Synthesis and Characterization of Thermoresponsive Graft Copolymers of NIPAAm and 2-Alkyl-2-oxazolines by the "Grafting from" Method

Juan Rueda,[§] Stefan Zschoche,[†] Hartmut Komber,[†] Dirk Schmaljohann,[‡] and Brigitte Voit*,[†]

Leibniz Institute of Polymer Research Dresden, Hohe Strasse 6, D-01069 Dresden, Germany, and Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff, CF10 3XF, Wales UK

Received March 17, 2005; Revised Manuscript Received June 15, 2005

ABSTRACT: New thermoresponsive graft copolymers were synthesized by the cationic ring-opening polymerization of 2-methyl-2-oxazoline (MeOxa) or 2-ethyl-2-oxazoline (EtOxa), initiated by the random copolymers of chloromethylstyrene (CMS) and *N*-isopropylacrylamide (NIPAAm) using the "grafting from" method with a yield of 66−94%. The polymers were characterized by NMR, GPC, and DSC, and the conformational transition (lower critical solution temperature, LCST) of macroinitiators and graft copolymers was determined by the turbidity and DSC measurements. The transition temperature of the graft copolymers could be fine-tuned through the composition of the macroinitiator and the graft copolymer. An increasing quantity of the hydrophobic comonomer chloromethylstyrene in the macroinitiator lowered its LCST, while in the graft copolymer an increasing content of the hydrophilic segment of poly(2-methyl-2-oxazoline) or poly(2-ethyl-2-oxazoline) raised the transition temperature. For graft copolymers with a high content of long poly(2-alkyl-2-oxazoline) grafts, stabilized aggregates with a thermoresponsive core can be formed at the LCST instead of precipitation of the material.

Introduction

Polymers are finding increasing applications in the area of biomaterials. Polymer therapeutics[1] and synthetic polymeric scaffolds for tissue engineering[2,3] are only two areas, where a particular design of polymer properties can lead to materials with advantageous response to e.g. injured or dysfunctional tissue. The use of stimuli-responsive polymers and hydrogels is of particular interest because it allows the design of material which responds to the change in environmental conditions by an abrupt change in physicochemical properties.[4,5] The homopolymers and copolymers of poly(*N*-isopropylacrylamide) (PNIPAAm) show a conformational transition or volume phase transition in a short interval of temperature, called lower critical solution temperature (LCST), which changes drastically the physical and chemical properties.[6,7] Thus, in aqueous solution, PNIPAAm shows a sudden coil-to-globule transition upon heating, which leads to a precipitation or dispersion of the polymer.[8,9] The LCST of the PNIPAAm is with 32 °C near to the human body temperature so that one can take advantage of this fact to design materials which can be used in living systems as biomaterials, such as thermoresponsive cell culture carriers and thermogelling tissue scaffolds.[10−13]

The LCST of PNIPAAm can be varied by the copolymerization of the NIPAAm monomer with hydrophilic or hydrophobic comonomers[6,14−22] as well as through addition of electrolytes or a cosolvent.[23,24] The variation of the transition temperature LCST in a relatively wide range allows the application of this material not only as biomaterial but also in industrial systems of rheology control, membranes, valves, and sensors.[16]

The 2-oxazolines are heterocyclic substances (endo iminoethers) whose polymerization has been researched intensively.[25−28] The 2-oxazolines can be polymerized through a ring-opening cationic polymerization producing poly(*N*-acylalkyl imines), and this polymerization is started by electrophilic species such as methyl triflate, methyl tosylate, alkyl halides, and Lewis and Brönsted acids. Under appropriate reaction conditions, such as monomers, initiators, and solvents, pure and dry, the polymerization of the 2-oxazolines shows a behavior of "living" type, such as linear time−conversion relation and the potential to synthesize block copolymers. In this way, from the beginning of the polymerization not only the degree of polymerization and its polydispersity but also the functionality of the polymeric chains can be predefined. Thus, different macromolecular architectures, such as block and graft copolymers, telechelics, and star polymers, can be easily obtained.[29−34]

Polyoxazolines show hydrophilic or hydrophobic character depending on the nature of acyl group. For example, poly(2-methyl-2-oxazoline) (polyMeOxa) and poly(2-ethyl-2-oxazoline) (polyEtOxa) are soluble in water, whereas polyoxazolines with longer aliphatic chains and aromatic or fluorinated groups show solubility only in organic solvents. However, the poly(2-ethyl-2-oxazoline) and its copolymers already show an LCST behavior over a wide temperature range, indicating an increasing hydrophobicity compared to that of poly(2-methyl-2-oxazoline).[35,36] Recently, poly(2-ethyl-2-oxazoline)-containing hydrogels have also been suggested as temperature-sensitive biomaterials.[37]

Through the use of the living characteristics of the polymerization and by varying the oxazoline monomer structure various functional polymeric materials, such as polymeric nonionic surfactants, compatibilizers, hydrogels, stabilizers for dispersion polymerization, bio-

[†] Leibniz Institute of Polymer Research Dresden.
[‡] Cardiff University.
[§] Present address: Laboratorio de Polímeros, Sección Física, DAI, Pontificia Universidad Católica del Perú, Box. 1761, Lima, Perú.
* To whom correspondence should be addressed: Ph ++49 351 4658 591; Fax ++49 351 4658 565; e-mail voit@ipfdd.de.

