Nanogel Star Polymer Nanostructures of Controlled Size, Molecular Architecture and Functionality: No Assembly Required

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ABSTRACT

We have developed versatile routes to nanogel core, multiarm stars and polymeric unimolecular amphiphiles that provide exquisite control over molecular size, polydispersity, chain and peripheral functionality etc. The number of arms is adjustable from 20 to more than 100. The synthetic route is cheap and scalable and purification is simple providing an intrinsic advantage over dendrimers. These materials readily encapsulate a variety of hydrophobic materials at reasonable loading levels (10-15%) without covalent binding. The cores and arms can be bio- stable, -degradable or –compatible. In addition to small molecules, nanoparticles including superparamagnetic materials can be encapsulated in the outer shell through ligand-mediated binding. The outer shell and periphery can also catalyze the controlled deposition of various inorganic shells including organosilicates and gold. The nanoparticles are multicompartmental and are useful as dual function reagents for therapeutics and imaging.

Keywords: nanogel stars, synthesis, characterization, encapsulation

Introduction:

Micellar nanostructures produced by the self assembly of block copolymers have been widely studied for the encapsulation of small molecules including drugs and therapeutic reagents [1]. These structures improve reagent solubility and provide a level of protection toward degradation under physiological conditions. The micellar structures are, however, dynamic assemblies which are easily destroyed. We are studying soft colloid, multiarm star polymers where the polymer arms emanate from a nanogel core (Figure 1). The topology of such nanogel star polymer materials can be similar to dendrimers providing a central core region, various molecular compartments for encapsulation and a plethora of functionality which can be distributed throughout the polymer arms and/or localized on the periphery [2]. These materials are easier and cheaper to synthesize on a large scale, simpler to purify and offer more functional variety and distribution than dendrimers. There are four main techniques that have been discussed for the preparation of star polymers using controlled polymerization techniques: (i) arm first/static core [3] (ii) core first/static core [4] (iii)core first/living core [5] and (iv) arm first/living core [6]. Polymer control has been achieved using anionic, controlled free radical, cationic and ring opening polymerization (ROP) techniques. In this discussion, we have focused on nanogel star polymers produced by the arm first/living core route utilizing both anionic and ring opening polymerization with organic catalysts (OROP) [7] to generate both the initial arms and the crosslinked core. Unimolecular amphiphiles are generated by postpolymerization transformation.

Discussion:

Recently we have described the preparation of nanogel star polymer utilizing anionic techniques to form the core and the initial arms [2]. The use of functionalized initiators allows the decoration of the polymers formed with other small molecules or polymers. When the peripherial functionality is converted to an initiator, polymeric arms can be grown fron the nanogel star using controlled radical polymerization usually atom transfer radical polymerization [8] to produce block copolymer arms. Alternatively, preformed polymers (e.g. PEG) can be appended through postpolymerization coupling to generate block copolymer arms. Anionic polymerization is usually initiated using styrene monomer at room temperature in cyclohexane and the core is generated with divinylbenzene as the crosslinking core forming reagent. In our hands, purified p-divinylbenzene yields the best and most dependable results. Polydispersities of the functionalized nanogel stars prepared using this process are typically in the 1.1-1.25 range. By varying the stoichiometry of the reagents , we can control the molecular size and number of arms in the initial star nanogel polymer. By using an anionic initiator such as 3-tert-butyldimethylsiloxy propyl lithium, hydroxyl functionality is installed at the ends of the star polymer chain. This permits further functionalization after the formation of the initial star polymer core (vide infra). The arms emanating from the nanogellated core can be homo or block copolymers which are prepared as a first step by anionic [6], controlled radical [9], cationic [10] or ring opening polymerization [11] and subsequently used to create the microgel core. For ROP, we have used organic catalysts (OROP) rather than the more traditional metallic catalysts. For the controlled ring opening of lactones such as valero- and capro-lactone, we have most often used 1,5,7-triaza-bicyclo[4.4.0]dec-5-ene (TBD) as the catalyst and an alcohol initiator (Scheme I). As a crosslinking

