Functional Nanoparticles from Dendritic Precursors: Hierarchical Assembly in Miniemulsion

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Introduction

Hyperbranched polymers,[1] as a result of inherent dendritic topology, attract considerable interest for a wide range of optical,[2] medical,[3] material,[4] and reagent immobilization applications.[5] Indeed, in most cases hyperbranched polymers can substitute the use of perfect dendrimer supports with the benefit of hyperbranched polymers having a considerably easier synthesis.

Hyperbranched polyglycerols (HPG) have been known since Vandenberg reported the anionic polymerization of glycidol in the 1980s, a process which was further refined to afford relatively monodisperse, highly branched products (degree of branching (DB) typically around 60%) with a controllable M_n in the range of a few thousand daltons.[6] Recently, Brooks extended this approach to HPGs with up to 1 MDa molecular weight by conducting the polymerization in bulk heterophase with an emulsifying nonsolvent preventing the formation of higher, insoluble products.[7] Such products are densely packed and have a diameter in the order of ∼10 nm. We here present a new approach, utilizing miniemulsion polymerization, to create larger HPG analogues, targeting an optimum diameter of 50 nm. This size range we consider to be ideal for drug delivery vehicles that may accumulate in tumor/inflamed tissue by the enhanced permeation and retention effect (EPR).[8] Supramolecular aggregates of HPG derivatives in this size range have been studied within our laboratory and have shown promise toward this aim.[9] Studies show that HPGs have favorably low toxicity and may be considered in the same class as poly(ethylene glycol)s in this regard.[10]

Miniemulsion polymerization makes use of surfactant stabilized dispersed droplets as "nanoreactors" in which it is possible to conduct reactions with fairly unlimited scope depending on the conditions selected.[11] The dispersed phase can be hydrophobic, in so-called direct miniemulsions, where the continuous phase is typically aqueous. Conversely, a hydrophilic phase dispersed in a hydrophobic medium is called an inverse miniemulsion. Since miniemulsion droplets are by definition in the size range of approximately 10−500 nm in diameter, they are suited to our purpose. Whereas the majority of miniemulsion reactions are reported between low molecular weight monomers, giving rise to linear polymers via radical or polyaddition processes, we envisaged an approach to cross-link existing (2−3 nm diameter, $M_n \approx 5$ kDa) HPG dendritic macromonomers to higher homologues using the "nanoreactor" template to control size. For this aim we chose a facile cross-linking "click" reaction, the Huisgen alkyne/azide cycloaddition.[12] This general concept of utilizing dendritic building blocks to produce nanoscale objects termed as "megamers" was recently highlighted by Tomalia,[13] A similar approach has been reported with miniemulsion ATRP cross-linking of macromoners by Matyjasewski.[14] The Huisgen reaction has been used in macroemulsion to cross-link dextran macromoners, but this approach is not widely realized in miniemulsion.[15]

Results and Discussion

Following our approach, we successfully prepared both hydrophobic and hydrophilic HPG-based nanoparticles in direct and inverse miniemulsion, respectively. The route to hydrophobic particles is shown in Scheme 1. 5000 g/mol in molecular weight, 60% DB HPG[16] was functionalized on 80% of the free hydroxyl groups with propargyl bromide. The subsequent poly(propargyl ether) (**1**) and decane diazide (**2**) as a cross-linker (3:1 alkyne/azide ratio) were dissolved in chloroform and miniemulsified in water with sodium dodecyl sulfate (SDS) as surfactant. Catalytic $CuSO_4 \cdot 5H_2O$/sodium ascorbate was added to the aqueous phase, and the system was heated to 60 °C for 3 h to remove chloroform and initiate the cross-linking polymerization in bulk nanodeposits. Spherical particles were obtained as a colloidal suspension in water stabilized by the remaining surfactant. As evidenced by dynamic light scattering (DLS) and transmission electron microscopy (TEM) measurements, the nanoparticles can be precipitated by acetone addition and fully resuspended in dilute SDS solution; suspensions were stable for several months.