10.1021/ma050570p CCC: $30.25
Published on Web 07/27/2005

Scheme 1. Synthesis of Copolymer of NIPAAm and CMS (Macroinitiators)

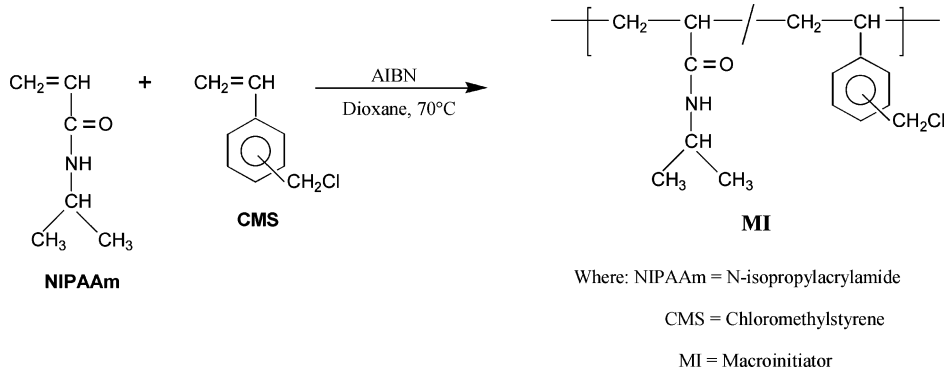


Table 1. Synthesis of the Macroinitiators: Experimental Details and Results (Solution Polymerization, T = 70 °C, t = 24 h, 0.5 mol % AIBN, Solvent: Dioxane 60 mL)

	feed compositions					content of CMS	
macroinitiator	NIPAAm (mmol)	CMS (mmol)	yield[a] (%)	M_n[b] (g/mol)	PD[c]	feed (mol %)	copolym[d] (mol %)
MI1	48.02	0.49	85	29 250	4.9	1.01	1.0
MI2	55.20	1.42	84	27 900	4.5	2.49	2.6
MI3	43.66	2.30	81	29 000	3.7	5.0	5.3
MI4	43.66	4.85	83	24 150	3.4	10.0	10.0
MI5	26.50	7.00	55	13 700	2.6	20.9	26.0

[a] Yield of the polymerization. [b] Number-average molar mass M_n determined by GPC. [c] Polydispersity (PD = M_w/M_n) determined by GPC. [d] Mole percentage of initiator groups ($-CH_2Cl$) per polymer chain of the macroinitiator determined by ^1H NMR spectroscopy.

catalyst modifiers, and supramolecular assemblies, have been developed.[25,33]

Changing the hydrophilic or hydrophobic characteristic of the polyoxazolines, the transition temperature LCST of the copolymers of NIPAAm can be varied. David et al. have synthesized block and graft copolymers of NIPAAm and 2-methyl-2-oxazoline through the macromonomer method obtaining polymers, which showed a LCST in relatively narrow temperature range from 33 to 36.5 °C.[38] It was found that a higher degree of polymerization of the polyoxazoline macromonomer and a higher polymethyloxazoline content in copolymer increase the LCST.

This paper describes the synthesis of graft copolymers of NIPAAm and 2-methyl- and 2-ethyl-2-oxazoline through the "grafting from" method (Scheme 1). We have studied, in an ample range, the influence of the hydrophobic initiating units inside of macroinitiator, the polymerization degree of poly(2-alkyl-2-oxazoline) side chains, and the total content of polyMeOxa or polyEtOxa on the LCST behavior of graft copolymers.

Experimental Part

Materials. *N*-Isopropylacrylamide (Aldrich) was purified by recrystallization from ethanol and dried in a vacuum. Chloromethylstyrene (CMS) is a mixture of isomers (30% *meta* and 70% *para*) and was distilled twice before used. 2,2′-Azobis(isobutyronitrile) (AIBN) was recrystallized twice from methanol. 2-Methyl-2-oxazoline (MeOxa) and 2-ethyl-2-oxazoline (EtOxa) were purchased from Aldrich and distilled twice from CaH_2 and stored under a dry nitrogen atmosphere. Diethyl ether, dioxane, chloroform, and methanol were distilled before used. Potassium iodide was used as received.

The water used for all the measurements was purified and deionized in an Millipore Milli-Q purification system.

Synthesis of Copolymers of Chloromethylstyrene and *N*-Isopropylacrylamide ("Macroinitiators"). A typical procedure was the following (MI3): 4.93 g of NIPAAm, 0.350 g of chloromethylstyrene, and 0.040 g of 2,2′-azobis(isobutyronitrile) were added to the reaction vessel and dissolved in 60 mL of dioxane. Oxygen was removed by flushing with nitrogen for 15 min, and then the reaction vessel was closed and heated at 70 °C for 24 h. At the end of this time, the polymeric product was obtained by slow precipitation of the reaction mixture in diethyl ether. The precipitation was repeated twice, using the solvent/nonsolvent system dioxane/diethyl ether. Finally, the polymer was dried at 40 °C in a vacuum for 24 h. The experimental details and results are summarized in Table 1.

NMR of MI5 in $CDCl_3$ (δ, ppm): ^1H: 1.1 (CH_3, NIPAAm), 1.2−2.5 (CH, CH_2, backbone), 3.7−4.1 (CH, NIPAAm), 4.5 (CH_2Cl, CMS), 5.5−6.6 (NH, NIPAAm), 6.6−7.5 (H_{Ar}, CMS); ^{13}C: 22.5 (CH_3, NIPAAm), 30−45 (CH, CH_2, backbone), 41.1 (CH, NIPAAm), 45.9 (CH_2Cl, *p*-CMS), 46.2 (CH_2Cl, *m*-CMS), 125−130 (CH_{Ar}, CMS), 135.4 (*p*-C, *p*-CMS), 137.5 (*m*-C, *m*-CMS), 144.7 (*ipso*-C, CMS), 173.9 (C=O, NIPAAm).

Synthesis of Graft Copolymers Poly(chloromethylstyrene-*co*-*N*-isopropylacrylamide-*graft*-2-alkyl-2-oxazoline). A typical example was the following: 0.213 g of polymer MI3, 0.032 g of potassium iodide, and 6 mL of benzonitrile were added to a reaction vessel under flow of dry nitrogen. After the total dissolution of the copolymer and salt, it was added 0.30 g of 2-ethyl-2-oxazoline to the reaction mixture; the reaction vessel was closed and heated at 120 °C for 7 h. At the end of the graft copolymerization, 3 mL of a solution of KOH in methanol was added to the reaction mixtures to generate the hydroxy end groups. An excess of 5 mol % KOH relating to CMS content of the macroinitiators was used. After 15 h at room temperature the reaction mixtures were poured in 100 mL of diethyl ether to precipitate the polymer. The precipitation was repeated three times using the solvent/nonsolvent system chloroform/diethyl ether. Finally, the obtained polymer was dried at 40 °C with a vacuum of 0.1 mmHg for 24 h. The experimental details and results are summarized in Table 3.

