT end group during the ionization process since MALDI is a relatively tough ionization method, in particular when compared to electrospray ionization MS. The schematic representations of the ionized species resulting thereof are depicted in Figure 1C. Because of the m/z difference of 2, the isotopic patterns of these distributions partially overlap. Table 1 demonstrates that the deviation of the calculated and observed masses is within the experimental accuracy of the employed mass analyzer. A second polymer distribution can be found with m/z = 14 lower values than the described one resulting from AIBN instead of RAFT agent initiated chains.[34]

In order to obtain graft copolymers with different grafting densities but with the same length of the side chains, a stock solution was prepared for the CROP using MeTos as initiator and EtOx as monomer ([M]/[I] = 5) in a 4.0 M solution in acetonitrile. The polymerization was carried out until full monomer conversion in separate vials for 1 min at 140 °C using microwave irradiation as heating source. Because of the living nature of the CROP, the resulting solutions contain OEtOx oligomers with still active oxazolinium end groups and tosylate as counterion. A drop of water was added to one of these reaction solutions for characterization of the side chains before the grafting-onto process. The MALDI-TOF mass spectrum (see Figure 1B and Table 1) shows a single distribution that can be assigned to OEtOx with a methyl end group that is derived from the initiator and a hydroxyl end group resulting from the termination with water.

The final step of the grafting onto method is the connection of the side chains to the polymer backbone via direct end-capping of the living OEtOx chains with deprotonated PMAA. Therefore, varying amounts of solutions of PMAA, which was deprotonated using triethylamine as base, in DMF were added directly to each microwave vial and heated to 80 °C for 15 h. Using this synthetic procedure, the graft copolymers **P4−P13** were synthesized with a theoretical grafting density from 20% to 100%. The resulting SEC traces after purification of the polymers by precipitation are shown in Figure 3, and the values of M_n and PDI are provided in Tables 3 and 4. The advantage of this grafting-onto method is the possibility to characterize the used backbone and side chain units separately and, with respect to the future comparison of the polymer properties, the assurance of a constant backbone length. However, the challenge in this method is the determination of the grafting density.

The SEC traces that were taken from the reaction solutions of **P4−P8** reveal an almost quantitative disappearance of the OEtOx side chains after the grafting-onto process hinting toward a high grafting efficiency even for a feed

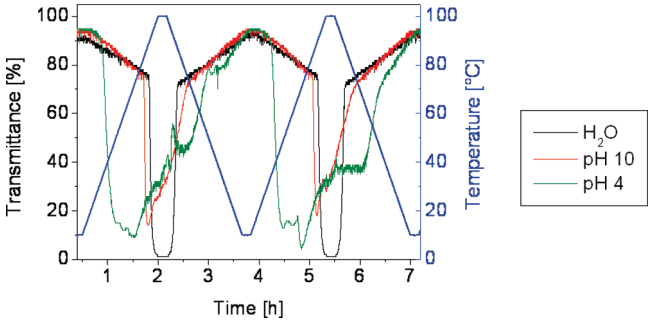


Figure 5. Turbidity curves for the determination of the cloud points of **P6** in water as well as in buffered solutions at pH 4 and pH 10, respectively.

grafting density of 80%. On the other hand, the interpretation of the ^{1}H NMR spectra is a difficult task for the polymers with higher grafting densities since the spectra are dominated by the large amount of protons in the OEtOx side chains. In addition, some of the MAA groups of the graft copolymers might still exist in a deprotonated form or could be involved in hydrogen bonding. Since the acid/base titration method provided reliable results for **P1**−**P3**, it was applied for the determination of the grafting densities of the polymers prepared by route B. The resulting data are presented in Tables 3 and 4 and show that a maximum degree of grafting of 92% could be obtained with a 5-fold excess of OEtOx in the case of **P9**. The high sterical hindrance of the already attached side chains presumably prevents full grafting of the backbone. However, comb polymers where every single methacrylate unit of the backbone is grafted with a OEtOx side chain can easily be obtained using the macromonomer approach,[26] and the advantage of the grafting-onto method is the same backbone length of all graft copolymers that were synthesized from the same batch of PMAA backbone.

