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Self-Assembly and Photo-Cross-Linking of Eight-Armed PEG-PTMC Star Block Copolymers

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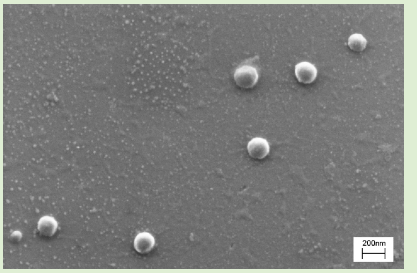
ABSTRACT: Eight-armed poly(ethylene glycol)-poly(trimethylene carbonate) star block copolymers (PEG-$(PTMC)_8$) linked by a carbamate group between the PEG core and the PTMC blocks were synthesized by the metal-free, HCl-catalyzed ring-opening polymerization of trimethylene carbonate using an amine-terminated eight-armed star PEG in dichloromethane. Although dye solubilization experiments, nuclear magnetic resonance spectroscopy, and dynamic light scattering clearly indicated the presence of aggregates in aqueous dispersions of the copolymers, no physical gelation was observed up to high concentrations. PEG-$(PTMC_9)_8$ was end-group-functionalized using acryloyl chloride and photopolymerized in the presence of Irgacure 2959. When dilute aqueous dispersions of PEG-$(PTMC_9)_8$-Acr were UV irradiated, chemically cross-linked PEG-PTMC nanoparticles were obtained, whereas irradiation of more concentrated PEG-$(PTMC_9)_8$-Acr dispersions resulted in the formation of photo-cross-linked hydrogels. Their good mechanical properties and high stability against hydrolytic degradation make photo-cross-linked PEG-PTMC hydrogels interesting for biomedical applications such as matrices for tissue engineering and controlled drug delivery systems.



INTRODUCTION

Hydrogels are polymer networks that receive much interest for their use in biomedical applications because they generally exhibit excellent biocompatibility as a result of their high water content.[1−3] Chemically cross-linked hydrogels are generally more stable and have better mechanical properties compared with physically cross-linked systems. Poly(ethylene glycol) (PEG) is a widely used hydrophilic component in hydrogels because of its good biocompatibility. As a hydrophobic component, aliphatic polyesters such as poly(lactide) (PLA) and poly(ε-caprolactone) (PCL) have been investigated extensively in block copolymers with PEG for the preparation of physically cross-linked hydrogels. Chemically cross-linked hydrogels are often prepared by photocuring of polymers that are functionalized with, for example, acrylic end groups.[4] Hubbell et al. prepared photo-cross-linked hydrogels from end-acrylated PLA-PEG-PLA and PLGA-PEG-PLGA triblock copolymers.[5] The degradation times of the hydrogels varied from 0.3 to 120 days depending on the molecular weight of the PEG and the length and nature of the hydrophobic polyester block. A drawback of hydrogels based on PEG-polyester copolymers is the formation of acidic components upon degradation, which may deactivate pH-sensitive compounds such as growth and differentiation factors.[6] In this respect, poly(trimethylene carbonate) (PTMC) is an interesting alternative because it is enzymatically degraded in vivo with the formation of water-soluble and nonacidic products such as 1,3-propanediol, carbon dioxide, and PTMC oligomers.[7] During the past decade, much effort has been devoted to the synthesis of PEG-PTMC block copolymers. Most often, stannous octoate is used as the catalyst for the ring-opening polymerization of TMC initiated by hydroxyl end groups of PEG. Recently, low-molecular-weight PEG-PTMC block copolymers were prepared by the ring-opening polymerization of TMC from hydroxyl functional PEGs and HCl as a catalyst via an activated monomer mechanism.[8] This method avoids the use of potentially cytotoxic organometallic catalysts and is preferred when the resultant polymers are to be used in biomedical applications.[9] Using mono-, di-, or multifunctional PEGs, polymers with one, two, four, or eight PTMC arms were prepared with constant hydrophobic content (50 wt % PTMC) and a total molecular weight of 4000 g/mol.[8] The critical association concentration (CAC) of these polymers as well as the aggregate diameter in aqueous solution increased with increasing arm number, which was attributed to hampered hydrophobic interactions. Temperature-dependent gelation of PEG-PTMC diblock and PTMC-PEG-PTMC triblock copolymers has been shown by Kim et al.[10] and Bat et al.,[11] respectively. For the diblock polymers, the transition temperature could be controlled in a range of 20−75 °C by varying the polymer concentration, molecular weight, and composition of the

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polymer. In the triblock system, sol-to-gel transitions were observed for polymers with a PEG content between 68 and 82 wt %. Gelation occurred only for polymers with a M_n of 14 kg/mol and higher, indicating that the total molecular weight is an important polymer property for the temperature-dependent gelation behavior. Whereas diblock copolymers afford hydrogels with low storage moduli, PTMC-PEG-PTMC triblock copolymer hydrogels at similar polymer concentrations showed storage moduli up to 3 kPa.

Chemically cross-linked hydrogels based on PEG and PTMC have been prepared only from low-molecular-weight triblock copolymers by end-group acrylation and subsequent photopolymerization.[12] Upon immersion in PBS at 37 °C, a photo-cross-linked PEG-PTMC triblock copolymer (600−1000−600 g/mol) exhibited a mass loss of 7% after 4 weeks as a result of bulk degradation.

In previous research, we have shown that eight-armed PEG-PLA star block copolymers yield physically cross-linked hydrogels at much lower concentrations compared with linear PLA-PEG-PLA triblock copolymers.[13] This prompted us to investigate the aggregation behavior of analogous PEG-PTMC star block copolymers in aqueous solutions. In this Article, we describe the synthesis of eight-armed PEG-PTMC star block copolymer by metal-free ring-opening polymerization of TMC initiated by amine end-functionalized eight-armed PEG. The self-assembly of the star polymers in water was studied. It was found that stable bridging between polymer aggregates, necessary to form a hydrogel, is disfavored because of the high mobility of the PTMC blocks. We show that interaggregate bridging can, however, be stabilized by UV cross-linking; an acrylated eight-armed PEG-PTMC star block copolymer was used to prepare a photo-cross-linked hydrogel or nanoparticles by UV irradiation. The physical, mechanical, and degradation properties of the hydrogel were investigated as well as its biocompatibility.

EXPERIMENTAL SECTION

Materials. Hydroxyl-terminated eight-armed poly(ethylene glycol) (PEG-$(OH)_8$, $M_{n,NMR}$ = 21 400 g/mol) was purchased from Jenkem (Allen, TX) and purified before use by dissolution in dichloromethane and precipitation in cold diethyl ether. The PEG-$(OH)_8$ was converted in PEG-$(NH_2)_8$ as previously described.[14] Trimethylene carbonate (TMC) was obtained from Boehringer (Ingelheim, Germany) and used as received. HCl (1.0 M solution in diethyl ether), acryloyl chloride, methanesulfonyl chloride (mesyl chloride), triethylamine (TEA), and 25% aqueous ammonia solution were from Sigma Aldrich (St. Louis, MI). Toluene, hexane, diethyl ether, methanol, and dichloromethane were all from Biosolve (Valkenswaard, The Netherlands). Dichloromethane, TEA, and toluene were dried over calcium hydride, potassium hydroxide, and sodium, respectively, and distilled prior to use. Irgacure 2959 was obtained from Ciba (Basel, Switzerland). Lipase from porcine pancreas was purchased from Sigma Aldrich. Chondrocytes from bovine cartilage were used for cytotoxicity measurements in this study. The cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air in 175 cm^2 flasks containing Dulbecco's modified eagle medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Gibco). The medium was refreshed twice a week until cells reached confluency. When confluent, cells were detached using trypsin solution (0.25% trypsin/0.05% EDTA, Invitrogen) and diluted 1:5 in a fresh culture flask. In this study, cells from passage 2 were used.