NMR of GC9 in $CDCl_3$ (δ, ppm): ^1H: 1.1 (CH_3, NIPAAm and EtOxa), 1.3−2.5 (CH, CH_2, MI-backbone), 2.3 and 2.4 ($C(O)CH_2$, EtOxa), 3.3−3.5 (NCH_2, EtOxa), 4.0 (CH, NIPAAm), 5.5−6.7 (NH, NIPAAm), 6.7−7.5 (H_{Ar}, CMS), ^{13}C: 9.4 (CH_3, EtOxa), 22.5 (CH_3, NIPAAm), 26.0 ($C(O)\mathbf{C}H_2$, EtOxa), 32−40 (CH_2, NIPAAm), 41.2 (NHCH, NIPAAm), 42.2 (CH, NIPAAm), 43−49 (NCH_2, EtOxa), 125−131 (CH_{Ar}, CMS), 174.0 and 174.4 (C=O, NIPAAm and EtOxa).

Determination of the Transition Temperature. The transition temperature of the synthesized polymers was determined by the UV/vis turbidity measurements and the

Table 2. T_{tr}[a] Determination of Macroinitiators

macroinitiator[b]	T_g[c] (°C)	ΔC_p[d]	T_{tr}(UV/vis)[e] (°C)	T_{tr}(DSC)[f] (°C)
MI1	n.m.[i]	n.m.	29.5	32.0
MI2	n.m.	n.m.	27.5	31.0
MI3	136.6	0.54	23.0	29.0
MI4	133.2	0.51	[j]	[j]
MI5	124.4	0.43	[j]	[j]
poly(NIPAAm) [g]	135.7	0.54	32	32
poly(CMS) [h]	74.2	0.29		

[a] Transition temperature. [b] Macroinitiators. [c] Glass transition temperature determined by DSC method. [d] ΔC_p of glass transition temperature. [e] T_{tr} determined by UV/vis method (600 nm, 1 wt % polymer solution in water). [f] T_{tr} determined by the DSC method. [g] Poly(*N*-isopropylacrylamide), Polysciences, M_n 40 000 g/mol, PD 2.4. [h] Poly(chloromethylstyrene) synthesized, M_n 20 100 g/mol, PD 2.5. [i] Not measured. [j] For MI4 and MI5 T_{tr} could not be determined.

DSC methods according to the procedure described in the literature.[6]

UV/vis turbidity measurement: 30 mg of graft copolymer was dissolved in 3 mL of bidistilled water, and then the solution was filtered. For the measurement in the UV/vis spectrometer, the sample was placed in a cells system, whose temperature was controlled exactly by a temperature-regulated bath containing a thermometer with thermocouple. The specific heating rate was 0.05 °C/min. The transmittance was measured at a wavelength of 600 nm. Other wavelengths have been checked to notice any influence of the particle size. The transition temperature was determined as the inflection point of the transmittance vs temperature curve.

DSC method: thermograms of a 10 wt % aqueous polymer solution were taken at a heating rate of 1 °C/min in a temperature range of 20−70 °C. The onset temperature was taken as the transition temperature.

Analytical Measurements. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 spectrometer using $CDCl_3$, D_2O, or CD_3OD as solvents. The spectra were referenced on the solvent peak for $CDCl_3$ and CD_3OD or on internal sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 ($\delta(^1H) = 0$ ppm) for D_2O.

Gel permeation chromatography (GPC) were measured on a Waters system equipped with a 510 pump, detector UV-486, detector RI-410, and ultrastyragels columns 7 μm (500, 10^3, 10^4, 10^5 Å). Chloroform or dimethylacetamide was used as elution solvents with a flow rate of 0.5 mL/min. Polystyrene standards were used for calibration.

Differential scanning calorimetry (DSC) were carried out on a Perkin-Elmer DSC-7 to determine the glass transition temperature (T_g) of the polymers and the transition temperature of the polymer solutions. The T_g were measured at a heating rate of 10 K/min.

The UV/vis spectra for the transition temperature determination were obtained from a spectrometer Varian 100, equipped with a thermostatically regulated bath.

Results and Discussion

Synthesis of Copolymers of Chloromethylstyrene and *N*-Isopropylacrylamide. The radical copolymerization of chloromethylstyrene and *N*-isopropylacrylamide was carried out in dioxane at 70 °C for 24 h being started by 2,2′-azobis(isobutyronitrile) (Scheme 1). The polymerization yields were relatively high with >80%, except for the MI5 which was only 55%. In Table 1 the details of the synthesis of the copolymers of CMS and NIPAAm as well as the obtained results are shown.

The copolymer structure was confirmed by NMR spectroscopy. The signals of NIPAAm, *p*-CMS, and *m*-CMS units of the copolymer could be assigned both in ^{1}H and in ^{13}C NMR spectra (see Experimental Part).

The comonomer content of MI1−MI5 was determined from the integrals of the isopropyl-CH (NIPAAm) and the CH_2Cl (CMS) signal in the ^{1}H NMR spectra. The obtained CMS comonomer contents varying from 1 to 26 mol % are in good agreement with the initial molar compositions (Table 1). For the polymerization system CMS/NIPAAm no corresponding values of the reactivity ratios (r_1 and r_2) are reported in the literature. However, for the analogous polymerization system styrene/*N*,*N*-diethylacrylamide, the copolymerization parameters are $r_1 = 1.23$ and $r_2 = 0.39$, respectively,[39] indicating a higher styrene reactivity and thus a favored incorporation of the styrene in the polymeric chain. From that one would expect CMS to polymerize faster compared to NIPAAm, which would lead to copolymers with a higher CMS content at low conversion. At high conversion, which most of our samples reached, this could in principle lead to a certain heterogeneity within the sample by the formation of longer sequences formed by one monomer, since the NIPAAm will be predominantly consumed at high conversion (Table 1). However, this was never observed for our samples probably due to the use of CMS as the minor component.