reagent, we have utilized 5,5' bis(oxepanyl-2-one) although other polyfunctional, polymerizable lactones will work as well. The efficacy of OROP [11b] vs more traditional tin-catalyzed processes [11a] is shown in Figure 2 The nanogel star polymer from OROP is of higher molecular weight and the the polydispersity is substantially reduced in the crude product. In addition, recent FDA restrictions on residual tin to <20 ppm [12] coupled with the difficulty of removal of highly oxophilic tin residues from multiarm star polymers validates the organic catalyst approach. The lactone derived polymers are degradable under physiological conditions, particularly at elevated pH. Figure 3 shows the degradation of a valerolactone-derived nanogel star polymer containing peripherial PEG blocks (Mn~ 2kDa) attached by postpolymerization functionalization at 37°C and a pH of 7.4 . SEC analysis shows the degradation of the high molecular weight star and the regeneration of the PEG appendages. For biomedical applications, the core and the inner shell (block copolymer arms) are usually hydrophobic and provide a compartment for the solvophobic encapsulation of cargo while the outer shell is usually hydrophilic to provide compatibility with aqueous solutions. Materials ranging in size from 10-40nm with arm numbers from 20 to more than 100 have been prepared. The outermost periphery from the initially formed nanogel stars displays the functionality of the initiator used to produce the arms.

For example, a typical nanogel polystyrene star of molecular weight 104 KDa, PDI=1.16 and arm number of 31 containing hydroxyl terminal functionality was quantitatively acylated with alfa bromoisobutryl chloride to produce the multiarm nanogel star polymer initiator. This could be decorated with poly(dimethylaminoethyl methacrylate) under ATRP conditions [8]. The nanogel star block copolymer produced typically contained about 19 DMAEA/polystyrene arms as determined by 1H NMR integration. The overall molecular weight of the nanogel star block copolymer was 274 kDa and the PDI= 1.22. The hydrodynamic radius was ~ 12nm in THF measured by dynamic light scattering. The presence of the DMAEMA blocks conferred water solubility on the star polymer. These unimolecular micellar materials will encapsulate hydrophobic reagents quite readily. In this particular case the hydrophobic porphyrin (5,10,15,20-(3,5-di-tertbutylphenyl) porphyrin is encapsulated to the extent of~10wt. % based on the polymer weight. Figure 4 shows the absorption spectrum of the dye in THF compared with that of the occlusion complex in water. Spontaneous update of this mater by mouse myoblast primary cells is shown in Figure 5. Unimolecular micellar nanogel star polymers of this type can readily accommodate cargo loads of 9-15% without covalent bonding. Additional loading capacity may be realized if desired through other specific interactions between polymer and cargo (e.g hydrogen bonding, electrostratic, acid-base etc.).

We have primarily studied nanogel cores of polystyrene or aliphatic polyesters and arms which are composed of functionalized poly-styrene, -acrylates , - aliphatic esters or - ethers either as homo or random or block copolymers. The structures may not only be used for encapsulation of small molecules/dyes but also to provide catalytic sites for the formation of inorganic shells or to encapsulate nanoparticles including magnetic materials. In this regard, Figure 6 shows a core shell system comprised of the DMAEMA decorated star discussed with a silica shell grown in situ in ethanol -water with triethoxyorthosilicate [12]. This procedure is shown in Scheme III. The thickness of the silica-like shell can be controlled by varying reagent concentrations, temperature, addition rate stiochiomretries, pH etc. (thicknesses 5-30nm). A TEM micrograph of some silica-coated star polymer nanoparticles prepared in this fashion is shown in Figure 6. The shell can be functionalized after formation using standard techniques or the functionality can be introduced during shell formation by the use of functionalized organosilicates (e.g aminopropyltriethoxysilane). Similarly the ligating capability of the outer shell ligands can be used to bind various nanoparticles and sequester them in the polymer shell. An example is shown in Figure 7 which shows ~7nm Fe_3O_4 nanoparticles incorporated into the nanogel star polymer. This particular example also has the porphyrin dye occluded in a dual function capacity as shown by the absorption-emission spectra in the Figure 7. Finally, we mention that the peripheral amino functionality of the nanogel stars can also be used for electroless deposition (Scheme IV [13] of a gold shell of controllable thickness. A example of these materials and some spectral properties are shown in Figure 8. The strong plasmon resonance absorption coupled by the shift to longer wavelengths of nanoshell derivatives suggests potentional applications for hyperthermic therapy[14].