Synthesis. The eight-armed poly(ethylene glycol)-poly(trimethylene carbonate) star block copolymer (PEG-$(PTMC_9)_8$) was synthesized by the ring-opening polymerization of trimethylene carbonate using amine-terminated eight-armed star PEG (PEG-$(NH_2)_8$) as an initiator and HCl as a catalyst in dichloromethane at room temperature.

PEG-$(NH_2)_8$ (5 g, 0.23 mmol) and toluene (15 mL) were introduced in a round-bottomed flask. The toluene was distilled off, and CH_2Cl_2 (5 mL) and TMC (2.04 g, 20 mmol) were added. The polymerization was started by the addition of 1 M HCl solution in diethyl ether (8 mL, 8 mmol) at 25 °C in a nitrogen atmosphere. After 48 h, the product was purified by precipitation in a 20-fold excess of a mixture of diethyl ether and methanol (40/1 v/v). PEG-$(PTMC_9)_8$ was obtained by filtration and dried under vacuum at ambient temperature overnight. ^{1}H NMR ($CDCl_3$, δ): 7.92 (s, CH_2NHCOO), 4.28 (t, CH_2NHCOO), 4.22 (t, $OCH_2CH_2CH_2O$), 3.72 (t, CH_2CH_2OH), 3.63 (PEG protons), 2.03 (q, $OCH_2CH_2CH_2O$), 1.90 (q, CH_2CH_2OH).

PEG-$(PTMC_9)_8$ star block copolymer was functionalized with acrylate end groups (PEG-$(PTMC_9)_8$-Acr) according to a procedure described in literature.[12] PEG-$(PTMC_9)_8$ (6.70 g, 0.24 mmol) was dissolved in 15 mL of dichloromethane. Acryloyl chloride (3.23 mL, 40 mmol) was added, and the reaction mixture was stirred under a N_2 atmosphere for 16 h at 50 °C. The product was precipitated in a 20-times excess of cold hexane and dried under vacuum for 1 day. ^{1}H NMR ($CDCl_3$, δ): 6.83 (s, CH_2NHCOO), 6.41 (dd, $OCOCHCH_2$), 6.10 (dd, $OCOCHCH_2$), 5.84 (dd, $OCOCHCH_2$), 4.23 (t, $OCH_2CH_2CH_2O$), 3.80 (t, CH_2CH_2OH), 3.63 (PEG protons), 2.79 and 2.65 (t, CH_2CH_2OH), 2.04 (q, $OCH_2CH_2CH_2O$).

Chemically cross-linked PEG-PTMC nanoparticles were prepared by UV irradiation of PEG-$(PTMC_9)_8$-Acr star block copolymer. In a typical procedure, a 1% w/v mixture of PEG-$(PTMC_9)_8$-Acr in water containing 10 mol % Irgacure 2959 photoinitiator relative to acrylate groups was prepared. The mixture was irradiated by UV light (∼5 mW/cm^2) at 365 nm for 2 h. Chemically cross-linked PEG-PTMC hydrogels were similarly prepared. Typically, a 40% w/v mixture of PEG-$(PTMC_9)_8$-Acr in water containing 10 mol % photoinitiator relative to acrylate groups was prepared. The mixture was irradiated by UV light at 365 nm for 2 h.

Characterization. ^{1}H NMR (300 MHz) spectra were recorded on a Varian Inova 300 NMR spectrometer. Polymers were dissolved in $CDCl_3$ at a concentration of 15 mg/mL. For NMR analysis of PEG-PTMC nanoparticles, an aqueous PEG-PTMC nanoparticle dispersion (1% w/v) was freeze-dried, and the particles were redispersed in $CDCl_3$ at a concentration of 15 mg/mL.

Aqueous Solution Properties. The CAC value of the PEG-$(PTMC_9)_8$ star block copolymer in water was determined using the 1,6-diphenyl-1,3,5-hexatriene (DPH) dye solubilization method.[15] Mixtures of PEG-$(PTMC_9)_8$ in distilled water were prepared in the concentration range of 1×10^{-6} to 5% w/v. A solution of DPH was prepared in methanol at a concentration of 0.5 mM. Approximately 1 mL of the polymer dispersion was added to a polystyrene vial, followed by the addition of 10 μL of the DPH solution. The samples were allowed to equilibrate for at least 3 h in the dark, after which the absorption at 357 nm relative to a blank (polymer dispersion containing no DPH) was measured using a Varian Cary 300 Bio UV−visible spectrophotometer. The absorption was plotted against the logarithm of the polymer concentration, and the intercept of the extrapolated straight lines was taken as the CAC.

Dynamic light scattering (DLS) of the PEG-$(PTMC_9)_8$ star block copolymer in water (1×10^{-4} − 10% w/v) was performed to determine aggregate sizes. Experiments were carried out between 25 and 55 °C using a Malvern Nano ZS, a laser wavelength of 633 nm, and a scattering angle of 173°. At each temperature, the sample was allowed to equilibrate for 5 min.

NMR experiments were carried out on a Bruker AMX-300 WB spectrometer working at 300.13 MHz for proton and at 75.47 MHz for carbon-13 using either a 5 mm probe head or a 4 mm CP/MAS probe

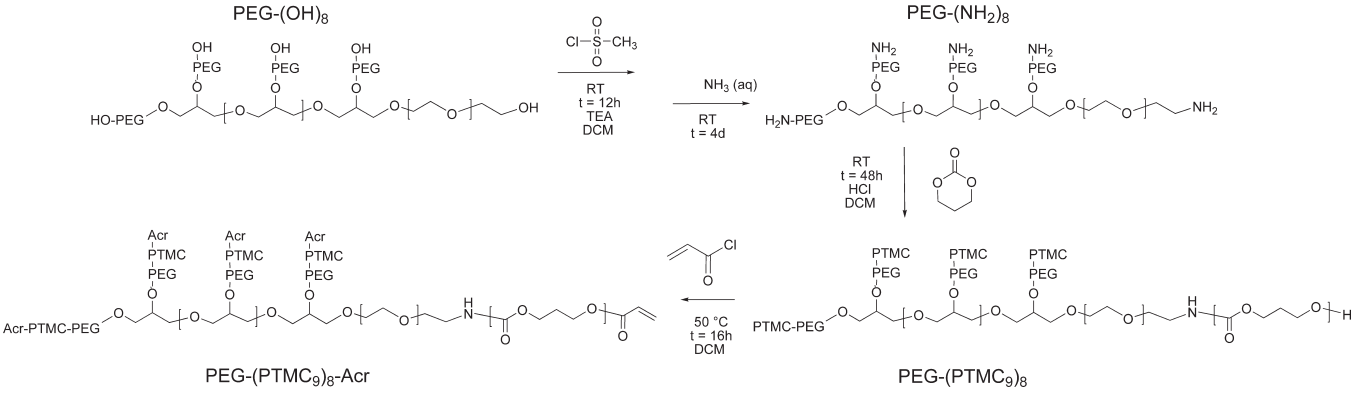

Figure 1. Synthesis scheme for the preparation of PEG-$(PTMC_9)_8$ and PEG-$(PTMC_9)_8$-Acr star block copolymers.

head for solution-state and solid-state measurements, respectively. The temperature was controlled within 0.1 °C.