The GPC measurements show a monomodal distribution of molecular weight for the copolymers of NIPAAm and CMS. The obtained molecular weight values and the polydispersities are typical for polymer synthesized by free radical polymerization (Table 1).

The DSC thermograms of copolymers of NIPAAm and CMS show only one glass transition temperature (T_g), which is slightly lower than the T_g of the polyNIPAAm homopolymer with a T_g of 135 °C.[35] The T_g of copolymers decrease with increasing CMS content in the copolymer because the T_g of the polyCMS homopolymer is only 75 °C[40] (Table 2). The observed T_g behavior of copolymers of NIPAAm and CMS is typical of random copolymers. As conclusion, regarding the macromolecular structure of copolymers of NIPAAm and CMS, we can assume a rather random distribution of the CMS units inside of the polyNIPAAm segments.

As mentioned before, polyNIPAAm homopolymers show a conformational transition (LCST) at 32 °C in aqueous solutions. In the NIPAAm/CMS copolymers this transition was observed as well, which was stated by UV/vis spectrometry and DSC measurements. The obtained values for transition temperatures of copolymers of NIPAAm and CMS are listed in Table 2. As was expected, the inclusion of the hydrophobic comonomer CMS inside the polymer structure decreases the transition temperature with respect to the polyNIPAAm homopolymer. For a CMS molar content above 10%, it is no longer possible to observe the transition temperature, and the water solubility was significantly decreased to the point that the polymers are soluble in water only at 5 °C.

It should be noted that the LCST refers to the minimum in the phase diagram. Thus, we will present our measured data as transition temperature T_{tr} (under the applied conditions).

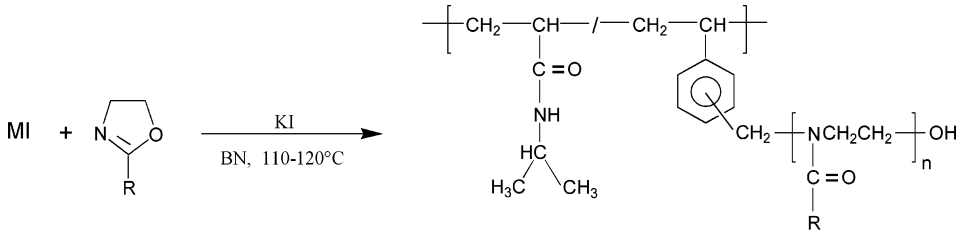
Synthesis of Graft Copolymers Poly(chloromethylstyrene-*co*-*N*-isopropylacrylamide-*graft*-2-alkyl-2-oxazoline). The synthesis of the graft copolymers was carried out by the ring-opening cationic polymerization of 2-methyl-2-oxazoline or 2-ethyl-2-oxazoline, started by the random copolymer of chloromethylstyrene and *N*-isopropylacrylamide using the "grafting from" method. Macroinitiators M1, M2, and M3 were chosen as initiators because these macroinitiators exhibit a volume phase transition, whereas no demixing

Table 3. Synthesis of Graft Copolymers of NIPAAm and 2-Alkyl-2-oxazoline (AlkOxa): Experimental Details and Results (Solution Polymerization: T = 110 °C for 2-Methyl-2-oxazoline, 120 °C for 2-Ethyl-2-oxazoline; t = 7 h, Solvent: Benzonitrile 6 mL, under Nitrogen, Molar Relation [KI]/[Ar-CH_2Cl] was 2, 4, and 8 for GC1−9, GC10−12, and GC13,14, Respectively)

graft copolym	macroinitiator	[AlkOxa]/[CMS][a] (mol/mol)	alkyloxazoline	yield[b] (%)	units[c] of side chain	[NIPAAm]/[AlkOxa][d] (mol/mol)	T_{tr}[e] (°C)
GC 1	MI3	10.0	MeOxa	66	7	2.55	28
GC 2	MI3	19.0	MeOxa	78	20	0.89	34
GC 3	MI3	23.3	MeOxa	89	28	0.64	42
GC 4	MI3	83.2	MeOxa	94	82	0.22	not found
GC 5	MI3	13.25	EtOxa	67	8	2.23	29
GC 6	MI3	19.6	EtOxa	76	17	1.05	36
GC 7	MI3	30.3	EtOxa	83	28	0.64	40
GC 8	MI2	14.9	EtOxa	64	8	4.68	29
GC 9	MI2	26.9	EtOxa	75	20	1.87	31
GC10	MI2	34.8	EtOxa	83	30	1.25	33
GC11	MI2	45.9	EtOxa	87	45	0.83	38
GC12	MI2	70.9	EtOxa	72	70	0.54	not found
GC13	MI1	28.1	EtOxa	68	21	4.71	32
GC14	MI1	51.8	EtOxa	75	45	2.20	34

[a] Mole ratio for graft polymerization. [b] Yield of the graft polymerization. [c] Number-averaged degree of polymerization of polyAlkOxa side chains determined by ^{1}H NMR. [d] Mole ratio of NIPAAm and AlkOxa of the graft copolymers. [e] T_{tr} determined by the UV/vis method (600 nm, 1 wt % polymer in water solution). T_{tr}s determined by the DSC method are the following: 31.3, 30.9, 31.3, and 31.8 °C for GC1, GC5, GC8, and GC9, respectively.

Scheme 2. Synthesis of Graft Copolymers



was observed for M4 and M5 due to the relatively high CMS content. The polymerization was carried out in benzonitrile at 110 °C (MeOxa) or 120 °C (EtOxa) for 7 h, and potassium iodide was used as activator (Scheme 2). Graft copolymers were obtained having a polyNIPAAm main chain and poly(2-alkyl-2-oxazoline) side chains. In Table 3 the details of the synthesis and the results obtained are shown.