Summary

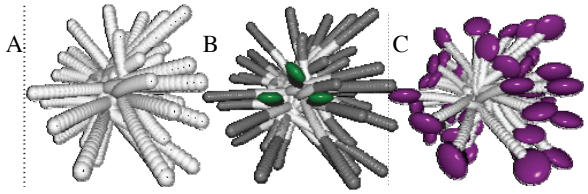
In summary, we have described an efficacious route to nanogel star polymers of controlled size and functionality by controlled polymerization for which the products are easily purified and scale up is quite simple. The star polymer products may be degradable or non-degradable as desired. Hydrophobic materials may be readily encapsulated and released. Peripherial functionality may also be utilized to make a variety of materials containing functionalized inorganic shells. This materials have various potential biomedical applications.

References:

1. (a) J. H. Park, S. Lee, J.-H Kim, K. Park, I.C. Kwon Prog. In Polym. Sci. (2008), 33(1), 113; (b) H. M. Aliabadi, A. Lavasanufar Expert Opinion on Drug Delivery (2006), 3(1) 139
2. V.Y. Lee, K. Havenstrite, M. Tjio, M. McNeil, H.M. Blau, R.D. Miller, J. Sly Adv. Mater. (2011), 23, 4509
3. J.R. Scaefgen, P.J. Flory J. Am. Chem. Soc (1948) 70, 2709

4. M. Nagasawa, T. Fujimoto Prog. Polym. Sci. Japan (1972) 2, 263
5. H. Eschwey, M.L. Hallensleben, W. Burcgard Makromol. Chem. (1973), 173, 235
6. J.G. Zilliox, P. Rempp, J. Parrod J. Polym. Sci. Symposia (1967), No 22 (Pt.1), 145
7. (a) J. L. Hedrick, R. M. Waymouth Chem. Rev. (2007), 107(12), 5813; (b) J.L. Hedrick, R.M. Waymouth Macromol. (2010), 43(5), 2093
8. "Handbook of Radical Polymerization" K. Matyjaszewski, T.P. Davis Eds., John Wiley & Sons Inc. Hoboken NJ (2002), Chapt. 11
9. A. Blencowe, J. F. Tan, T. K. Goh, G.G. Qiao Polymer (2009), 50, 5
10. S. Kanoka, M. Sawamoto, T. Higashimura Macromolecules (1991), 24, 2309
11. (a) J. T. Wiltshire, G.G. Qiao Macromolecules (2006), 39, 4282 (b) E. A. Appel, V.Y. Lee, T. T. Nguyen, M. McNeil, J.L. Hedrick, F. Nederberg, W.C. Swope, J. E. Rice, R.D. Miller, J. Sly. Chem. Comm. (2012), in press
12. (a) The United States Pharmaopeil Convention USP Heavy Metals, USP34-NF29, (2011) edn. (b) A. Stjerndahl, A.F. Wistrand, A.-C. Albertsson, Biomacromol. (2007), 8, 937
13. (a) Y.D. Jin, X. F. Kang, Y. H. Song, B.L. Zhang, G.J. Cheng, S.J. Dong Anal. Chem.(2001), 73(13), 2843; (b) T. Pham, J.B. Jackson, N.J. Halas, T.R. Lee (1999), 15(3) , 844
14. (a) S.J. Oldenburg, R.D Averitt, S. L. Westcott, N. J. Halas Chemical Physics Lett. (1998), 288, 243; (b) D. K. Chatterjee, P. Diagaradjane, S. Krishnan Therapeutic Delivery (2011), 2(8), 1001