Solution state ^{1}H NMR experiments were performed on samples of 0.1, 1, and 10% w/v PEG-$(PTMC_9)_8$ in D_2O (99.98% D, Eurisotop) with a 90° pulse of 5.7 μs and a recycle delay of 10 s; 8 to 4000 scans were acquired depending on sample concentration. The samples were prepared by dissolving the appropriate amount of copolymer in D_2O. Relative peak intensities within each spectrum were determined by integration of the peaks obtained by spectral deconvolution using the SPORT-NMR software[16] and were calibrated using 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) at a known concentration as internal standard. DSS was also used as reference for the ^{1}H and ^{13}C chemical shifts. Solution state ^{13}C NMR experiments were performed on the 10% w/v sample of PEG-$(PTMC_9)_8$ in D_2O with a pulse of 4 μs, corresponding to a 40° pulse and a recycle delay of 30 s and acquiring 2400 scans.

Solid-state ^{13}C magic-angle spinning (MAS) NMR experiments were performed under proton decoupling conditions on a dry PEG-$(PTMC_9)_8$ sample and on a 50% w/v sample of PEG-$(PTMC_9)_8$ in D_2O. In the ^{13}C direct excitation (DE) experiments, the 90° pulse length was 4 μs, and recycle delays of 12 and 120 s were used. In the ^{1}H−^{13}C cross-polarization (CP) experiments, the ^{1}H 90° pulse length was 4 μs, and a contact time of 1 ms and a recycle delay of 12 s were used. The MAS spinning rate was 3 and 1 kHz for the dry and hydrated samples, respectively.

Hydrogel Properties. Oscillatory rheology experiments were performed to determine the mechanical properties of the PEG-PTMC hydrogels. The storage (G') and loss (G'') modulus of hydrogels were monitored for 12.5 min at 25 °C on an Anton-Paar Physica MCR 301 rheometer. Experiments were performed using a flat plate measuring geometry (diameter 25 mm, gap 0.3 mm) utilizing a strain of 1% and a frequency of 1 Hz. To prevent water evaporation, we placed a solvent trap over the geometry.

Hydrolytic and enzymatic degradation experiments were performed to determine the stability of the hydrogels. Freshly prepared hydrogel samples were dried in air, and the initial weight W_0 was determined. The samples were immersed in PBS at 37 °C. To prevent bacterial growth, 0.02% w/v NaN_3 was added to the buffer solution. At regular times, samples were taken out and wiped with tissue paper, and their mass in the swollen state (W_s) was determined. The degree of swelling was calculated from

$$\text{degree of swelling} = \frac{(W_S - W_0)}{W_0} \times 100\%$$

Subsequently, the samples were allowed to dry in air overnight to yield the dry weight (W_D). The remaining relative polymer mass was calculated from

$$\text{relative polymer mass} = \frac{W_D}{W_0} \times 100\%$$

Enzymatic degradation was studied by immersing hydrogel samples in PBS containing 1% w/v lipase. The remaining relative polymer mass was determined as described above. All degradation experiments were performed in duplicate.

The direct cell contact method was employed to assess the cytotoxicity of photo-cross-linked PEG-PTMC hydrogels.[17] Chondrocytes from bovine cartilage (6×10^5 cells/mL) were seeded in 50 μL of DMEM in a 96-well culture plate. Plates were incubated at 37 °C in a humidified 5% CO_2 atmosphere until the cells formed a monolayer. The culture medium was aspirated, and hydrogel pieces ($1 \times 1 \times 1$ mm), preswollen in culture medium, were placed carefully on the monolayer in 200 μL of culture medium. The cell viability after 20 h was determined by the colorimetric MTS assay, according to the manufacturer's instructions (CellTiter 96 aqueous one solution cell proliferation assay, Promega). In brief, MTS solution was added to the wells (20 to 100 μL of medium) and incubated for 2 h at 37 °C in the dark. Afterward, the supernatant of each sample was collected, and its absorbance was measured at 540 nm using a Tecan Safire microplate reader. Data (n = 3) are expressed as mean ± standard deviation (SD).

The morphology of chemically cross-linked PEG-PTMC nanoparticles was evaluated by scanning electron microscopy (SEM). One drop of a nanoparticle dispersion in water was applied to a carbon-coated grid, and the water was evaporated overnight in air. Images were obtained with a scanning electron microscope (LEO Gemini 1550 FEG-SEM) at 1.6 kV.

RESULTS AND DISCUSSION

Synthesis. The acid-catalyzed ring-opening polymerization of TMC in the presence of alcohols as initiators is a highly efficient method recently evaluated.[18] Moreover, this method avoids the use of metal-based catalysts, which may cause adverse effects when these materials are used in biomedical applications. The HCl-catalyzed ring-opening polymerization of TMC also appeared to be a convenient method to prepare PEG-PTMC block copolymers when amine end-group-functionalized eight-armed star PEG was used as an initiator (Figure 1). In this case, the blocks are connected through carbamate bonds. The ring-opening polymerization was performed in dichloromethane at room temperature in high yield (>95%).

The ^{1}H NMR spectrum of the PEG-$(PTMC_9)_8$ star block copolymer shows the methylene protons of the PTMC blocks at 4.22 and 2.03 ppm (Figure 2A). The signal of the methylene protons next to the amine functional groups (CH_2−NH_2) of the starting PEG amine completely disappeared, revealing full end-group conversion. Signals of the carbamate NH and methylene