The ^{1}H NMR spectra of the graft copolymers, e.g., that of GC6 depicted in Figure 1, clearly show the characteristic signals of the NIPAAm units of the macroinitiator and the signals of polyEtOxa (or polyMeOxa). Unfortunately, the connecting unit between the macroinitiator and the polyoxazoline chain cannot be identified unequivocally because of both its low concentration and signal broadening after incorporation in a polymer backbone. From the initiation reaction and from evaluating the properties of the products there is no doubt that the polyoxazoline chains are really grafted onto the MI. The averaged number of AlkOxa units (Table 3) in the grafted polyoxazoline chains was calculated from the signal intensities of the methyl signal of the NIPAAm units and the NCH_2 signal of the polyoxazoline taking into account the composition of the macroinitiator. The calculated side-chain polymerization degrees vary from 7 to 82 monomeric units of MeOxa or EtOxa (Table 3). The yield of graft polymerizations was between 66 and 94%.

In general, the results for the composition of the graft copolymers synthesized with M1 are in good agreement with initial molar relation of the comonomers at the beginning of the polymerization (Table 3). This indicates that the size of the side chains of the grafted copolymers can be controlled by the initial molar ratio of the oxazoline and the CMS units inside of the macroinitiator due to the "living" character of the polymerization of 2-oxazolines. As already discussed in previous papers on similar polymerization systems,[31,32,41] the benzyl chloride functional groups inside of macroinitiators in the presence of potassium iodide form in situ the benzyl iodide functional groups, which start the polymerization of MeOxa or EtOxa by an ionic mechanism.

The results of GPC measurements for graft copolymers are difficult to interpret since they show a relatively broad distribution and unexpected values probably caused by the amphiphilic character of this copolymers and the inadequacy of the GPC method for that type of polymers.[42,43] The graft copolymers can have a interaction with the GPC column material or they can form aggregates or micelles in the GPC−solvent mixture (dimethylacetamide/water/LiCl).

DSC measurements show for some graft copolymers the presence of two glass transition temperatures. For example, for GC2, T_gs are observed at 76 and 119 °C, which correspond approximately to the side chains of poly(2-ethyl-2-oxazoline) with a T_g of 80 °C[40] and to the main chain of polyNIPAAm with T_g of 135 °C,[35] respectively. This allows the assumption that GC2 is phase-separated in the solid state.

Determination of the Transition Temperature. The transition temperature of these graft copolymers was determined by turbidity measurements and in some

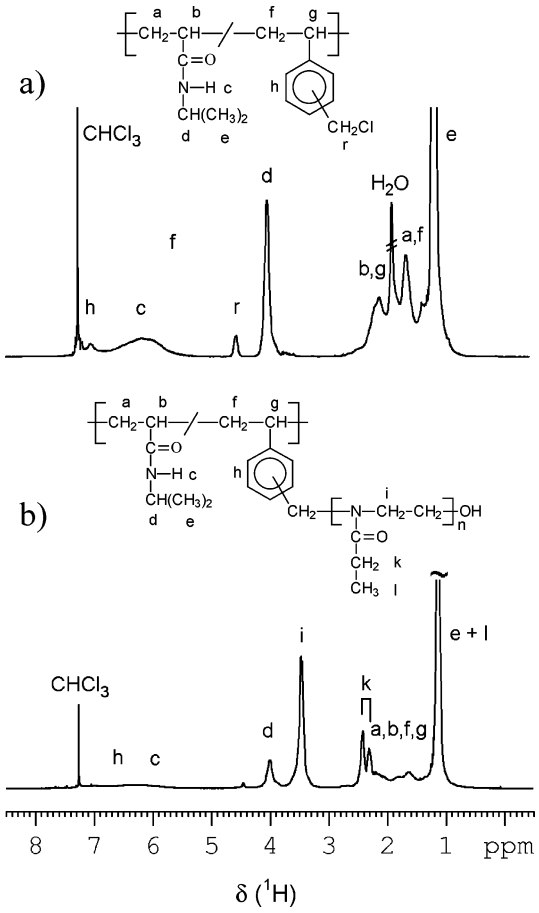


Figure 1. ^{1}H NMR spectra of the macroinitiator MI3 (a; 5.3 mol % CMS) and the graft copolymer GC6 (b; prepared from MI3 and EtOxa, $n = 17$) in $CDCl_3$.

cases by the DSC method. The first method consists of measuring the average of the transmittance of the aqueous polymer solutions vs the temperature. As was expected, the inclusion of the hydrophilic segments of poly(2-methyl-2-oxazoline) or poly(2-ethyl-2-oxazoline) increases the value of the T_{tr} of the respective graft copolymers of the macroinitiators of NIPAAm and CMS. This increase is proportional to the content of alkyloxazoline units in the side chains relative to the number of NIPAAm units in the main chain within a series using the same macroinitiator. Furthermore, the transition broadens with increasing poly(2-alkyl-2-oxazoline) chain length, and it reduces the change in transmittance in the UV/vis spectra (Figures 2 and 3). Moreover, when the degree of polymerization is high enough, T_{tr} is no longer detectable by the UV/vis method.

The observed facts can be explained as follows: when the length of poly(2-alkyl-2-oxazoline) side chains is relatively short in relation to the content of NIPAAm in the main chain, a change of conformation of graft copolymers occurs, leading first to an intramolecular collapse of the polymeric backbone followed by an intermolecular aggregation of the polymeric chains as the solution reaches the transition temperature.

This leads then to the precipitation of the graft copolymer from the aqueous solution. However, when the former relation is large enough, only the intramolecular conformation change occurs but not the intermolecular one because the hydrophilic side chains of the poly(2-alkyl-2-oxazoline) stabilize the collapsed macromolecules in aqueous solution and prevent the precipitation (Figure 4). In this way only molecular aggregates will be formed which are undetectable by the turbidity measurement. It has to be noted that at poly(2-ethyl-2-oxazoline) also exhibits an transition temperature at 62 °C (minimum in phase diagram) as a homopolymer, and the demixing or cloud point temperature is strongly dependent on the concentration and the molecular weight (type I behavior).[36] During our studies, only the transition temperature of the NIPAAm-containing backbone has been investigated since the temperature was kept well below the expected transition of polyEtOxa. Thus, the volume phase transition was not observed for graft copolymers with long poly(2-ethyl-2-oxazoline) chains, and no thermogelling was observed in the aqueous system.

