Macromol. Symp. **2004**, *210*, 427-436

Synthesis and Aqueous Solution Properties of Functionalized and Thermoresponsive Poly(D,L-lactide)/Polyether Block Copolymers

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Summary: Tri- and pentablock amphiphilic copolymers containing hydrophobic poly(D,L-lactide) block(s) and hydrophilic polyethers were synthesized in order to obtain new precursor architectures suitable for drug delivery systems. Polyglycidol-*b*-poly(ethylene oxide)-*b*-poly(D,L-lactide) possess high hydroxyl functionality provided by the linear polyglycidol block. Thus very stable hydroxyl functionalized micelles in aqueous media were obtained. On the other hand poly(D,L-lactide)-*b*-poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide)-*b*-poly(D,L-lactide) form temperature sensitive aggregates. The copolymers obtained were analyzed by SEC and NMR, and their aqueous solution properties were followed by cloud point measurements and determination of critical micellization temperature. TEM was used for particles visualization.

Keywords: biopolymers; block copolymers; polyethers

Introduction

Amphiphilic block copolymers containing poly(D,L-lactide) (PL) hydrophobic blocks are promising materials for advanced drug delivery systems.[1-5] Recently block copolymers of PL and poly(ethylene oxide) (PEO) have attracted great attention due to the unique biocompatibility of PEO combined with an absolute biocompatibility and biodegradability of PL.[6-15] Accordingly, Kataoka et al. have developed a new approach to aldehyde-functionalized PL-PEO micelles in order to engineer their surface properties.[16-18] Thus drug targeting agents can be easily attached to micelles consisting of hydrophilic PEO shell and a drug-loaded hydrophobic PL core.

Polyglycidol (PG) is a very hydrophilic and highly hydroxyl-functional polymer.[19-21] Linear PG is obtained by anionic polymerization of ethoxyethyl glycidyl ether (EEGE) followed by cleavage of the ethoxyethyl groups.[22-24] The PG block could be relatively easily

DOI: 10.1002/masy.200450648

incorporated into a common PEO-PL copolymer thus obtaining a precursor material for highly functional micelles.

It is well-known that PL-PEO copolymers form very stable micelles in aqueous media and in most cases temperature has no significant effect on the equilibrium of the system.[8,12,15] Kohori et al. have reported on thermo-sensitive micelles comprised of diblock copolymers of PL and poly(N-isopropylacrylamide) (PNIPAM).[1] This strategy can be extended by use of other than the PNIPAM polymers, which exhibit lower critical solution temperature (LCST) such as the oligomeric poly(propylene oxide).[25-27] The micellization of PEO-PPO-PEO (Pluronic, BASF; Synperonic, ICI) copolymers is a temperature induced process, which begins at a given critical micellization temperature (*cmt*) or critical micellization concentration (*cmc*).[28] The incorporation of PL outer blocks into a PEO-PPO-PEO macromolecule represents a convenient way of obtaining new PL-based thermosensitive materials. In this system the PEO blocks will provide kinetic stability for the aggregates above the LCST of PPO thus extending the micellar region.

The main goal of the present work is to expand the concept of PL-based amphiphilic block copolymers by introducing new multiblock copolymers containing highly functional or thermo-responsive blocks. PL-PEO-PPO-PEO-PL and PG-PEO-PL copolymers were synthesized with the aid of anionic polymerization. Their self-association properties in aqueous media were studied by cloud point measurements, determination of *cmc*, and transmission electron microscopy (TEM).

Experimental

A. Materials. All solvents were purified by standard methods. D,L-lactide (L) was purchased from Polysciences and purified by recrystallization from toluene. Pluronic P123 with the composition $EO_{20}PO_{70}EO_{20}$ (Aldrich) was freeze-dried before use. $Sn(Oct)_2$ (Aldrich), Ca (Aldrich), CaH_2 (Aldrich) and $CsOH.H_2O$ 99.5 % (Acros Organics) were used as received. EEGE was synthesized according to procedure, described elsewhere[29] and purified by vacuum distillation. Fractions of purity exceeding 99.0% (GC) were used for polymerizations. 1-methoxy-2-ethanol was purified by vacuum distillation. Ethylene oxide (EO) (Clariant) was used as received.