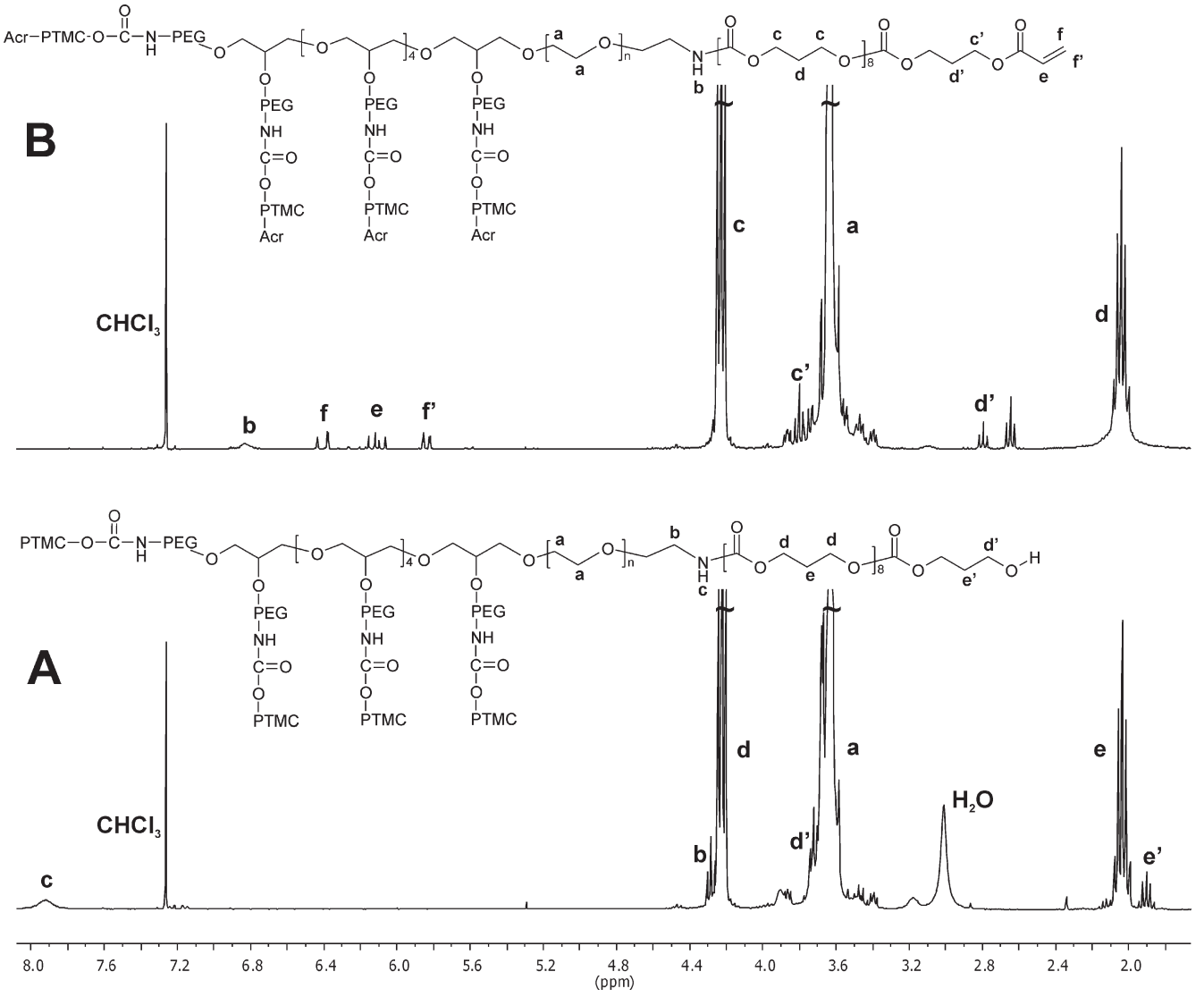

Figure 2. ^{1}H NMR spectra of PEG-$(PTMC_9)_8$ (A) and PEG-$(PTMC_9)_8$-Acr (B). Solvent: $CDCl_3$.

$-CH_2-NH-COO-$ protons appeared at 7.92 and 4.28 ppm, respectively. The degree of polymerization of the PTMC blocks was calculated using the integrals of peaks corresponding to the methylene protons of the TMC units and the main chain protons of PEG and was close to the ratio based on the feed composition.

Acrylation of PEG-based block copolymers has proven to be an efficient way to prepare chemically cross-linked hydrogels through photopolymerization.[19] The PEG-$(PTMC_9)_8$ star block copolymer was end-group-functionalized using acryloyl chloride in dichloromethane. In the ^{1}H NMR spectrum of the PEG-$(PTMC_9)_8$-Acr, new signals appeared at 6.41, 6.10, and 5.84 ppm corresponding to the vinylic protons (Figure 2B). Furthermore, signals correlating to protons of the terminal PTMC repeating unit shifted downfield (**c**′ and **d**′ in Figure 2B). The degree of acrylation was calculated to be >90% by comparing the respective areas of the peaks corresponding to the acryloyl groups and the PEG methylene groups.

Self-Assembly of PEG-$(PTMC_9)_8$ in Water. In water, PEG-$(PTMC_9)_8$ star block copolymers gave turbid dispersions, and no gelation was observed at room temperature, even for a polymer concentration of 60% w/v, despite the fact that they have hydrophobic and hydrophilic block lengths ($M_{n,PTMC}$ = 7400 g/mol, $M_{n,PEG}$ = 21 400 g/mol) similar to those of eight-armed PEG-PLA star block copolymers ($M_{n,PLA}$ = 6300 to 8600 g/mol, $M_{n,PEG}$ = 23 800 g/mol), which have been observed to give thermosensitive hydrogels at relatively low concentration.[20] PEG-$(PTMC_n)_8$ star block polymers with longer PTMC blocks (n up to 23) showed the same behavior.

The CAC of the PEG-$(PTMC_9)_8$ star block copolymer as determined with the DPH solubilization method, afforded a value of 0.004% w/v. This value is of the same magnitude as previously reported CAC values of multiarmed PEG-PTMC block copolymers of low molecular weight ($M_{n,PEG}$ = 2000 g/mol, $M_{n,PTMC}$ = 2000 g/mol).[8] However, it is two orders of magnitude lower than the CAC value (0.19% w/v) of PEG-PLA star block copolymer of similar molecular weight and hydrophobic content.[20] Using DLS, the aggregate size and aggregate size distribution of PEG-$(PTMC_9)_8$ in water was investigated at different concentrations and temperatures. At concentrations of 1×10^{-4} w/v % up to the CAC, aggregates with an average dimension of 300 nm were observed. At a concentration of 0.1% w/v, a broad distribution around a mean aggregate size of 234 nm

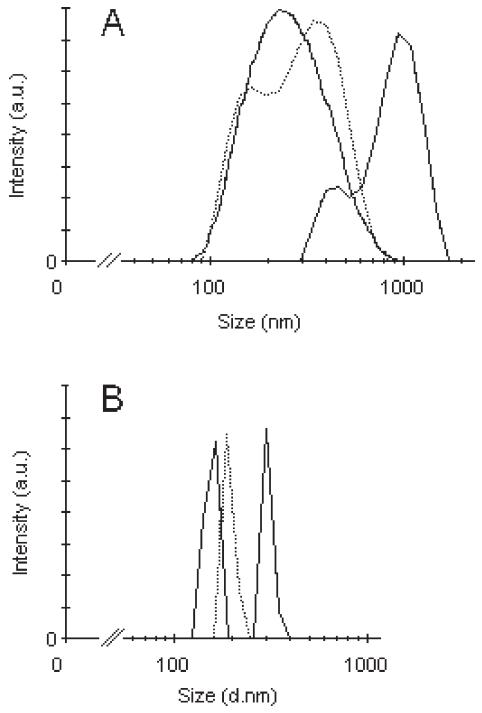

Figure 3. (A) Aggregate size distributions of PEG-$(PTMC_9)_8$ star block copolymers in water at 25 °C. 0.1% w/v (gray line), 1% w/v (dotted line), and 10% w/v (black line). (B) Aggregate size distributions of PEG-PTMC triblock and star block copolymers in water at 25 °C. 1×10^{-4} w/v % $(PTMC_{10})$-PEG-$(PTMC_{10})$ (gray line), 1×10^{-4} w/v % PEG-$(PTMC_9)_8$ (black line), and their mixed (1/1 v/v) solution (dotted line).

was found; with increasing PEG-$(PTMC_9)_8$ concentration, the aggregate size distribution became clearly bimodal with maximum dimensions increasing, reaching 1 μm at a concentration of 10% w/v (Figure 3A). The size of the aggregates was independent of the temperature between 25 and 55 °C. Aggregates with average sizes on the order of a few hundred nanometers were reported for other eight-armed PEG-PTMC copolymers[8] and were also formed by a $(PTMC_{10})$-PEG-$(PTMC_{10})$ triblock (M_n 1000−8000−1000 g/mol) copolymer. (See Figure 3B.) Immediately after mixing (1/1 v/v) of dispersions of $(PTMC_{10})$-PEG-$(PTMC_{10})$ and PEG-$(PTMC_9)_8$, aggregates with a size in between the values of the two separate dispersions were detected by DLS. This indicates that the aggregates quickly reorganize, that is, that the individual PEG-PTMC molecules are able to exchange between aggregates and to form new aggregates with another dimension. These results suggest that because of the instability of the highly constrained supramolecular aggregates a new thermodynamic equilibrium is rapidly formed, even at high concentrations.