Solubility of the Graft Copolymers in Aqueous Media. In order to investigate the solubility behavior of the synthesized polymers in aqueous media at different temperatures, turbidity measurements at a wavelength of 500 nm were performed using a polymer concentration of 5 mg mL^{-1} and a heating ramp of 1 K min^{-1}. Since the polymers contain acid functions, the measurements were carried out in deionized water as well as under acidic and basic conditions. At a pH value of 4 the methacrylic acid moieties of the polymer should be protonated whereas they should exist in a deprotonated form at a pH value of 10. A typical result from those turbidity measurements is shown in Figure 5 for **P6**.

At the beginning of the measurement the polymer is dissolved due to the formation of hydrogen bonds with the surrounding water molecules. Upon increase in temperature these bonds are weakened, and when the cloud point of the solution is reached, the polymer precipitates from the solution, resulting in a decrease of transmittance. As soon as the mixture is cooled below the cloud point, the polymer redissolves and the transmittance increases again. The transmittance drops rapidly when the polymer precipitates in all investigated solutions. However, in some of the systems the polymers form larger sticky particles leading to an unexpected increase in transmittance after the cloud point and a slower redissolution of the polymer upon cooling. The reversibility of this LCST behavior is proven by repeating such a heating−cooling cycle twice. The results of the solubility screening are depicted in Figure 6A.

The solubility behavior of the polymers with a lower grafting density, which were synthesized via grafting-onto

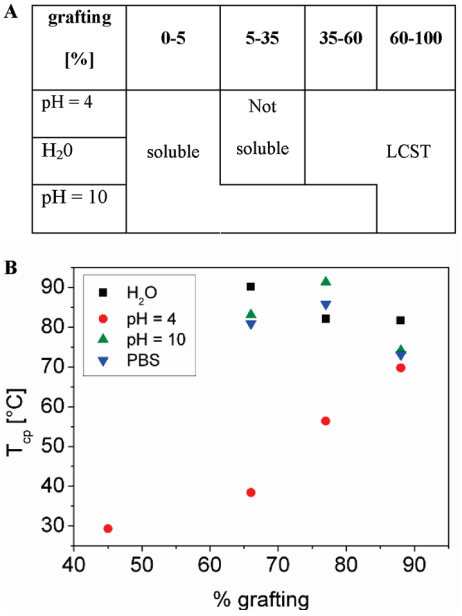


Figure 6. (A) Results of the solubility screening of the graft copolymers in aqueous media. (B) Detected cloud points for aqueous solutions of the POEtOx−MAA graft copolymers (c = 5 g L^{-1}, heating rate 1 K min^{-1}).

B2, is dominated by the large amount of carboxylic acid within the polymer. The polymer with the lowest grafting density (7%) **P10** remains soluble at all investigated pH values and temperatures. This observation can be ascribed to the fact that the solubility of the PMAA backbone (which also does not show an LCST behavior) is very little affected by the small amount of incorporated OEtOx. **P11**−**P13** (with grafting densities from 20% to 35%) show similar characteristics in basic media, where the deprotonated acidic moieties cause such a high hydrophilicity of the polymer that it remains soluble until 100 °C. In contrast, the protonated MAA functions seem to "occupy" all hydrogen bond accepting binding sites of the OEtOx in such a strong way that the graft copolymers are not soluble in water and buffered solution at a pH value of 4 anymore. This hypothesis is supported by the calculated ratios of [MAA]:[EtOx] in the polymer (see Tables 3 and 4) that are indeed close to one. A similar explanation can be applied to the solubility behavior of **P5**, where the deprotonated acidic functions provide solubility in basic medium whereas the protonated acidic functions cause LCST behavior of the polymer in acidic medium.