B. Synthesis of Block Copolymers. 1. PEEGE-PEO-PL. To $CsOH.H_2O$ (9 mmol), magnetically stirred in a reaction vessel equipped with argon and vacuum line, an equimolar amount of 1-methoxy-2-ethanol was added at 90°C. After stirring for 1 hour, the system was switched to the vacuum line for 2 hours. After flushing the vessel with argon another 9 mmol of additional alcohol were added. The polymerization of the PEEGE block was started by adding an appropriate amount of the EEGE monomer to the fresh initiator mixture at 60°C. The formation of the first block was completed in 24 hours. After removing a sample for analysis, the reaction temperature was raised to 90°C and EO was bubbled through the system for 2, 3, or 5 hours, depending on the desired length of the PEO block. After EO polymerization the PEEGE-PEO diblock copolymers were carefully purified and analyzed. The polymerization of L was performed in THF at 40 °C for 4 hours. The PEEGE-PEO-$CaNH_2$ macroinitiator was obtained by the procedure, described by Piao et al.[30]

2. PL-PEO-PPO-PEO-PL. The building of the PL outer blocks was performed by polymerization of L initiated by P123 precursor with one of the following reagents: $Sn(Oct)_2$, CaH_2, $CsOH.H_2O$, and $Ca(NH_3)_6$. To 1 mmol of P123, magnetically stirred in a reaction ampoule equipped with argon and vacuum line, $Sn(Oct)_2$, CaH_2, or $CsOH.H_2O$ were added at 90°C. The molar ratio $[OH]/Sn(Oct)_2$ was $1x10^3$,[31] whereas it was 1 for $[OH]/CaH_2$[6] and $[OH]/CsOH.H_2O$. After stirring for 1 hour, the system was switched to the vacuum line for 2 hours. An appropriate amount of L was added and the polymerization continued for 24 hours at 120°C. The polymerization of L via calcium hexaammoniate was performed in THF at 40 °C for 4 hours.

Copolymers Purification. The samples were dissolved in methylene chloride and filtered through Hylfo Super Cel® (diatomaceous earth). After precipitation from freshly distilled dry diethyl ether the copolymers were extensively dried under vacuum.

C. Cleavage of Ethoxyethyl Protective Groups. The described procedure below is similar to a recent method for deprotection of tetrahydropyranyl ethers.[32] A given amount of PEEGE-PEO-PL was dissolved in MeOH. Then $AlCl_3.6H_2O$ was added and the reaction was kept for 0.5 hours at room temperature. The EEGE:$AlCl_3$:MeOH molar ratio was 100:1:800. The reaction product was filtered through diatomaceous earth and the solvents were evaporated under reduced pressure.

D. Analyses. SEC analyses were performed on Waters system equipped with four Styragel columns with nominal pore sizes of 100, 500, 500, and 1000 Å and with a differential refractometric detector. THF was used as the solvent at 40 °C at an elution rate of 1ml/min.

Toluene was used as an internal standard for indication of elution volumes. Polystyrene standards were used for the molecular weight calibration.

The ^1H and ^{13}C NMR spectra were recorded at 250 MHz and 62.5 MHz respectively, on a Bruker WM 250, using $CDCl_3$ as solvent.

Cloud point (CP) transitions of 2% aqueous solutions of the samples were followed on a Specord UV-VIS spectrometer (Carl Zeiss, Jena) switched to transmittance regime at λ = 500 nm using a thermostated cuvette holder. The solutions were initially equilibrated at 0 ° C before heating them gradually (0.1 °C/min).

The determination of *cmc* by 1,6-diphenyl-1,3,5-hexatriene (DPH) dye solubilization was done in a way similar to Ref. 24 and 28.

Samples for TEM were prepared by direct dissolution of the samples in bidistilled water (C=10 g/l) followed by slow evaporation of the solvent. Measurements were performed on JEM 200 CX apparatus.

Results and Discussion

Synthesis of Multiblock Copolymers. The cesium initiating system was preferred for the sequential polymerization of EEGE and EO leading to a PEEGE-PEO precursor. It is well-known that PL is not stable in strong basic media.[33] By the formation of the third block of PL a dark brown tint was observed which was most likely due to unwanted side reactions. Hence the formation of the PL block was initiated by $Ca(NH_3)_6$, which was found to be very efficient for polymerization of cyclic esters.[30,34-36] In general Ca initiators are favored for the ring-opening polymerization of lactides, because they show: i) very high efficiency at temperature as low as 40°C; ii) low toxicity, which is very important in drug delivery.