The formation of aggregates of PEG-$(PTMC_9)_8$ in water was confirmed by solution-state ^{1}H and ^{13}C NMR spectroscopy measurements on samples at several concentrations and temperatures. In fact, in the ^{1}H NMR spectra of 0.1, 1, and 10% w/v PEG-$(PTMC_9)_8$ samples in D_2O at room temperature, the methylene protons of PEG (peak a in Figure 4) and those of PTMC resonating at 1.91 ppm, belonging to the terminal PTMC units (peak f in Figure 4), were fully detected, whereas the PTMC protons resonating at 2.06 and 4.17 ppm (peaks e and d in Figure 4) gave signals with lower intensity than expected on the basis of stoichiometry. In particular 50, 66, and 74% of the latter protons were observable in the spectrum of the 10, 1, and 0.1% w/v sample, respectively. Furthermore, the ^{1}H signals of PTMC were much broader than those of PEG. These observations on relative intensities and line widths of PTMC and PEG signals are similar to those reported for linear, Y-shaped, and star-shaped PEG-PTMC block copolymers.[8,21−25] They are evidence that the PEG chains are well swollen in water and undergo fast motions, whereas the PTMC chains are aggregated in hydrophobic domains. The PTMC chain mobility was reduced at room temperature and increased upon heating, as highlighted by the increase in intensity and the narrowing of the PTMC ^{1}H signals with increasing temperature (Figure 4). However, whereas the line width progressively decreased with increasing temperature up to 70 °C, the intensities reached a plateau value at 40 °C corresponding to 65, 90, and 100% of PTMC protons for the 10, 1, and 0.1% w/v samples, respectively. The intensity and line width of the PEG ^{1}H signal did not change throughout the temperature range investigated. The solution state ^{13}C NMR spectra, although acquired with experimental parameters that do not guarantee quantitative results, showed temperature trends similar to those described for the ^{1}H spectra. (See Figure 4.) The increase in undetected PTMC units with increasing concentration suggests an increase in aggregate dimensions.

Solid-state ^{13}C NMR experiments were performed on a 50% w/v sample of PEG-$(PTMC_9)_8$ in D_2O to investigate the degree of rigidity of the PTMC aggregates (Figure 5). The DE spectra recorded with a short and a long recycle delay were identical, with the relative intensities of the different peaks reflecting the copolymer stoichiometry; furthermore, the CP spectrum showed very little intensity. These findings concur to put in evidence a high mobility of the PTMC chains, differently from what was observed for PEG-PLA star block copolymers.[20] This behavior is not surprising because it reflects the high mobility of PTMC in the solid state, as shown by the DE and CP spectra recorded on a sample of dry PEG-$(PTMC_9)_8$ (Figure 5). In fact, as in the hydrated sample, the DE spectra recorded with a short and a long recycle delay were identical, with peaks relative to PTMC at 28.02, 31.54, 64.74, and 155.18 ppm. The CP spectrum showed for PTMC only narrow peaks ascribable to a minor crystalline component, at 25.58, 31.10, 62.41, 63.45, and 154.62 ppm, not distinguishable in the DE spectra because of their very small intensity with respect to the overlapping broader resonances of the dominant amorphous component.

The aggregation behavior of PEG-$(PTMC_9)_8$ in water was further investigated using oscillatory rheology.[26] In Figure 6, the plateau value of G', denoted as G'_0, which is the value determined in the region where the storage modulus is nearly independent of frequency, is plotted as a function of concentration. For comparison, $(PTMC_{30})$-PEG-$(PTMC_{30})$ triblock (M_n 3000−8000−3000 g/mol) and PEG-$(PLA_{13})_8$ star block copolymers were investigated as well. First of all, PEG-$(PTMC_9)_8$ showed weak elasticity with G'_0 values on the order of 10−100 Pa, significantly lower than those found for the other systems. Moreover, a very different dependence of G'_0 on concentration was found for the three copolymers. In fact, the PEG-$(PLA_{13})_8$ star block copolymer showed a linear dependence, whereas the other copolymers showed a power law dependence of the type $G'_0 \approx C^a$, with $a = 5.2$ for $(PTMC_{30})$-PEG-$(PTMC_{30})$ and $a = 13.8$ for PEG-$(PTMC_9)_8$. Indeed a linear dependence of G'_0 on concentration is predicted by the transient network theory, according to which the plateau modulus is given by $G'_0 = gNk_BT$,

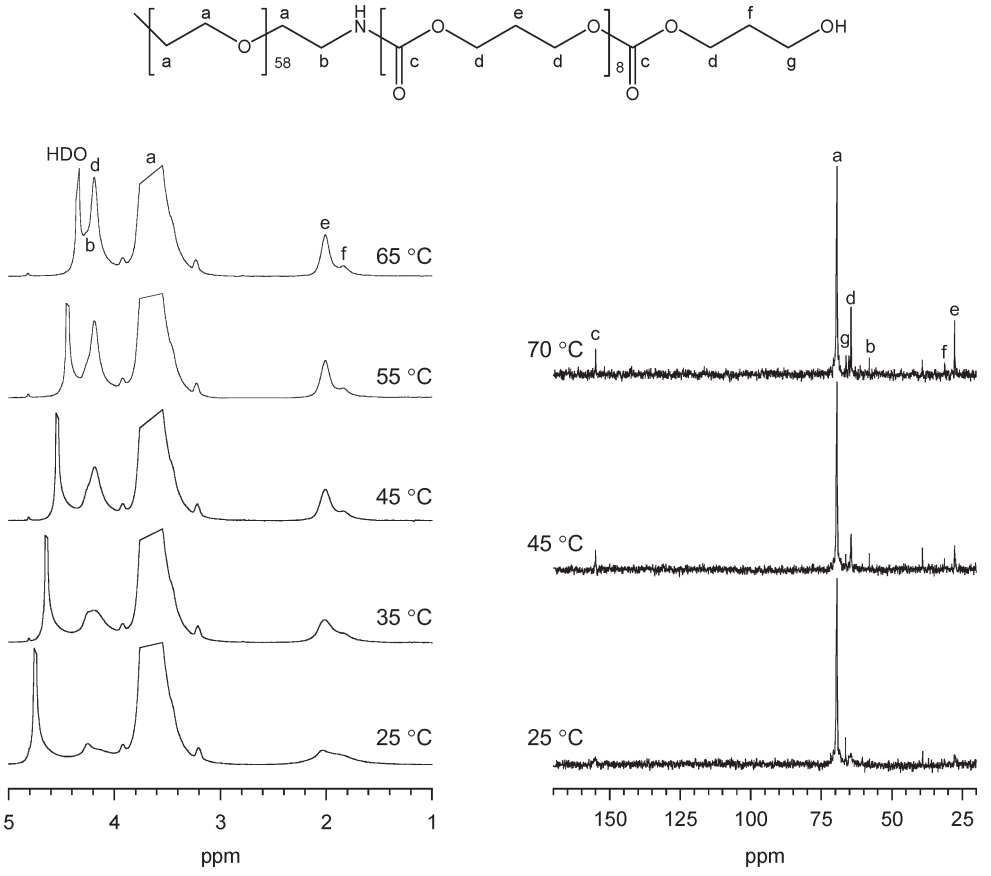

Figure 4. ^{1}H (left) and ^{13}C (right) NMR spectra of 10% w/v PEG-$(PTMC_9)_8$ in D_2O at different temperatures with peak assignment. Unassigned small peaks belong to impurities.