In contrast **P6**−**P8**, which were synthesized via grafting-onto **B1**, have a much lower content of methacrylic acid and exhibit LCST behavior in all aqueous solutions. Figure 6B illustrates the dependence of the cloud points of the aqueous polymer solutions on the grafting density of the polymers. In the range from 60% to 100% grafting density, the cloud point of the solutions containing the deprotonated form of the polymer increases at pH 10 and in water slightly with increasing content of acidic functions and, thus, decreasing grafting density. This increasing of the cloud points is most likely due to the fact that the more hydrophilic deprotonated acidic moieties in the polymer enhance the solubility of the entire graft copolymer. However, the observed effect occurs only in a temperature range of less than 10 °C and might also be influenced by the presence of cations and anions in solution due to the use of buffers. To evaluate the effect of a buffered solution on the cloud point, the measurements were also performed in phosphate buffered saline (PBS). Indeed, the cloud points in PBS deviate from the values in

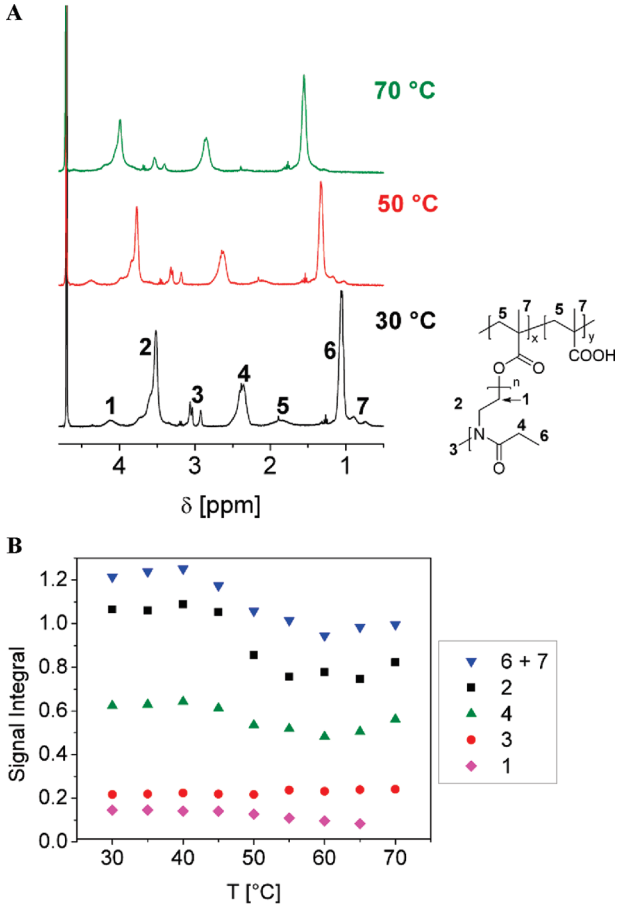


Figure 7. (A) ^{1}H NMR spectra of **P2** in D_2O at different temperatures. (B) Integral values of the distinct polymer signals after normalization according to the residual solvent signal.

deionized water in a similar temperature range, obstructing a conclusive interpretation of the somewhat scattered cloud points of the deprotonated polymers with 60%−100% grafting density. Obviously, the solubility of the graft copolymers is affected in a stronger way in acidic media (pH 4) when the MAA groups of the polymer are protonated. In this state the MAA moieties serve as hydrogen bond donating units to form hydrogen bonds with the OEtOx chain and, thus, lower the energy gain for the formation of hydrogen bonds with surrounding water molecules. Therefore, the cloud points in buffered solutions at pH 4 are tremendously decreased when compared to those at higher pH values. Apparently, an increasing amount of MAA functions in the polymer intensifies this effect as the cloud points decrease further with lower grafting densities of the polymer.

These findings comply with the properties of other pH- and thermoresponive polymers such as copolymers of oligo(ethylene glycol)methacrylate (OEGMA) and MAA where the LCST behavior of the P(OEGMA) polymers could be tuned in a similar way by the incorporation of MAA.[36,37] In addition, pH sensitivity has been introduced into the most popular thermoresponsive polymer PNiPAm by various copolymerizations with acidic monomers[38−40] either offering the possibility to trigger its drug delivery[41] as well as for the stabilization of gold nanoparticles[42] or emulsions[43] by changes of the pH values.