For the synthesis of PL-PEO-PPO-PEO-PL, CsOH, $Sn(Oct)_2$, CaH_2 and $Ca(NH_3)_6$ initiators were used for the purpose of comparison. As seen from Table 2, L polymerization initiated by $Ca(NH_3)_6$ excels the other systems, proceeding at the lowest temperature and giving 100% yield. The copolymers molecular weight characteristics, determined by SEC and ^1H NMR, and their compositions determined by ^1H NMR are summarized in Table 1 and Table 2.

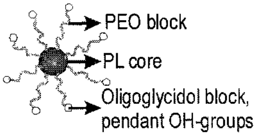
The ethoxyethyl groups of the PEEGE block were successfully cleaved to hydroxyl ones (Figure 1), thus converting it to a linear PG block, which number of functionalities is equal to its degree of polymerization. Apparently the PG-PEO-PL copolymers represent very suitable precursors to hydroxyl-functional micelles composed of a biodegradable PL core and a highly hydrophilic PG-PEO shell (Scheme 1).

Table 1. Composition and molecular weight characteristics of the PEEGE block copolymers.

Composition (NMR)	Mn (NMR)	MWD (SEC)
$EEGE_7$	1000	1.25
$EEGE_7EO_{29}$	2300	1.10
$EEGE_7EO_{29}L_{13}$	3200	1.18
$G_7EO_{29}L_{13}$	2700	-
$EEGE_{16}$	2300	1.18
$EEGE_{16}EO_{137}$	8300	1.13
$EEGE_{16}EO_{137}L_{17}$	9500	1.32
$EEGE_{16}EO_{137}L_{40}$	11000	1.24
$G_{16}EO_{137}L_{40}$	9500	
$EEGE_{16}EO_{246}$	13000	1.35
$EEGE_{16}EO_{246}L_{44}$	16000	1.33
$EEGE_4$	600	1.48
$EEGE_4EO_{159}$	7500	1.45
$EEGE_4EO_{159}L_{25}$	9300	1.22

Table 2. Composition and molecular weight characteristics of pentablock copolymers of PL and Pluronic P123.

Composition (NMR)	Ininitiator	L Conversion (%, NMR)	Mn (NMR)	MWD (SEC)
L_4P123L_4	CsOH	66	6400	1.4
L_5P123L_5		83	6500	1.3
$L_{10}P123L_{10}$		100	7200	1.5
$L_{22}P123L_{22}$		73	8900	1.7
L_3P123L_3	$Sn(Oct)_2$	50	6200	1.4
L_4P123L_4		66	6400	1.3
L_8P123L_8		80	7000	1.2
L_5P123L_5	CaH_2	83	6500	1.5
L_6P123L_6		60	6700	1.3
$L_{10}P123L_{10}$	$Ca(NH_3)_6$	100	7200	1.5
$L_5EO_{30}PO_{34}EO_{30}L_5$		100	5400	1.4



Scheme 1. Model of the PG-PEO-PL micelle

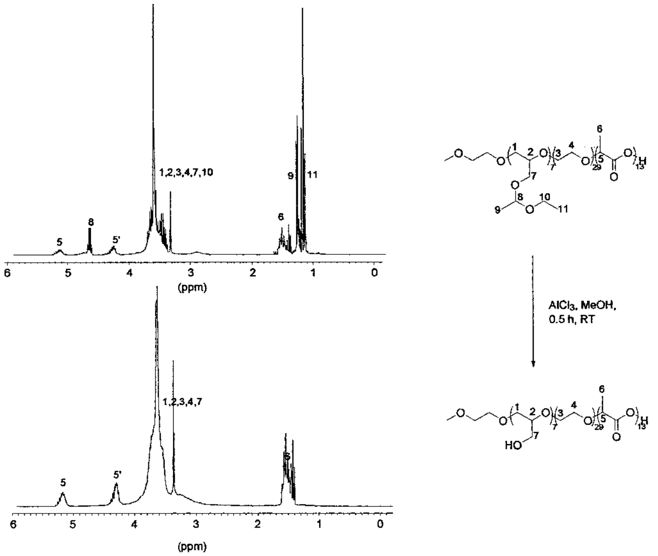


Figure 1. ^{1}H NMR Spectra of EEGE$_7$EO$_{29}$L$_{13}$ (1) and G$_7$EO$_{29}$L$_{13}$ (2)

Aqueous Solution Properties

Cloud Points. Clouding of aqueous dispersions of PG-PEO-PL series was not observed in the temperature range accessible for measurements. The same was observed for PEO-PL copolymers by other authors.[8] The stabilizing role of the protective PEO shell, which prevents dispersion particles from collapsing, is emphasized here by the presence of highly hydrophilic PG blocks positioned at the shell surface.