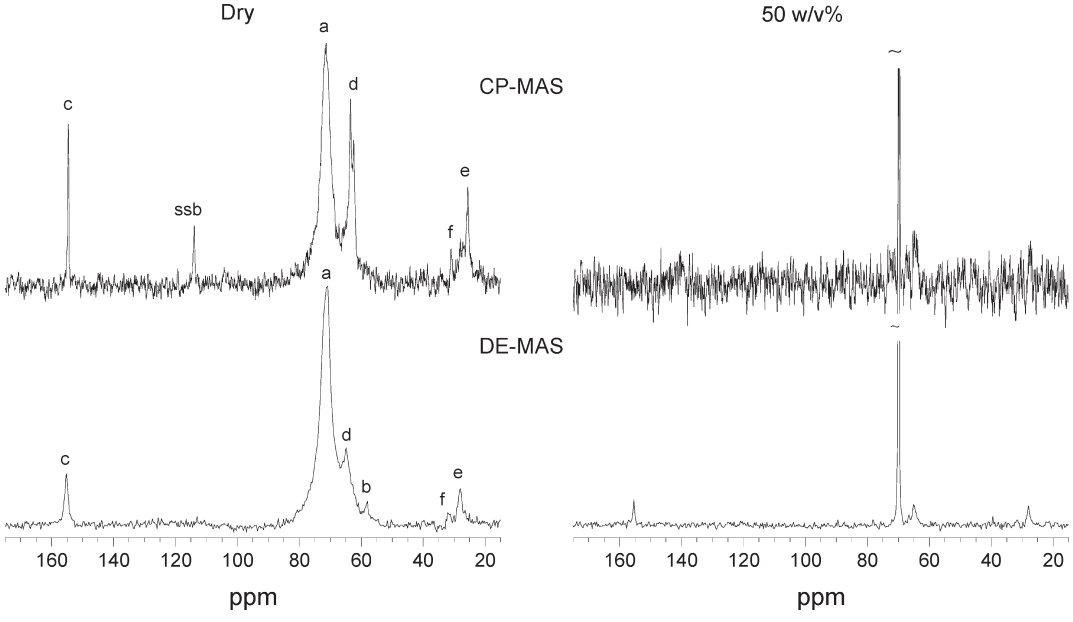

Figure 5. ^{13}C CP and DE MAS NMR spectra of dry PEG-$(PTMC_9)_8$ and of a 50% w/v PEG-$(PTMC_9)_8$ sample in D_2O at room temperature. CP and DE spectra were recorded acquiring 1600 and 800 scans, respectively. Letters refer to the formula in Figure 4.

where N is the number density of polymer chains and g is a correction factor denoting the fraction of elastically active chains, which does not change with concentration.[27] In the case of PEG-$(PLA_{13})_8$ star block copolymer, the application of this theory gave $g = 0.13$, corresponding to one bridging chain per molecule. The power law found for the other systems suggests a

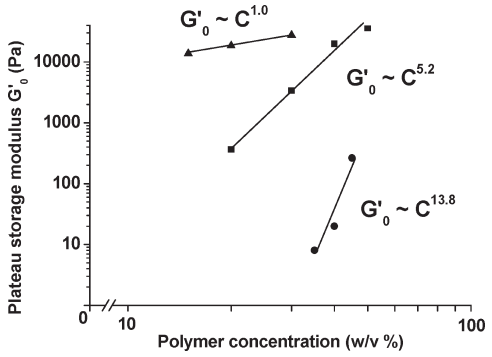

Figure 6. Plateau storage modulus G'_0 as a function of concentration at 25 °C. PEG-$(PTMC_9)_8$ (●), $(PTMC_{30})$-PEG-$(PTMC_{30})$ (■), and PEG-$(PLA_{13})_8$ (▲). Lines represent fitting curves.

different network topology in which the fraction of elastically active chains increases with concentration. Values of *a* similar to that found for $(PTMC_{30})$-PEG-$(PTMC_{30})$ are reported in the literature for ABA telechelic polymer and polyampholyte hydrogels,[26,28] protein gels,[29] and poly(*N*-isopropylacrylamid) (PNiPAM) microgels.[30] The value determined for PEG-$(PTMC_9)_8$ is exceptionally high. The high exponent found for these systems could be better rationalized within theories for aggregation of colloidal particles in terms of either fractals[31] or soft spheres.[30]

Combining results from DLS, NMR, and rheology measurements, a tentative description of the aggregation behavior of PEG-$(PTMC_9)_8$ in water can be given. On the basis of DLS findings, PEG-$(PTMC_9)_8$ forms aggregates with minimal dimensions of ~200 nm, already well below the CAC. The aggregate size distribution broadens with increasing concentration, becoming clearly bimodal for concentrations >1% w/v. Moreover, the average size increases with concentration, reaching micrometer dimensions (Figure 3). In contrast with the analogous eight-armed PEG-PLA star block copolymers,[20,32] no aggregates with dimensions on the order of a few tens of nanometers, compatible with core−shell type micelles or flower-like micelles, were detected. This result, added to the very low CAC value, is evidence of the formation at low concentration of large micelles with dimensions of ~200 nm, probably resulting from secondary aggregation.[33] Although with increasing concentration further aggregation of these large micelles occurs and the system becomes weakly elastic, there is no evidence of gel formation up to high concentrations (60% w/v). Indeed, the observed rheological properties are similar to those reported for microgel and colloid dispersions.[30] The very different aggregation behavior observed for PEG-$(PTMC_9)_8$ with respect to PEG-$(PLA_n)_8$ can be ascribed to the differences in interactions between chains displayed by PTMC and PLA. Evidence of this was obtained from NMR experiments, which clearly showed that PTMC chains are quite mobile within the aggregates also at high concentration (Figure 5), whereas PLA chains form rigid aggregates, even at concentrations below the CAC.[32] The close packing of micelles or aggregates, which is required for gelation, is possibly hampered by the amorphous nature of and possibly low van der Waals interactions between the PTMC blocks in comparison with PLA blocks.

Chemically Cross-Linked Nanoparticles and Hydrogels. When dilute aqueous PEG-$(PTMC_9)_8$-Acr dispersions (<5% w/v) were subjected to UV irradiation, covalently cross-linked nanoparticles were obtained. An SEM image of the nanoparticles,

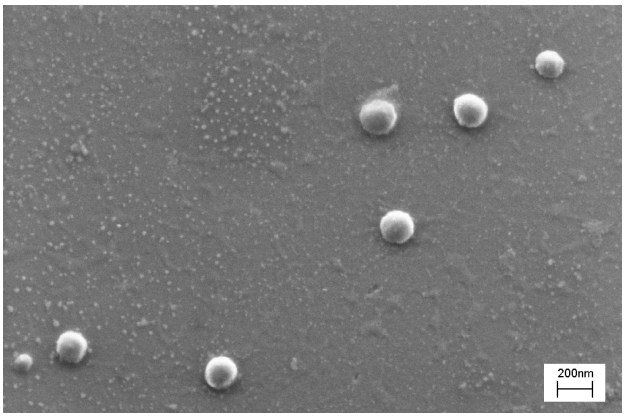

Figure 7. SEM image of PEG-PTMC nanoparticles prepared by UV irradiation of a 1% w/v mixture of PEG-$(PTMC_9)_8$-Acr in water.

which have a diameter of ~200 nm, is presented in Figure 7. The average particle size, determined by DLS, yielded an average diameter of 240 nm. Comparing the slightly higher value in the hydrated state to the value determined by SEM (200 nm) suggests that these particles do not swell considerably in water.

NMR spectroscopy was used to investigate the formation of the covalently cross-linked PEG-PTMC nanoparticles in more detail. An aqueous dispersion (1% w/v) of PEG-$(PTMC_9)_8$-Acr was UV-irradiated in the presence of Irgacure 2959 as a photoinitiator. The nanoparticles were subsequently lyophilized and redispersed in $CDCl_3$. The ^1H NMR spectrum ($CDCl_3$) of the covalently cross-linked PEG-PTMC nanoparticles revealed that the vinylic protons disappeared upon UV irradiation (data not shown). Furthermore, the relative intensity of the PTMC methylene signal at 4.28 ppm decreased three-fold in comparison with the starting PEG-$(PTMC_9)_8$-Acr star block copolymer. In aqueous environment, the hydrophobic PTMC blocks and the acrylate end groups are condensed into hydrophobic domains. Upon UV irradiation, the free radical polymerization of acrylate groups into polyacrylate chains will mainly occur in these domains. It is expected that after UV irradiation because of the cross-links introduced the PTMC domains are confined to the interior of the particle even in a good solvent such as $CDCl_3$, which results in a decreased PTMC signal intensity. The absence of any signals typical for polyacrylate chains may be explained analogously. The results show that UV irradiation of dilute aqueous PEG-$(PTMC_9)_8$-Acr dispersions represents a facile method for the preparation of chemically cross-linked PEG-PTMC nanoparticles. Studies exploring the applicability of covalently cross-linked PEG-PTMC nanoparticles as drug delivery vehicles are currently in progress.