As shown in Table 1, the size of particle obtained could be tuned in a rational manner by varying the ratio of surfactant to dispersed phase volume. The diameter size range could be varied between 25 and 85 nm with low polydispersity satisfactory for most further applications. Recovery yields varied from quantitative for the small particle cases to somewhat lower (65%) when preparing larger particles. Lost material precipitated from the aqueous suspension presumably due to droplet coalescence and the formation of larger aggregates.

Hydrophilic HPG-based nanoparticles were prepared by a slightly modified procedure outlined in Scheme 2. Complementary macromonomers, 5000 g/mol in molecular weight, 60% DB HPG with in one case 30% (relative to free OH groups) azido (**7**) and in the other case 30% propargyl (**8**) functionality, were mixed in 1:1 ratio in DMF and miniemulsified in cyclohexane with the aid of a poly(ethylene-*co*-butylene)-*block*-poly(ethylene oxide) surfactant (P(B/E−EO)).[17] A small amount of propargyl-functionalized hemicyanine dye (**9**) was added to the system as a proof of concept that other moieties could be incorporated into nanoparticle formation; omission of this dye was found to have no discernible effect on the product structure, however.[18] The $CuSO_4 \cdot 5H_2O$/sodium ascorbate system was ineffective as catalyst in this heterogeneous system; however, our experiments showed that efficient copper-free "click" coupling can occur using thermal initiation in miniemulsion conditions. After heating to 80 °C for 16 h in a sealed tube, stable nanoparticles are formed which are precipitated by

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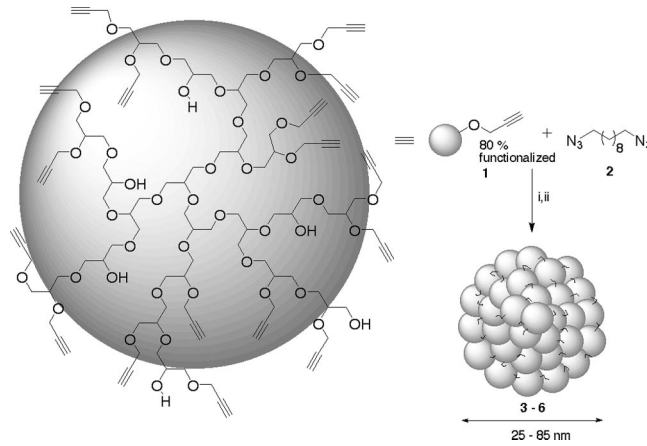
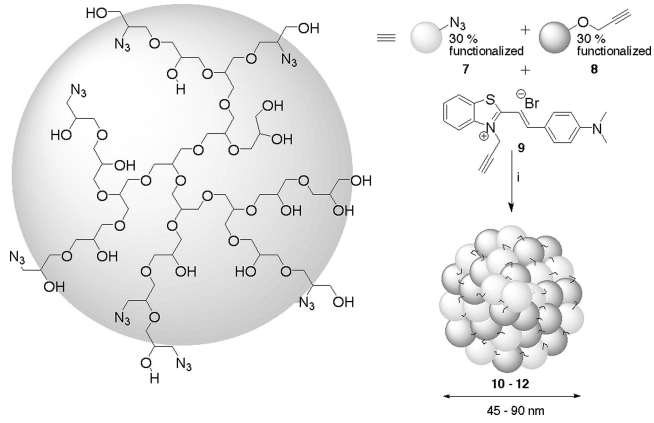
Scheme 1. Procedure Employed in the Formation of Hydrophobic Nanoparticles[a]



[a] (i) $CHCl_3/H_2O$/SDS miniemulsification; (ii) $CuSO_4$/sodium ascorbate, 60 °C, 3 h and then rt, 16 h.

Scheme 2. Procedure Employed in the Formation of Hydrophilic Nanoparticles[a]



[a] (i) DMF/cyclohexane/P(B/E−EO) miniemulsification, 80 °C 16 h.

Table 1. Reaction Conditions for the Formation of Hydrophobic Nanoparticles in Direct Miniemulsion[a]

product	monomers (mg)[b]	$CHCl_3$ (mL) dispersed phase	av diameter (nm)[c]	recovery (%)
3	50	1	25	>95
4	100	2	45	90
5	150	3	65	75
6	250	5	85	65

[a] Constant parameters: H_2O continuous phase = 15 mL. Surfactant (SDS) = 30 mg. More details in the Experimental Section. [b] Sum of 4:1 w/w ratio of HPG:diazide (**1**:**2**). [c] Number-average determined by DLS.