On the contrary, all the copolymers of the PL-PEO-PPO-PEO-PL series clouded at some temperature, which depended on the length of the PL blocks (Table 3). As seen from Figure 2, the copolymers with higher L content clouded at lower temperatures. The relatively long PPO block plays an important role for the phase separation since it is water soluble at low temperature only. The presence of a PPO block provides the system with thermo-sensitive properties, similar to PNIPAM block in PNIPAM-PL copolymers, described recently.[1]

Table 3. *Cmc* and CP data for tri- and pentablock copolymers

Composition	CP	cmc	
		20°C	37°C
$EEGE_7EO_{29}L_{13}$		3.46	3.46
$G_7EO_{29}L_{13}$		5.00	5.00
$EEGE_{16}EO_{137}L_{17}$		6.10	3.97
$EEGE_{16}EO_{137}L_{40}$		1.10	1.10
$G_{16}EO_{137}L_{40}$		4.50	4.50
$EEGE_{16}EO_{246}L_{44}$		2.67	2.67
$EEGE_4EO_{159}L_{25}$		4.69	3.86
$L_5EO_{30}PO_{34}EO_{30}L_5$	45	4.60	1.14
L_3P123L_3	36	0.40	0.09
L_4P123L_4	23	0.24	0.11
L_5P123L_5	16	0.33	0.13
L_6P123L_6	13	0.10	0.05
L_8P123L_8	12	0.18	0.11

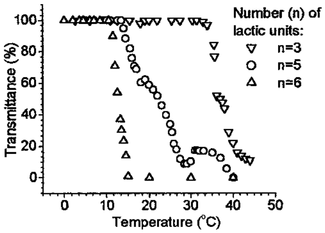


Figure 2. Clouding Curves of $L_nEO_{20}PO_{70}EO_{20}L_n$, C= 20 g/l

Cmc Measurements. Hydrophobic dye solubilization method was used extensively for the determination of *cmc*[8] and *cmt*[15] of amphiphilic copolymers containing hydrophobic PL block(s). The experimental *cmc* values of our multiblock copolymers are summarized in Table 3. *Cmc* of the copolymers depended predominantly from the length of the PL blocks – the higher the DP of the PL block(s), the lower the *cmc*.

PG-PEO-PL. The *cmc* values of the PEEGE-PEO-PL and the PG-PEO-PL copolymers were not affected by temperature, thus indicating for the appearance kinetically "frozen" systems due to the glassy state of the PL core.[4] The *cmc* of PG-PEO-PL are consistently higher than *cmc* of PEEGE-PEO-PL as a result of the increased hydrophilicity of the PG block (Figure 3a). By varying the length of the PEO blocks, one can adjust the hydrophilic-hydrophobic balance of the system. Copolymers with longer hydrophilic PEO blocks have weaker self-association tendency, hence their *cmc* appear at higher concentrations.

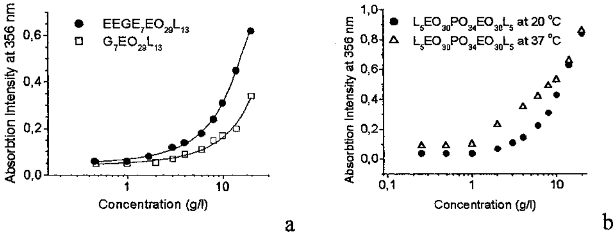


Figure 3. a) *cmc* curves of $EEGE_7EO_{29}L_{13}$ and $G_7EO_{29}L_{13}$ at 37°C ; b) *cmc* curves of $L_5EO_{30}PO_{34}EO_{30}L_5$ at 20 and 37°C

PL-PEO-PPO-PEO-PL. As a consequence of the PPO thermosensitive block, the micellization of PL-PEO-PPO-PEO-PL was observed to be temperature dependent in all cases (Figure 3b). Obviously the fluctuations of the *cmc* values listed in Table 3 are most probably due to the relatively high polydispersities of the samples. As can be seen from the proposed model in Scheme 2 the temperature-driven aggregation of PL-PEO-PPO-PEO-PL proceeds through two stages at least. Above *cmc* and at temperatures below the LCST of PPO flower-like micelles having hydrophobic PL and PEO-PPO-PEO shell are formed. Above the LCST, PPO becomes dehydrated, and as a result the PPO chains form a second hydrophobic domain.