Photopolymerization of an aqueous dispersion of PEG-$(PTMC_9)_8$-Acr at concentrations >5% w/v yielded chemically cross-linked star block copolymer hydrogels. In preliminary experiments, the effects of UV wavelength, reaction time, and initiator concentration on the gel formation and the mechanical properties were determined. The PEG-$(PTMC_9)_8$-Acr was dispersed in water at a concentration of 40% w/v and photopolymerized under various conditions. Oscillatory rheology measurements were then performed at 25 °C by monitoring the storage (G') and loss (G'') modulus of the preformed hydrogels. It followed that an initiator concentration of 10 mol % relative to acrylate groups, an UV irradiation time of 120 min, and an UV irradiation wavelength of 365 nm resulted in the

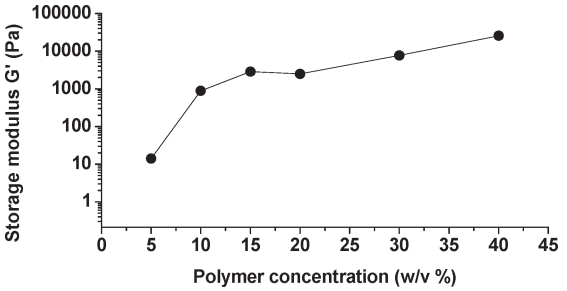

Figure 8. Storage modulus G' of PEG-$(PTMC_9)_8$-Acr hydrogels as a function of the initial polymer concentration at 25 °C.

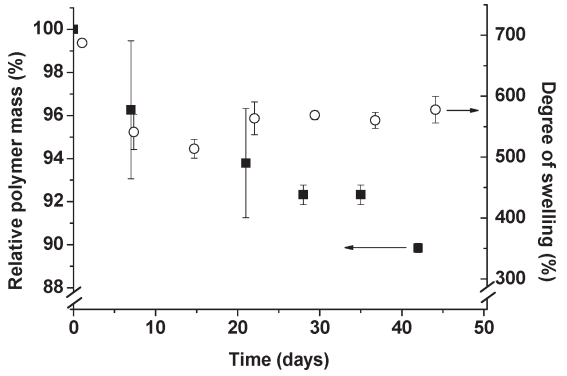

Figure 9. Relative polymer mass loss and degree of swelling of PEG-$(PTMC_9)_8$-Acr hydrogels in PBS at 37 °C.

highest hydrogel stiffness. The effect of the polymer concentration on hydrogel stiffness was investigated using these optimized irradiation conditions (Figure 8). The increase in G' with concentration can be ascribed to the formation of a more densely cross-linked network. These rheological experiments indicate that hydrogels can be designed with storage moduli up to 26 kPa.

Hydrogel Degradation and Cytotoxicity. The in vitro degradation of chemically cross-linked PEG-$(PTMC_9)_8$-Acr hydrogels was investigated by a gravimetrical procedure. The swelling of hydrogels during degradation as well as the polymer mass loss were monitored up to 6 weeks (Figure 9). Hydrogel samples were prepared at a 40% w/v polymer concentration in water and then immersed in PBS at 37 °C. At regular time intervals, samples were taken out and their mass in the swollen state was determined. Subsequently, the samples were allowed to dry overnight to yield the dry polymer weight after degradation. It follows from Figure 9 that the PEG-$(PTMC_9)_8$-Acr hydrogels possess excellent stability in PBS, showing only 10% mass loss after 6 weeks. Such a relatively slow degradation rate is commonly observed for PTMC homopolymers[34] and PEG-PTMC block copolymers[12] in buffer solution and can be ascribed to the high hydrolytic stability of the carbonate linkage at physiological pH. The swelling of the hydrogels remains constant at ~550%. The slight decrease in swelling during the first week of immersion may be due to the loss of non-cross-linked material. The observed mass loss is likely due to bulk degradation as a result of the slow hydrolysis of carbonate groups in the PTMC domains. An adequate ^{1}H NMR end-group analysis could not be performed because the expected hydroxy propyl end groups coincide with the PEG signal.

The addition of lipase, an enzyme that has been shown to degrade PTMC polymers,[35] did not significantly alter the

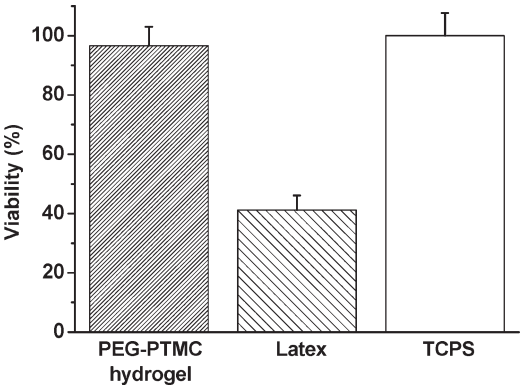

Figure 10. Viability of chondrocytes in contact with PEG-$(PTMC_9)_8$-Acr hydrogel or latex for 20 h. Control viability (cells grown on TCPS) was taken as 100%.

degradation behavior of the PEG-$(PTMC_9)_8$-Acr networks (data not shown). This is possibly due to the low molecular weight of the PTMC blocks in these PEG-$(PTMC_9)_8$-Acr networks because it was reported that the enzymatic degradation rate of PTMC films decreases with decreasing PTMC molecular weight.[36] On water-insoluble substrates, at water−lipid interfaces, and on hydrophobic supports, the conformation of the lipase molecule is open and the structure is hyperactive.[37,38] In the absence of such an interface, however, the active center of the lipase molecules is buried under a short helical segment.[39] Because of the high PEG content of the PEG-$(PTMC_9)_8$-Acr hydrogels, the slow enzymatic degradation may therefore also be the result of a low lipase activity.

The direct contact method was used to assess the cytotoxicity of photo-cross-linked PEG-$(PTMC_9)_8$-Acr hydrogels. The viability of bovine cartilage chondrocytes in contact with the PEG-$(PTMC_9)_8$-Acr hydrogels for 20 h is similar to the viability of cells grown on a TCPS substrate (Figure 10). Cells grown in contact with latex rubber, a positive control, exhibited a significant reduction in viability. The obtained results indicate that photo-cross-linked PEG-$(PTMC_9)_8$-Acr hydrogels are nontoxic to bovine cartilage chondrocytes.

■ CONCLUSIONS

A PEG-$(PTMC_9)_8$ star block copolymer was prepared by ring-opening polymerization of TMC initiated by PEG-$(NH_2)_8$ in the presence of HCl as a catalyst. Dye solubilization experiments showed that aqueous dispersions of the copolymer form aggregates at very low concentrations, and DLS experiments on PEG-$(PTMC_9)_8$ in water afforded aggregate sizes with a minimum dimension of 200 nm, corresponding to large micelles resulting from secondary aggregation. Smaller micelles with dimensions on the order of a few tens of nanometers, present in hydrogel-forming systems such as PEG-$(PLA_n)_8$, were not detected. Oscillatory rheology measurements at high concentrations showed properties similar to those reported for microgel and colloid dispersions. NMR experiments clearly showed that PTMC chains are quite mobile within the aggregates, and DLS experiments revealed fast diffusion of macromolecules between aggregates. The short residence time of the PTMC blocks in the hydrophobic regions appears thus too short to obtain a stable network.