The former hypothesis indicated in Figure 4 is supported by results obtained from temperature-dependent ^{1}H NMR experiments. Figure 5 depicts the ^{1}H NMR spectra of copolymer GC2 in D_2O at different temperatures. The first spectra up to 32 °C are identical and

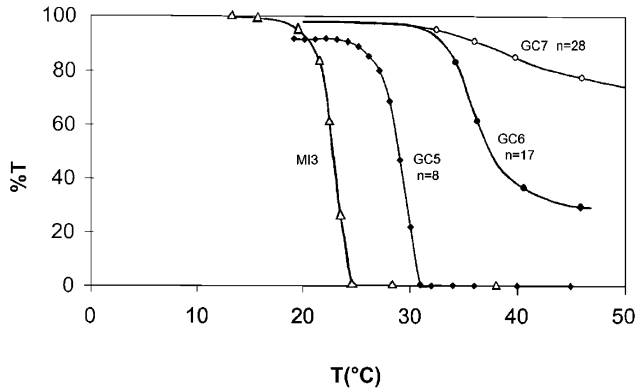


Figure 2. T_{tr} determination by the UV/vis method of graft copolymer synthesized with EtOxa and MI3 (transmittance (%)−temperature (°C) plot, 600 nm, 1 wt % polymer solution).

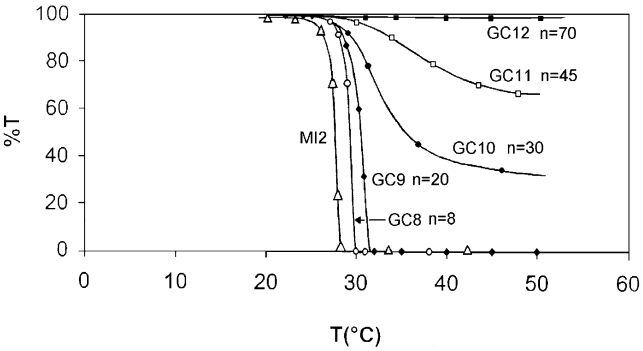


Figure 3. T_{tr} determination by the UV/vis method of graft copolymer synthesized with EtOxa and MI2 (transmittance (%)−temperature (°C) plot 600 nm, 1 wt % polymer solution).

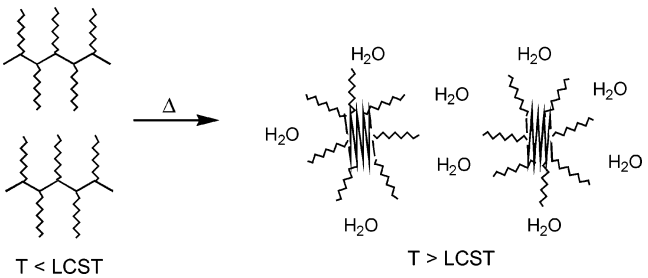


Figure 4. Conformational behavior of graft copolymer with a relative high ratio of poly(2-alkyl-2-oxazoline) side chains to NIPAAm backbone.

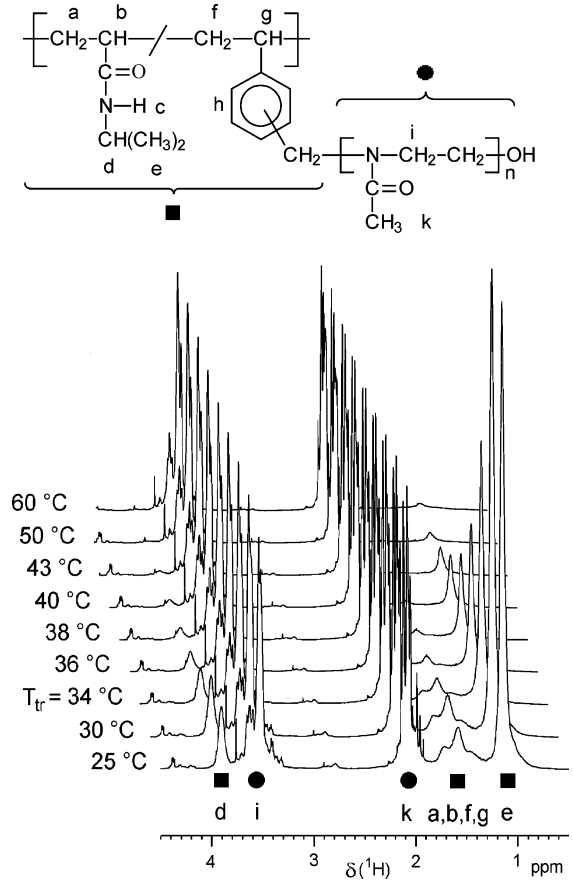


Figure 5. Temperature dependence of the ^{1}H NMR spectrum (region) of GC2 in D_2O showing the decrease in signal intensity of backbone signals (■) above T_{tr} and the unaffected signals of the polyoxazoline grafts (●).

show the signals of both the NIPAAm units in the backbone and the polyMeOxa graftings as assigned. At about 34 °C a process starts, resulting in decrease in intensity for the signals of the NIPAAm units as can be well observed for the signals of the isopropylamide group at about 1.1 and 4.0 ppm. The transition from a solvated NIPAAm backbone to a collapsed solidlike situation is connected with a drastic reduction in chain mobility. These regions cannot be detected in a solution-state NMR experiment which results in the observed signal broadening. The onset temperature of 34 °C is in excellent agreement with the T_{tr} determined by the turbidity measurements. A plot of the content of remaining mobile regions against the sample temperature resembles the intensity curve of the turbidity measurement. At about 50 °C the MI backbone signals nearly disappeared, indicating a complete transition from the solvated to a solidlike state.