Scheme 2.

TEM Measurements

Figure 4a shows a micrograph of aggregates prepared from the copolymer $G_7EO_{29}L_{13}$ by the direct dissolution method. Granular PL cores of 40 to 50 nm are built of spherical particles of 15-30 nm of diameter. Most probably a secondary aggregation process occurred during the solvent evaporation. Particles obtained by $L_{10}EO_{20}PO_{70}EO_{20}L_{10}$ (Figure 4b) are considerably larger (from 100 to 170 nm) and are also of complex architecture.

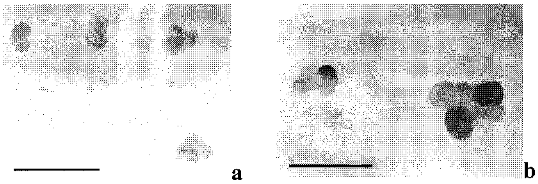


Figure 4. TEM micrographs of a) $G_7EO_{29}L_{13}$, (bar is 100 nm) and b) $L_{10}EO_{20}PO_{70}EO_{20}L_{10}$, bar is 200 nm.

Conclusions

Novel tri- and pentablock copolymers comprised of hydrophobic PL and hydrophilic polyoxiranes were synthesized mainly with the aid of anionic polymerization.

PG-PEO-PL copolymers formed stable functionalized micelles in aqueous media, while PL-PEO-PPO-PEO-PL solutions clouded, and cmc values were affected by the temperature. The hydrophilic-hydrophobic balance may be effectively varied by altering the number of L units.

The copolymers described in the present work are candidates for carriers in drug delivery systems. The hydroxyl - functionalized PG block opens an attractive approach for attachment of drug-targeting agents on PG-PEO-PL micelle surface. Thermosensitive PL-PEO-PPO-PEO-PL micelles can participate in immobilizing of two drugs – one in the PPO domains and the other – in PL core.

Acknowledgement

A.P. would like to acknowledge Macedonian Academy of Sciences as well as Bulgarian Academy of Sciences for the opportunity to collaborate and specialize in the Institute of Polymers, BAS.