PEG-$(PTMC_9)_8$ was functionalized with acrylate end groups and photopolymerized in the presence of Irgacure 2959. UV irradiation of dilute (<5% w/v) PEG-$(PTMC_9)_8$-Acr dispersions resulted in covalently cross-linked PEG-PTMC nanoparticles, whereas more concentrated dispersions yielded chemically cross-linked PEG-PTMC hydrogels. Rheological experiments showed that hydrogels can be designed with storage moduli up to 26 kPa by varying the precursor concentration and the irradiation conditions. Gravimetrical degradation experiments revealed that PEG-PTMC star block copolymer hydrogels possess excellent in vitro stability with 10% mass loss after 6 weeks. Furthermore, cytotoxicity experiments indicated that photo-cross-linked PEG-PTMC hydrogels are nontoxic to chondrocytes. This study shows that photo-cross-linkable PEG-$(PTMC_9)_8$-Acr represents a versatile system that holds promise for biomedical applications such as controlled drug delivery systems and matrices for tissue engineering.

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REFERENCES

(1) Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 27–46.
(2) Drury, J. L.; Mooney, D. J. *Biomaterials* **2003**, *24*, 4337–4351.
(3) Hennink, W. E.; van Nostrum, C. F. *Adv. Drug Delivery Rev.* **2002**, *54*, 13–36.
(4) Ifkovits, J. L.; Burdick, J. A. *Tissue Eng.* **2007**, *13*, 2369–2385.
(5) Sawhney, A. S.; Pathak, C. P.; Hubbell, J. A. *Macromolecules* **1993**, *26*, 581–587.
(6) Jain, A. K.; Goyal, A. K.; Gupta, P. N.; Khatri, K.; Mishra, N.; Mehta, A.; Mangal, S.; Vyas, S. P. *J. Controlled Release* **2009**, *136*, 161–169.
(7) Matsumura, S.; Harai, S.; Toshima, K. *Macromol. Rapid Commun.* **2001**, *22*, 215–218.
(8) Kim, B. S.; Oh, J. M.; Cho, J. S.; Lee, S. H.; Lee, B.; Khang, G.; Lee, H. B.; Kim, M. S. *J. Appl. Polym. Sci.* **2009**, *111*, 1706–1712.
(9) Morinaga, H.; Ochiai, B.; Mori, H.; Endo, T. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 1985–1996.
(10) Kim, S. Y.; Kim, H. J.; Lee, K. E.; Han, S. S.; Sohn, Y. S.; Jeong, B. *Macromolecules* **2007**, *40*, 5519–5525.
(11) Bat, E.; Grijpma, D. W.; Feijen, J. *J. Controlled Release* **2008**, *132*, e37–e39.
(12) Matsuda, T.; Kwon, I. K.; Kidoaki, S. *Biomacromolecules* **2004**, *5*, 295–305.
(13) Hiemstra, C.; Zhong, Z.; Dijkstra, P. J.; Feijen, J. *Macromol. Symp.* **2005**, *224*, 119–131.
(14) Elbert, D. L.; Hubbell, J. A. *Biomacromolecules* **2001**, *2*, 430–441.
(15) Alexandridis, P.; Holzwarth, J. F.; Hatton, T. A. *Macromolecules* **1994**, *27*, 2414–2425.
(16) Geppi, M.; Forte, C. *J. Magn. Reson.* **1999**, *137*, 177–185.
(17) Risbud, M.; Saheb, D. N.; Jog, J.; Bhonde, R. *Biomaterials* **2001**, *22*, 1591–1597.
(18) Hyun, H.; Kim, M. S.; Khang, G.; Lee, H. B. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 4235–4241.
(19) Metters, A. T.; Anseth, K. S.; Bowman, C. N. *Polymer* **2000**, *41*, 3993–4004.
(20) Buwalda, S. J.; Dijkstra, P. J.; Calucci, L.; Forte, C.; Feijen, J. *Biomacromolecules* **2010**, *11*, 224–232.
(21) Kim, M. S.; Hyun, H.; Kim, B. S.; Khang, G.; Lee, H. B. *Curr. Appl. Phys.* **2008**, *8*, 646–650.
(22) Zhang, H.-H.; Huang, Z.-Q.; Sun, B.-W.; Guo, J.-X.; Wang, J.-L.; Chen, Y.-Q. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 8131–8140.
(23) Hyun, H.; Lee, J. W.; Cho, J. S.; Kim, Y. H.; Lee, C. R.; Kim, M. S.; Khang, G.; Lee, H. B. *Colloids Surf., A* **2008**, *313−314*, 131–135.
(24) Feng, J.; Su, W.; Wang, H.-F.; Huang, F.-W.; Zhang, X.-Z.; Zhuo, R.-X. *ACS Appl. Mater. Interfaces* **2009**, *1*, 2729–2737.
(25) Cho, J. S.; Kim, B. S.; Hyun, H.; Youn, J. Y.; Kim, M. S.; Ko, J. H.; Park, Y. H.; Khang, G.; Lee, H. B. *Polymer* **2008**, *49*, 1777–1782.
(26) Stavrouli, N.; Aubry, T.; Tsitsilianis, C. *Polymer* **2008**, *49*, 1249–1256.
(27) Tsitsilianis, C.; Iliopoulos, I. *Macromolecules* **2002**, *35*, 3662–3667.
(28) Bossard, F.; Sfika, V.; Tsitsilianis, C. *Macromolecules* **2004**, *37*, 3899–3904.
(29) Renkema, J. M. S.; van Vliet, T. *Food Hydrocolloids* **2004**, *18*, 483–487.
(30) Senff, H.; Richtering, W. *J. Chem. Phys.* **1999**, *111*, 1705–1711.
(31) Chae, B. S.; Lee, Y. S.; Jhon, M. S. *Colloid Polym. Sci.* **2004**, *282*, 236–242.
(32) Calucci, L.; Forte, C.; Buwalda, S. J.; Dijkstra, P. J.; Feijen, J. *Langmuir* **2010**, *26*, 12890–12896.
(33) Hong, H. Y.; Mai, Y. Y.; Zhou, Y. F.; Yan, D. Y.; Cui, J. *Macromol. Rapid Commun.* **2007**, *28*, 591–596.
(34) Kluin, O. S.; van der Mei, H. C.; Busscher, H. J.; Neut, D. *Biomaterials* **2009**, *30*, 4738–4742.
(35) Zhang, C.; Subramanian, H.; Grailer, J. J.; Tiwari, A.; Pilla, S.; Steeber, D. A.; Gong, S. *Polym. Adv. Technol.* **2009**, *20*, 742–747.
(36) Zhang, Z.; Kuijer, R.; Bulstra, S. K.; Grijpma, D. W.; Feijen, J. *Biomaterials* **2006**, *27*, 1741–1748.
(37) Winkler, F. K.; D'Arcy, A.; Hunziker, W. *Nature* **1990**, *343*, 771–774.
(38) Bastida, A.; Sabuquillo, P.; Armisen, P.; Fernandez-Lafuente, R.; Huguet, J.; Guisan, J. M. *Biotechnol. Bioeng.* **1998**, *58*, 486–493.
(39) Brady, L.; Brzozowski, A. M.; Derewenda, Z. S.; Dodson, E.; Dodson, G.; Tolley, S.; Turkenburg, J. P.; Christiansen, L.; Huge-Jensen, B.; Norskov, L.; Thim, L.; Menge, U. *Nature* **1990**, *343*, 767–770.