Table 2. Reaction Conditions for the Formation of Hydrophilic Nanoparticles in Inverse Miniemulsion[a]

product	monomers (mg)[b]	amount of surfactant (mg)	av diameter (nm)[c]	recovery (%)
10	70	30	45	>95
11	70	20	75	75
12	70	10	90	50

[a] Constant parameters: cyclohexane = 15 mL. DMF dispersed phase = 1 mL. Additive (**9**) = 1 mg. More details in the Experimental Section. [b] Sum of 1:1 w/w ratio of HPG polyazide:HPG polypropargyl (**7**:**8**). [c] Number-average determined by DLS.

acetone addition, washed several times (solid/liquid extraction) with hexane to remove surfactant, and may be redissolved fully in water and other polar solvents.

As shown in Table 2, the diameter of nanoparticles obtained could be varied between 45 and 90 nm by changing the amount of surfactant used. The products have a very narrow polydispersity due to the conditions employed, particularly the choice of the block copolymer surfactant, giving a highly homogeneous emulsion. Again the amount of recovered material lowers as one forms larger aggregates, however, but this could conceivably be remedied by choice of conditions; the reported conditions are optimized toward 50 nm diameter particles. In all cases the added dye was fully incorporated into the hence colored nanoparticles as washings during purification contained no trace of the strong red color of the dye.

Conclusion

Both of our approaches give reproducible access to large, homogeneously dispersible HPG analogues (20−90 nm) unattainable by previous methods. Further functionalization is expected to be facile as the products bear "click" substrates on the surface. Dye or drug molecules can be encapsulated in the nanoparticles either covalently as in the case of hemicyanine (**9**) or physically by incorporation of such species in the dispersed phase prior to cross-linking polymerization. Promising results toward this aim have been achieved with these systems and will be reported in due course. The privileged size and ease of preparation of these nanoparticles make them viable candidates as drug delivery vehicles. Further optimization is envisaged where the cross-links between HPG monomers are cleavable under controlled stimuli to facilitate drug release at target sites.