[1] F. Kohori, K. Sakai, T. Aoyagi, M. Yokoyama, Y. Sakurai, T. Okano, *J. Contr. Rel.,* **1998**, *55*, 87.
[2] P. Jarret, C. B. Lalor, L. Chan, M. P. Redmon, A. J. Hickey, *Coll. Surf. B: Biointerfaces,* **2000**, *17*, 11.
[3] H. S. Yoo, T. G. Park, *J. Contr. Rel.,* **2001**, *70*, 63.
[4] T. Riley, S. Stolnik, C. R. Heald, C. D. Xiong, M.C. Garnett, L. Illum, S. S. Davis, S. C. Purkiss, R. J. Barlow, P. R. Gellert, *Langmuir,* **2001**, *17*, 3168.
[5] T. Riley, C. R. Heald, S. Stolnik, M.C. Garnett, L. Illum, S. S. Davis, S. M. King, R. K. Heenan, S. C. Purkiss, R. J. Barlow, P. R. Gellert, C. Washington, *Langmuir,* **2003**, *19*, 8428.
[6] I. Rashkov, N. Manolova, S. M. Li, J. L. Espartero, M. Vert, *Macromolecules,* **1996**, *29*, 50.
[7] S. M. Li, I. Rashkov, J. L. Espartero, N. Manolova, M. Vert, *Macromolecules,* **1996**, *29*, 57.
[8] S. Tanodekaew, R. Pannu, F. Heatley, D. Attwood, C. Booth, *Macromol. Chem. Phys.,* **1997**, *198*, 927.
[9] S. Tanodekaew, J. Godward, F. Heatley, C. Booth, *Macromol. Chem. Phys.,* **1997**, *198*, 3385.
[10] X. Chen, S. P. McCarthy, R. A. Gross, *Macromolecules,* **1997**, *30*, 4295.
[11] T. Fujiwara, M. Miyamoto, Y. Kimura, S. Sakurai, *Polymer,* **2001**, *42*, 1515.
[12] T. Fujiwara, M. Miyamoto, Y. Kimura, T. Iwata, Y. Doi, *Macromolecules,* **2001**, *34*, 4043.
[13] C. R. Heald, S. Stolnik, K. S. Kujawinski, C. De Matteis, M. C. Garnett, L. Illum, S. S. Davis, S. C. Purkiss, R. J. Barlow, P. R. Gellert, *Langmuir,* **2002**, *18*, 3669.
[14] S. H. Lee, S. H. Kim, Y. K. Han, Y. H. Kim, *J. Polym. Sci. A: Polym. Chem.,* **2002**, *40*, 2545.
[15] S. Y. Park, B. R. Han, K. M. Na, D. K. Han, S. C. Kim, *Macromolecules,* **2003**, *36*, 4115.
[16] Y. Yamamoto, Y. Nagasaki, M. Kato, K. Kataoka, *Coll. Surf. B: Biointerfaces,* **1999**, *16*, 135.
[17] K. Emoto, Y. Nagasaki, M. Iijima, M. Kato, K. Kataoka, *Coll. Surf. B: Biointerfaces,* **2000**, *18*, 337.
[18] E. Jule, Y. Nagasaki, K. Kataoka, *Langmuir,* **2002**, *18*, 10334.
[19] R. Tokar, P. Kubisa, S. Penczek, A. Dworak, *Macromolecules,* **1994**, 27, 320.
[20] A. Dworak, W. Walach, B. Trzebicka, *Macromol. Chem. Phys.* **1995**, *196*, 1963.
[21] A. Sunder, R. Hanselmann, H. Frey, R. Mülhaupt, *Macromolecules,* **1999**, *32*, 4240.
[22] D. Taton, A. Le Borgne, M. Sepulchre, N. Spassky, *Macromol. Chem. Phys.* **1994**, *195*, 139.
[23] A. Dworak, G. Baran, B. Trzebicka, W. Walach, *React. Funct. Polym.,* **1999**, *42*, 31.
[24] Ph. Dimitrov, E. Hasan, S. Rangelov, B. Trzebicka, A. Dworak, Ch. B. Tsvetanov, *Polymer,* **2002**, 43, 7171.
[25] M. Malmsten, P. Linse, K.-W. Zhang, *Macromolecules,* **1993**,*26*, 2905.
[26] K. Mortensen, D. Schwahn, S. Janssen, *Phys. Rev. Lett.,* **1993**, *71(11)*, 1728.
[27] H.G. Schild, D.A. Tirell, *J. Phys. Chem.,* **1990**, *94*, 4352.
[28] P. Alexandridis, J.F. Holzwarth, T.A. Hatton, *Macromolecules,* **1994**, *27*, 2414.
[29] A. Fitton, J. Hill, D. Jane, R. Miller, *Synthesis,* **1987**, 1140.
[30] L. Piao, Z. Dai, M. Deng, X. Chen, X. Jing, *Polymer,* **2003**, *44*, 2025.
[31] G. Schwach, J. Coudane, R. Engel, M. Vert, *J. Polym. Sci. A: Polym. Chem.,* **1997**, *35*, 3431
[32] V. V. Namboodri., R.S. Varma, *Tetrahed. Lett.,* **2002**, *43*,1143.
[33] H. Tsuji, Y. Ikada, *J. Polym. Sci. A: Polym. Chem.,* **1998**, *6*, 59.
[34] L. Piao, M. Deng, X. Chen, L. Jiang, X. Jing, *Polymer,* **2003**, *44*, 2331.
[35] Z. Zhong, M. J. K. Ankone, P. J. Dijkstra, C. Birg, M. Westerhausen, J. Feijen, *Polym. Bull.,* **2001**, *46*, 51.
[36] Z. Zhong, M. J. K. Ankone, P. J. Dijkstra, C. Birg, M. Westerhausen, J. Feijen, *Macromolecules,* **2001**, *34*, 3863.