The polymers **P1**−**P3** that were synthesized via the macromonomer method (route A) also show an LCST behavior in water (T_{cp} = 17.0, 53.6, and 8.2 °C, respectively) and buffered solutions at pH 4 (T_{cp} = 27.1, 50.6, and 13.6 °C,

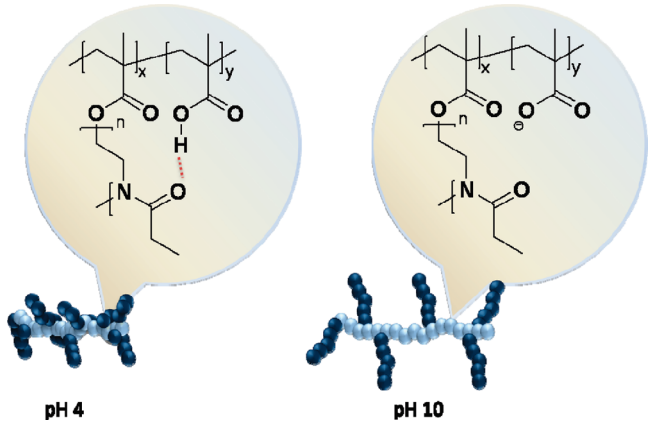


Figure 8. Schematic representation of the polymer structure under acidic and basic conditions indicating possible hydrogen bond binding sites.

respectively). However, these results are difficult to interpret due to the fact that the backbone length varies, which is known to have an effect on the LCST behavior of the graft copolymers.[26]

The LCST behavior of **P2** was further investigated by ^{1}H NMR spectroscopy in deuterium oxide at different temperatures below and above the cloud point of the solution (5 mg mL^{-1}) at 48 °C. The measurements were performed in a temperature range from 30 to 70 °C in steps of 5 °C using the residual solvent resonance as an internal standard. Selected ^{1}H NMR spectra are shown in Figure 7A.

Since it is known that the chemical shift of the H_2O signal in the ^{1}H NMR spectrum is dependent on the measurement temperature, TSP was added as standard for the measurements at 30 °C as well as at 70 °C. Indeed, the residual water signal was shifted by the amount of 0.5 ppm when compared to the signal of TSP, which is known to be unaffected by the measurement temperature. As a result, the observed temperature dependence of the chemical shift of the polymer signals is artificially caused by the temperature dependence of the chemical shift of the H_2O signal. However, the measurements that were evaluated were performed without TSP using the H_2O signal as internal reference since it is known that various additives can strongly affect the cloud point of a polymer solution. Surprisingly, the raise of the temperature above the cloud point of the solution did not lead to a complete disappearance and broadening of all the polymer signals in the spectra hinting toward the fact that some parts of the polymer remain mobile and solvated. Nevertheless, a general decrease of the integrals of the polymer signals after normalization according to the H_2O signal could be observed. The resulting values for the individual polymer signals are plotted as a function of temperature in Figure 7B. Neither do the signal integrals decrease in a linear way nor in the whole investigated temperature range. Changes in the polymer structure seem to occur between 45 and 55 °C, the temperature region where the cloud point of the solution was determined via turbidity measurements. Interestingly, not the entire polymer structure seems to be affected by the phase transition as the CH_3− signal of the methyl groups that can be found at the end of the OEtOx side chains remain constant. On the other hand, the signals that belong to the OEtOx parts close to the backbone as well as the backbone signals are significantly broadened causing them to disappear within the baseline, indicating a collapsed polymer structure in that region. These findings support the fact that hydrogen bonding between MAA

groups and amide hydrogen bond accepting moieties of the OEtOx induce the coil-to-globule transition of the copolymers as illustrated in Figure 8. A similar behavior of graft copolymers containing a PNiPAm backbone and mobile poly(vinylpyridine) side chains has been reported by Rusu et al.[44]

Conclusion

Graft copolymers containing a PMAA backbone and a varying amount of OEtOx side chains could be synthesized using two different synthetic approaches, the macromonomer method and a grafting-onto process, combining the CROP of EtOx and the RAFT polymerization technique in each of the two pathways.

