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Glycerol based polyether-nanogels with tunable properties via acid-catalyzed epoxide-opening in miniemulsion

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ABSTRACT

A series of defined nanogels of 20–120 nm in diameter were synthesized by acid-catalyzed epoxid-opening polymerization based on glycerol in miniemulsion. Multifunctional alcohols were used as monomers and di- and triepoxides as crosslinking agents. The properties of these nanogels, i.e., size, degree of branching, viscosity, and swelling behavior, can be controlled by varying the functionalities of the monomers and crosslinkers. Inverse gated ^{13}C NMR indicated that the addition of monomer occurred at both ends of the opened epoxide ring of the crosslinkers. This feature led to higher degree of branching and consequently to a lower viscosity of the resulting nanogels. The formation of some cycles as a possible side reaction was evidenced by different particle sizes in dry (TEM) and swollen states (DLS in water).

1. Introduction

Over the last decade hyperbranched aliphatic polyethers have gained widespread attention because of their high biocompatibility and solubility in water [1,2]. These macromolecules typically exhibit compact, globular structures in combination with a high number of functional groups. Therefore a wide range of applications have been foreseen for them in biomedical science, which include solubility enhancement, MRI contrast agents, neutron capture therapy, gene therapy, drug delivery, and photodynamic therapy [3,4]. Water soluble hyperbranched polyglycerols (hPGs), which are built of glycerol units, have played a major role here.

In 1999 Sunder et al. reported the use of latent AB_2 monomers for a ring-opening multi-branching polymerization following single monomer methodology (SMM) which is based on polymerization of AB_x-type monomers [5]. Polymerization of such monomers results in highly branched polymers, as long as A only reacts with B from another molecule. Intramolecular reaction between A and B results in termination of polymerization by cyclization [6]. This technique is interesting for polymer chemistry, because it hinders the formation of by-products. PG with the highest number-average molecular weight of 700 kDa and a particle size of approximately 10 nm were reported by Brooks et al. in 2006 [7]. In 2009 Frey et al. achieved a molecular weight of 24 kDa in a two-step approach using a low molecular weight PG with low polydispersity index (PDI) as a macroinitiator [8].

Particles ranging in size from 20 to 100 nm, however, are desired for several biomedical applications [1,3]. The above-mentioned approaches show that classical methods do not yield particles of the required size [9,10]. Thus the PG synthesis needs to be extended to larger polyether particles with controlled physical properties. Recently, our group reported polyglycerol nanogels with controllable sizes that were between 25 and 85 nm in diameter using inexpensive, commercially available monomers [11,12] or dendritic precursors [13] in miniemulsion. Degradable particles have also been obtained [14]. These nanogels are completely soluble in polar solvents including water and show promising results in cellular uptake studies due to their defined size and high biocompatibility.

Unlike the classical SMM based on glycidol, we followed the double monomer methodology (DMM), which was first reported in the work of Kakimoto [15] and Fréchet [16] on hyperbranched polymers via the $A_2 + B_3$ approach. We recently extended this approach to A_n and B_m. Copolymerization of A_n and B_m or other multifunctional monomers can also give rise to hyperbranched polymers, provided that the polymerization is kept below the gel point by limiting polymer conversion or by manipulation of the multifunctional monomer stoichiometry [6,17,18]. Exceeding the critical condition leads to undesired gelation, which can become beneficial when controlled by miniemulsion techniques.

Miniemulsions are specially formulated heterophase systems consisting of nanodroplets in a continuous phase. They can be

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fabricated by shearing a system containing two immiscible liquids, a surfactant, and an osmotic pressure agent, which is soluble in the dispersed phase but not in the continuous phase [19]. After rapid stirring and treatment with a sonicator the mixture becomes a dispersion containing rather monodisperse droplets of 50–500 nm in diameter [11,13]. Each droplet acts as a discrete nanoreactor vessel, in which polymerization takes place. The resulting polymer particles ought to represent 1:1 copies of the dispersed droplets [20]. This last feature allows a wide range of well-defined materials to be prepared in an efficient way (Fig. 1).

Nanoparticles with unique physical properties have been shown to influence many vital interactions with the body including phagocytosis, circulation, targeting, and adhesion [21]. We have recently demonstrated that PG nanogels of 20–50 nm have ideal particle sizes for cellular uptake.

Here we report on the physical properties of polyether-nanogels. For this purpose a series of branched polyethers has been synthesized by crosslinking a mixture of A_n and B_m building blocks as monomer and crosslinking agents respectively in miniemulsion in order to obtain nanogels with properties that are tunable and suited for different applications.

2. Experimental section

2.1. Materials

General chemicals were purchased from Acros Organics and Raschig. The water used was Millipore filtered.