However, the signals of the polyMeOxa segments at 2.1 (CH_3) and at 3.5 ppm (NCH_2) do not suffer a significant variation within the temperature range investigated. This clearly indicates that the mobility of the polyMeOxa side chains is hardly affected by the transition of the backbone. The grafted chains are solvated also above transition temperature. Summarizing, one can state that when the graft copolymer solutions reach T_{tr}, the backbone segments of poly(NIPAAm) suffer a collapse while the side chains of polyAlkOxa stay solvated and mobile.

The T_{tr}s for some macroinitiators and graft copolymers were obtained also by the DSC method (Figures 6 and 7). In the T_{tr}s of macroinitiators a correlation between the UV/vis and the DSC values is observed. The T_{tr} values obtained by DSC are always slightly higher than the T_{tr} values by UV/vis (Table 2). With a higher content of CMS in the macroinitiators, the T_{tr} of macroinitiators decreases and also the endotherm becomes broader. In the T_{tr} of graft copolymers the following is observed: again, in general, a certain correlation between the T_{tr} via UV/vis and DSC is noticed. The copolymers GC1 and GC5 were synthesized on the basis of MI3, and their endothermic peaks are similar to the peaks of this macroinitiator. They also show a broad volume phase transition. GC8 and GC9 have a more narrow endotherm peak, and this is in agreement with the UV behavior, which exhibits a sharp transition curve with high intensity.

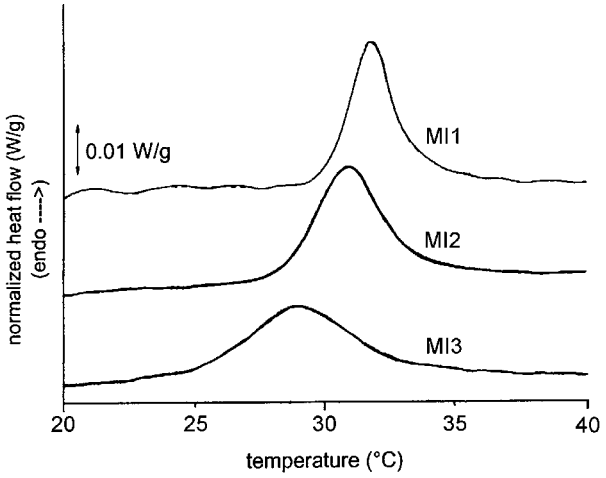


Figure 6. T_{tr} determination of copolymers of NIPAAm and CMS by the DSC method.

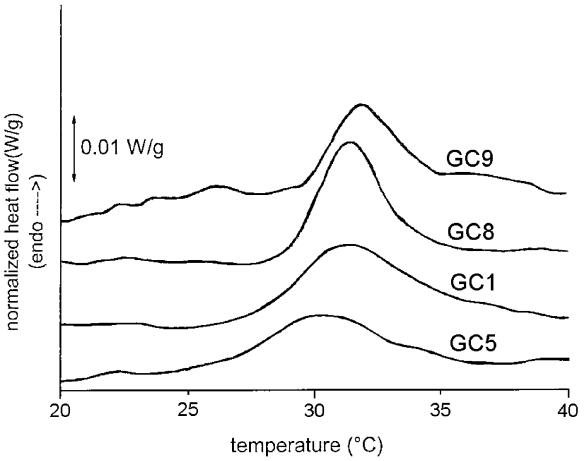


Figure 7. T_{tr} determination of graft copolymer by the DSC method.

Conclusions

Graft copolymers with a backbone of polyNIPAAm and polyMeOxa or polyEtOxa side chains can be synthesized by the ring-opening cationic polymerization of MeOxa or EtOxa initiated by benzyl chloride functional groups in a random copolymer of NIPAAm and chloromethylstyrene. The degree of polymerization of the side chains of polymethyloxazoline or polyethyloxazoline can be controlled by the initial molar ratio of 2-alkyl-2-oxazoline to benzyl chloride. This is possible because the polymerization of the oxazolines has "living" char-

acter without the occurrence of chain transfer or termination reactions.

The transition temperature of the graft copolymers can be controlled through the composition of the macroinitiator and the graft copolymer. An increasing quantity of the hydrophobic comonomer chloromethylstyrene in the macroinitiator decreases the transition temperature, while in a graft copolymer an increasing content of hydrophilic segment of poly(2-methyl-2-oxazoline) or poly(2-ethyl-2-oxazoline) raises the temperature of transition. When the content of the hydrophilic part is large enough, the transition of the copolymer cannot be observed any more, and it is possible that macromolecular aggregates are formed in aqueous solution stabilized by the polar polyoxazoline side chains. The NMR measurements indicate clearly that the mobility of the polyMeOxa side chains is hardly affected by the transition of the backbone. The grafted chains are solvated also above the transition temperature.

The grafted copolymers synthesized in this study, especially the stabilized aggregates with the thermoresponsive core and the water-soluble shell like side chains, could find a practical use as biomaterial for the application as cell cultivation carrier, and drug delivery systems with a stimuli-responsive release of bioactive substances, or in the rheology control of aqueous solutions.

Acknowledgment. Juan Carlos Rueda gratefully acknowledges the Deutscher Akademischer Austauschdienst (DAAD) for a fellowship for a research stay in the Leibniz Institute of Polymer Research Dresden, Germany. The help of L. Häussler and of D. Voigt in DSC and GPC measurements, respectively, is gratefully acknowledged.