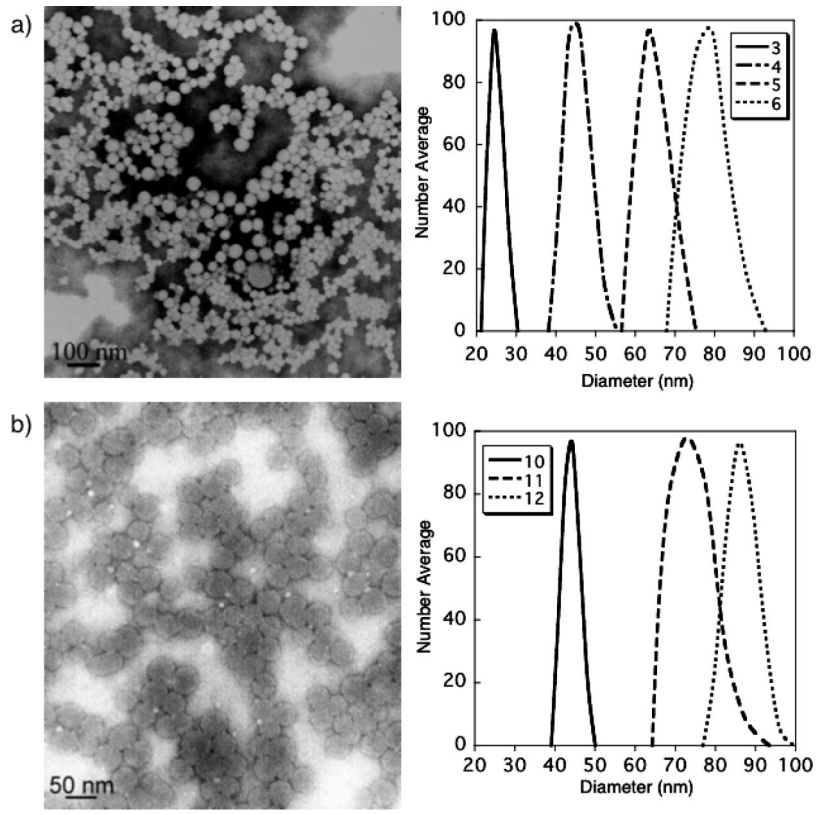


Figure 1. (a) TEM image (left, product **3**) and DLS size distributions (right, **3**−**6**) of hydrophobic particles obtained in direct miniemulsion. (b) TEM image (left, product **10**) and DLS size distributions (right, **10**−**12**) of hydrophilic particles obtained in inverse miniemulsion.

Experimental Section

Materials. General chemicals were purchased from Acros Organics and used reagent grade. Water used was Millipore filtered. SDS was purchased electrophoresis grade. P(B/E−EO) was kindly supplied by Helmut Schlaad (Max Planck Institute for Colloids & Interfaces, Golm, Germany) and is poly(ethylene-*co*-butylene)-*block*-poly(ethylene oxide): M_n = 8100; 41 wt % poly(ethylene oxide). Syntheses of polyglycerols **1**, **7**, and **8** are described in the Supporting Information; diazide **2** was synthesized using standard tosylation/azide substitution starting from decanediol. Hemicyanine dye (**9**) was synthesized following the literature procedure.[18] Emulsions were sonicated using a sonicator, W-220f, with microtip on 70% intensity (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.).

General Procedure for the Preparation of Particles 3−6 (Example Given for Product 3). A solution of SDS (30 mg, 0.104 mmol, 7 mM) in water (15 mL) was vigorously stirred in a cylindrical 30 mL vial for 30 min. A solution of poly(propargyl ether) (**1**) (40 mg, 0.266 mmol alkyne equivalents) and decane diazide (**2**) (10 mg, 0.045 mmol, 0.9 mmol azide equivalents) in $CHCl_3$ (1 mL) was added to the aqueous phase, and the whole was stirred vigorously for 1 h (after this time a milky macroemulsion is formed which would otherwise separate upon prolonged standing). The formed macroemulsion was ultrasonicated with a sonic tip apparatus 4 × 1 min under ice cooling to form a milky miniemulsion (the resultant miniemulsion is stable for periods of several weeks). Catalytic $CuSO_4 \cdot 5H_2O$ (10 mg) and sodium ascorbate (12 mg) were added to the stirring miniemulsion, and after heating in the open vial at 60 °C for 3 h, $CHCl_3$ evaporated to leave a transparent colloidal suspension of polymer. The system was stirred for a further 16 h at room temperature to ensure completeness of cycloaddition cross-linking reactions. The product was diluted with 1 mM SDS solution and analyzed by DLS, and TEM and was stable for months.

General Procedure for the Preparation of Particles 10−12 (Example Given for Product 10). A solution of the P(B/E−EO) surfactant (30 mg, 0.004 mmol) in cyclohexane (15 mL) was vigorously stirred in a cylindrical 30 mL vial for 30 min. A solution of polyazide (**7**) (35 mg, 0.301 mmol azide equivalents), poly(propargyl ether) (**8**) (35 mg, 0.340 mmol alkyne equivalents), and hemicyanine dye (**9**) (1 mg, 0.003 mmol) in DMF (1 mL) was added to the cyclohexane phase, and the whole was stirred vigorously for 1 h (after this time a slightly turbid macroemulsion is formed which would otherwise rapidly separate). The formed macroemulsion was ultrasonicated with a sonic tip apparatus 4 × 1 min under ice cooling to form a fully transparent miniemulsion (the resultant miniemulsion is stable for several hours). The miniemulsion was transferred to a resealable tube and heated with stirring at 80 °C for 16 h. After cooling to room temperature the red solid product nanoparticles were precipitated by addition of acetone (ca. 30 mL) and purified from surfactant by repeated (×3) solid/liquid extraction with *n*-hexane. The red solid product was dissolved in MeOH or water and analyzed by DLS and TEM.

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Supporting Information Available: Syntheses of polyglycerol macromonomers, UV−vis spectrum of nanoparticle dye conjugate **10**, and DLS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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