Poly(ethylene-*co*-butylene)-*block*-poly(ethylene oxide) (KLE = Kraton Liquid™-*block*-PEO, number-average molecular weight: 8.1 kDa, 41 wt.% PEO), was prepared and used according to published procedures [22,23]. Emulsions were sonicated using a sonicator, W-220f, with microtip on 70% intensity (company: Heat Systems-Ultrasonics, Inc.) Transmission electron microscopy samples were prepared on copper grids (200 mesh) by blotting samples in 1% aqueous phosphotungstic acid and visualized using a Philips CM12 electron microscope. Dynamic light scattering measurements were conducted using a BioDLS particle sizer (Brookhaven Instruments Corp.) The viscosity measurements of the nanogels were performed by a Schott Ubbelohde viscometer. The viscosities of aqueous solutions of concentrations ranging from 1 to 10 mg/mL were measured at 37 °C. Plotting η_{sp}/c versus c revealed their intrinsic viscosity [η]. Each measurement was repeated at least three times to calculate the average value. Estimation of OH amount were performed by titration according to DIN 53240-2.

^{1}H-NMR and inverse gated ^{13}C NMR spectra were recorded on a Bruker AV 700 (700 MHz for ^{1}H, 176 MHz for ^{13}C) instrument. Samples were dissolved in CD_3OD and measured at room temperature. Chemical shifts δ are given in ppm relative to TMS as an internal standard or relative to the resonance of the solvent (^{1}H NMR: methanol: δ = 3.34 ppm ^{13}C NMR: methanol: δ = 49.05 ppm). Inverse gated ^{13}C NMR were performed with following parameter: delay time = 10 s, acquisition time = 0.78 s, number of scans = 1024–4096, depending on sample concentration (80–250 mg in 0.7 mL solvent).

2.2. General procedure for the preparation of particles 1–7 (example given for product 1)

A solution of the KLE surfactant (20 mg, 0.0025 mmol) in cyclohexane (15 mL) was vigorously stirred in a cylindrical 30-mL vial for 30 min. A mixture of A_n, B_m, and 0.2 mL DMSO was added to the cyclohexane phase. The amount of A_n and B_m was calculated so that the ratio between the hydroxyl and epoxide groups was 3:2 and the total mass of A_n and B_m was 1 g. The mixture was stirred vigorously for 1 h.

Afterwards a slightly turbid macroemulsion was formed, which would have otherwise rapidly separated. The formed macroemulsion was ultrasonicated for one minute with a sonic tip apparatus four times under water cooling to form a fully homogenous miniemulsion. (The resultant miniemulsion was stable for several hours.) The miniemulsion was transferred to the resealable tube charged with a catalytic amount of para-toluene sulfonic acid (p-TSA). The miniemulsion was heated under stirring at 120 °C for 16 h. Finally the reaction was quenched with water and heated for 2 h.

After cooling to room temperature, the nanogels were precipitated as a honey like mass upon addition of *n*-hexane (30 mL). They were then purified from the surfactant by solid/liquid extraction, which was performed three times with *n*-hexane. Separation of the product from lower molecular impurities was carried out by ultracentrifugation in ultracentrifuge tubes with 10 kDa MWCO. Yields of isolated polymer ranged between 10% and 50%.

^{1}H NMR (CD_3OD, 700 MHz): δ (ppm) = 4.00–3.40 (m, PG backbone). ^{13}C NMR (CD_3OD, 176 MHz): δ (ppm) = 62.6 (L_{13}, CH_2OH); 64.4(T, CH_2OH); 70.6 (L_{13}, CH_2); 70.7 (L_{14}, CHOH); 72.2 (T, CHOH and CH_2); 72.2 (D, CH_2); 73.9 (L_{14}, CH_2); 80.3 (D, CH); 81.5 (L_{13}, CH).

3. Results and discussion

3.1. Synthesis

The synthesis of the nanogels was performed according to the procedure described above. The reaction is illustrated schematically in Fig. 2. Different combinations of A_n and B_m building blocks are listed in Table 1. The applied reaction is based on a polyaddition of an alcohol to an epoxide by acid catalysis. The nanogels were synthesized from cheap and commercially available glycerol/oligoglycerols as monomers and bis-/trisepoxides as crosslinker.

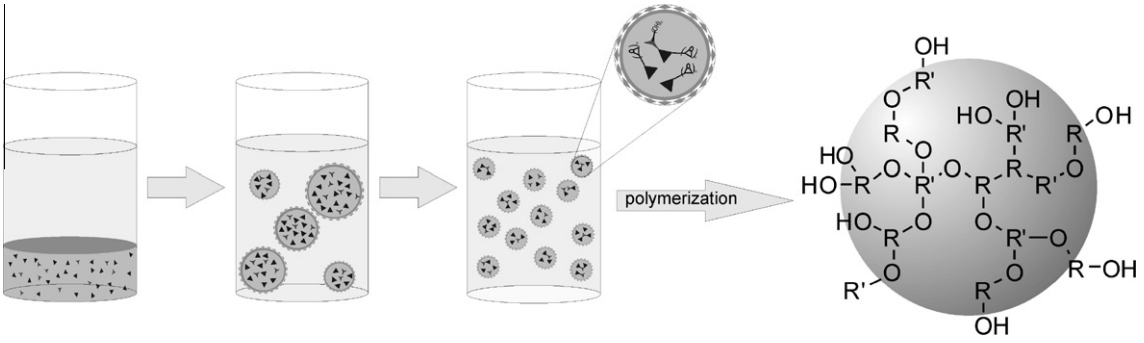

Fig. 1. Schematic representation of the synthesis of glycerol based nanogels via inverse miniemulsion.