References and Notes

(1) Duncan, R. *Nat. Rev. Drug Discovery* **2003**, *2*, 347−360.
(2) Hou, Q.; De Bank, P. A.; Shakesheff, K. M. *J. Mater. Chem.* **2004**, *14*, 1915−1923.
(3) Langer, R.; Tirrell, D. A. *Nature (London)* **2004**, *428*, 487−492.
(4) Miyata, T.; Uragami, T. In *Polymeric Biomaterials*, 2nd ed, Dumitriu, S., Ed.; Marcel Dekker: New York, 2002; pp 959−974.
(5) Ottenbrite, R. M., Kim, S. W., Eds. *Polymeric Drugs & Drug Delivery Systems*; CRC Press: Boca Raton, FL, 2001.
(6) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163−249.
(7) Okano, T., Ed.; *Biorelated Polymers and Gels*; Academic Press: San Diego, CA, 1998.
(8) Lifshitz, I. M.; Grosberg, A. Y.; Khoklov, A. R. *Rev. Mod. Phys.* **1978**, *50*, 683−713.
(9) Tanaka, T. *Phys. Rev. Lett.* **1978**, *40*, 820−823.
(10) Ebara, M.; Yamato, M.; Hirose, M.; Aoyagi, T.; Kikuchi, A.; Sakai, K.; Okano, T. *Biomacromolecules* **2004**, *5*, 505−510.
(11) Ebara, M.; Yamato, M.; Aoyagi, T.; Kikuchi, A.; Sakai, K.; Okano, T. *Biomacromolecules* **2003**, *4*, 344−349.
(12) Schmaljohann, D.; Oswald, J.; Jørgensen, B.; Nitschke, M.; Beyerlein, D.; Werner, C. *Biomacromolecules* **2003**, *4*, 1733−1739.
(13) Cheng, X. H.; Wang, Y. B.; Hanein, Y.; Bohringer, K. F.; Ratner, B. D. *J. Biomed. Mater. Res., Part A* **2004**, *70A*, 159−168.
(14) Kuckling, D.; Adler, H. J. P.; Arndt, K. F.; Ling, L.; Habicher, W. D. *Macromol. Chem. Phys.* **2000**, *201*, 273−280.
(15) Deng, Y.; Xiao, H.; Pelton, R. *J. Colloid Interface Sci.* **1996**, *179*, 188−193.
(16) Kuckling, D.; Richter, A.; Arndt, K. F. *Macromol. Mater. Eng.* **2003**, *288*, 144−151.
(17) Virtanen, J.; Baron, C.; Tenhu, H. *Macromolecules* **2000**, *33*, 336−341.
(18) Ringsdorf, H.; Venzmer, J.; Winnik, F. M. *Macromolecules* **1991**, *24*, 1678−1686.
(19) Mikosch, W.; Geissler, E. *Ber. Bunsen-Ges. Phys. Chem.* **1998**, *102*, 1589−1593.
(20) Nichifor, M.; Zhu, X. X. *Polymer* **2003**, *44*, 3053−3060.
(21) Nichifor, M.; Zhu, X. X. *Colloid Polym. Sci.* **2003**, *281*, 1034−1039.
(22) Chee, C. K.; Rimmer, S.; Shaw, D. A.; Sertar, I.; Swanson, L. *Macromolecules* **2001**, *34*, 7544−7549.
(23) Dhara, D.; Chatterji, P. R. *J. Macromol. Sci., Rev. Macromol. Chem. Phys.* **2000**, *C40*, 51−68.
(24) Winnik, F. M.; Ringsdorf, H.; Venzmer, J. *Macromolecules* **1990**, *23*, 2415−2416.
(25) Aoi, K.; Okada, M. *Prog. Polym. Sci.* **1996**, *21*, 151−208.
(26) Saegusa, T.; Kobayashi, S. *Makromol. Chem., Macromol. Symp.* **1986**, *1,* 23−37.
(27) Dworak, A. *Macromol. Chem. Phys.* **1998**, *199*, 1843−1849.
(28) Hoogenboom, R.; Fitjen, M. W. M.; Schubert, U. S. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 1830−1840.
(29) Velichkova, R. S.; Christova, D. C. *Prog. Polym. Sci.* **1995**, *20*, 819−887.
(30) Nuyken, O.; Rueda-Sanchez, J.; Voit, B. *Macromol. Rapid Commun.* **1997**, *18*, 125−131.
(31) Rueda, J.; Suica, R.; Komber, H.; Voit, B. *Macromol. Chem. Phys.* **2003**, *204*, 954−960.
(32) Rueda, J. C.; Komber, H.; Cedrón, J. C.; Voit, B.; Shevtsova, G. *Macromol. Chem. Phys.* **2003**, *204*, 947−953.
(33) Kobayashi, S.; Uyama, H. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 192−209.
(34) Weberskirch, R.; Hettich, R.; Nuyken, O.; Schmaljohann, D.; Voit, B. *Macromol. Chem. Phys.* **1999**, *200*, 863−873.
(35) Chiu, T. T.; Thill, B. P.; Fairchok, W. J. In *Water Soluble Polymers;* Advances in Chemistry Series 213; Glass, J. E., Ed.; American Chemical Society: Washington, DC, 1986; pp 425−433.
(36) Christova, D.; Velichkova, R.; Loos, W.; Goethals, E. J.; Du Prez, F. *Polymer* **2003**, *44*, 2255−2261.
(37) Wang, C.-H.; Hsiue, G.-H. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 1112−1121.
(38) David, G.; Alupei, V.; Simionescu, B. C.; Dinçer, S.; Piskin, E. *Eur. Polym. J.* **2003**, *39*, 1209−1213.
(39) Greenley, R. Z. Free Radical Copolymerization Reactivity Ratios. In *Polymer Handbook*, 3rd ed.; Brandrup, J., Immergut, E. H., Eds.; J. Wiley & Sons: New York, 1989; p II/225.
(40) Peyser, P. Glass Transition Temperature of Polymers. In *Polymer Handbook*, 3rd ed.; Brandrup, J., Immergut, E. H., Eds.; J. Wiley & Sons: New York, 1989; p VI/209.
(41) Saegusa, T.; Kobayashi, S.; Yamada, A. *Makromol. Chem.* **1976**, *177*, 2271−2283.
(42) Meijs, G.; Rizzardo, E. *J. Macromol. Sci., Rev. Macromol. Chem. Phys.*, **1990**, *C30*, 305−377.
(43) Weberskirch, R. PhD Thesis, Technological University Munich, Germany, 1998.

MA050570P