These polymers contain both hydrogen bond donating and accepting moieties in varying ratios revealing pH- as well as temperature-sensitive solubility properties in aqueous media. The hydrogen bonds that are formed inside the polymer compete with hydrogen bonding between the polymer and the solvent. Thus, the polymers with lower grafting density are not soluble at pH 4 but are soluble over the entire temperature range at pH 10, whereas the polymers with lower MAA content show an LCST behavior under acidic as well as basic conditions. The cloud points of these solutions are lowered with increasing MAA content due to the formation of an increased number of hydrogen bonds inside the polymer structure and can be varied in a temperature range from 8 to 90 °C by adjustment of the polymer composition and the pH of the solution.

^{1}H NMR measurements revealed a signal broadening at the cloud point of the polymer solution in D_2O but also showed that some parts of the polymer side chains still remain mobile even above the cloud point. These results indicate that the EtOx units close to the PMAA backbone collapse due to hydrogen bonding while the outer part of the OEtOx chain remains in solution.

The pH- and temperature-responsive behavior of the graft copolymers that is widely tunable opens a new avenue toward special biomedical applications, particularly due to the fact that the side chains still remain mobile as well as solvated and therefore can be used for targeted drug delivery of these systems, e.g., via attachment of suitable sugar moieties.

Acknowledgment. This work forms part of the research program of the Dutch Polymer Institute (DPI), project number 612. The authors thank the Fond der chemischen Industrie (FCI) and the Thüringer Kultusministerium for the financial support of this study.

References and Notes

(1) Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci.* **2004**, *29*, 1173–1222.
(2) Dimitrova, I.; Trzebickab, B.; Müller, A. H. E.; Dworak, A.; Tsvetanova, C. B. *Prog. Polym. Sci.* **2007**, *32*, 1275–1343.
(3) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163–249.
(4) Rzaev, Z. M. O.; Dinçer, S.; Piskin, E. *Prog. Polym. Sci.* **2007**, *32*, 534–595.
(5) Aoshima, S.; Kanaoka, S. *Adv. Polym. Sci.* **2008**, *210*, 169–208.
(6) Eggenhuisen, T. M.; Becer, C. R.; Fijten, M. W. M.; Eckardt, R.; Hoogenboom, R.; Schubert, U. S. *Macromolecules* **2008**, *41*, 5132–5140.
(7) Meyer, M.; Antonietti, M.; Schlaad, H. *Soft Matter* **2007**, *3*, 430–431.
(8) Park, J.-S.; Akiyama, Y.; Winnik, F. M.; Kataoka, K. *Macromolecules* **2004**, *37*, 6786–6792.
(9) Diab, C.; Akiyama, Y.; Kataoka, K.; Winnik, F. M. *Macromolecules* **2004**, *37*, 2556–2562.
(10) Hoogenboom, R.; Thijs, H. M. L.; Wouters, D.; Hoeppener, S.; Schubert, U. S. *Soft Matter* **2008**, *4*, 103–107.
(11) Park, J.-S.; Kataoka, K. *Macromolecules* **2006**, *39*, 6622–6630.
(12) Huber, S.; Jordan, R. *Colloid Polym. Sci.* **2008**, *286*, 395–402.
(13) Hoogenboom, R.; Thijs, H. M. L.; Jochems, M. J. H. C.; van Lankvelt, B. M.; Fijten, M. W. M.; Schubert, U. S. *Chem. Commun.* **2008**, 5758–5760.
(14) Diehl, C.; Schlaad, H. *Macromol. Biosci.* **2009**, *9*, 157–161.
(15) Christova, D.; Velichkova, R.; Loos, W.; Goethals, E .J.; Du Prez, F. *Polymer* **2003**, *44*, 2255–2261.
(16) Lin, P.; Clash, C.; Pearce, E. M.; Kwei, T. K. *J. Polym. Sci., Part B: Polym. Phys.* **1988**, *26*, 603–619.
(17) Hoogenboom, R. *Angew. Chem., Int. Ed.* **2009**, *48*, 7978–7994.
(18) Hoogenboom, R. *Macromol. Chem. Phys.* **2007**, *208*, 18–25.
(19) Adams, N.; Schubert, U. S. *Adv. Drug Delivery Rev.* **2007**, *59*, 1504–1520.
(20) Litt, M.; Levy, A.; Herz, J. *J. Macromol. Sci., Pure Appl. Chem.* **1975**, *A9*, 703–727.
(21) Kobayashi, S. *Prog. Polym. Sci.* **1990**, *15*, 751–823.
(22) Aoi, K.; Okada, M. *Prog. Polym. Sci.* **1996**, *21*, 151–208.
(23) Kobayashi, S.; Uyama, H. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 192–209.
(24) Hoogenboom, R. In *Handbook of Ring-Opening Polymerization*; Dubois, P., Degée, P., Coulembier, O., Raquez, J.-M., Eds.; Wiley-VCH: Weinheim, 2009; Chapter 6.
(25) Moad, G.; Rizzardo, E.; Thang, S. H. *Polymer* **2008**, *49*, 1079–1131.
(26) Weber, C.; Becer, C. R.; Hoogenboom, R.; Schubert, U. S. *Macromolecules* **2009**, *42*, 2965–2971.
(27) Weber, C.; Becer, C. R.; Hoogenboom, R.; Baumgaertel, A.; Schubert, U. S. *Des. Monomers Polym.* **2009**, *12*, 149–165.
(28) Poe, G. D. M.; McCormick, C. L. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 2520–2533.
(29) Alberti, A.; Benaglia, M.; Laus, M.; Sparnacci, K. *J. Org. Chem.* **2002**, *67*, 7911–7914.
(30) Wiesbrock, F.; Hoogenboom, R.; Schubert, U. S. *Macromol. Rapid Commun.* **2004**, *25*, 1739–1764.
(31) Hoogenboom, R.; Schubert, U. S. *Macromol. Rapid Commun.* **2007**, *28*, 368–386.
(32) Wiesbrock, F.; Hoogenboom, R.; Leenen, M.; van Nispen, S. F. G. M.; van der Loop, M.; Abeln, C. H.; van den Berg, A. M. J.; Schubert, U. S. *Macromolecules* **2005**, *38*, 7957–7966.
(33) Hoogenboom, R.; Wiesbrock, F.; Leenen, M. A. M.; Meier, M. A. R.; Schubert, U. S. *J. Comb. Chem.* **2005**, *7*, 10–13.
(34) Fijten, M. W. M.; Meier, M. A. R.; Hoogenboom, R.; Schubert, U. S. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 5775–7583.
(35) Jiang, X.; Schoenmakers, P. J.; van Dongen, J. L. J.; Lou, X.; Lima, V.; Brokken-Zijp, J. *Anal. Chem.* **2003**, *75*, 5517–5524.
(36) Becer, C. R.; Hahn, S.; Fijten, M. W. M.; Thijs, H. M. L.; Hoogenboom, R.; Schubert, U. S. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 7138–7147.
(37) Jones, J. A.; Novo, M.; Flagler, K.; Pagnucco, C. D.; Carew, S.; Cheong, C.; Kong, X. Z.; Burke, N. A. D.; Stöver, H. D. H. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 6095–6104.
(38) Huang, J.; Wu, X. Y. *J. Polym. Sci., Part A: Polym. Chem.* **1999**, *37*, 2667–2676.
(39) Velada, J. L.; Liu, Y.; Huglin, M. B. *Macromol. Chem. Phys.* **1998**, *199*, 1127–1134.
(40) Dai, S.; Ravi, P.; Tam, K. C. *Soft Matter* **2008**, *4*, 435–449.
(41) Bertrand, N.; Fleischer, J. G.; Wasan, K. M.; Leroux, J.-C. *Biomaterials* **2009**, *30*, 2598–2605.
(42) Nuopponen, M.; Tenhu, H. *Langmuir* **2007**, *23*, 5352–5357.
(43) Brugger, B.; Richtering, W. *Langmuir* **2008**, *24*, 7769–7777.
(44) Rusu, M.; Wohlrab, S.; Kuckling, D.; Moehwald, H.; Schoenhoff, M. *Macromolecules* **2006**, *39*, 7358–7363.