Figure 1: (A) Cartoon representation of microgel star polymer with homopolymer arms; (B) Microgel star with copolymer arms and encapsulated cargo (C) Microgel star with peripheral functionalization



Scheme I: Nanogel star polymer synthesis using OROP

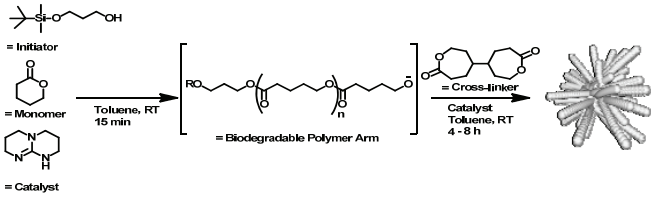


Figure 2: GPC analyses of (A) linear poly (valerolactone arm and nanogel star polyester so derived using TBD catalyst at room temperature ; (B) same product using tin (II) catalyst at elevated temperatures

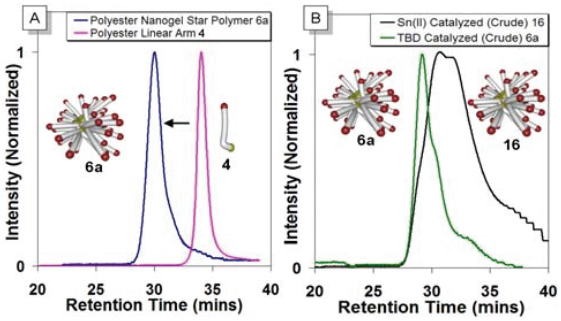


Figure 3: Nanogel star polymer composed of polyester core and PEG-b-PVL arms (PEG 2K) before and after hydrolysis

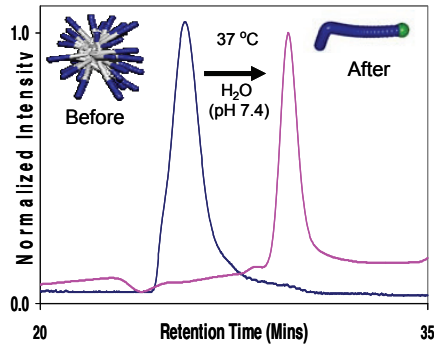


Figure 4: Occulsion complex of nanogel star Poly(styrene-b-DMAEMA) with porphyrin derivative in water vs the dye in THF

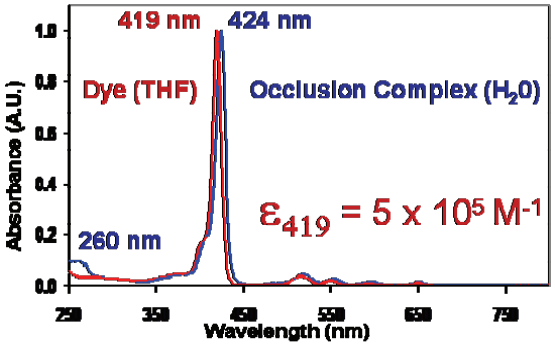
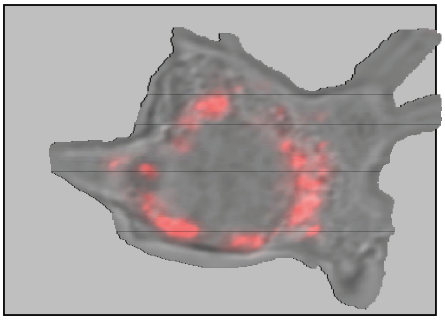
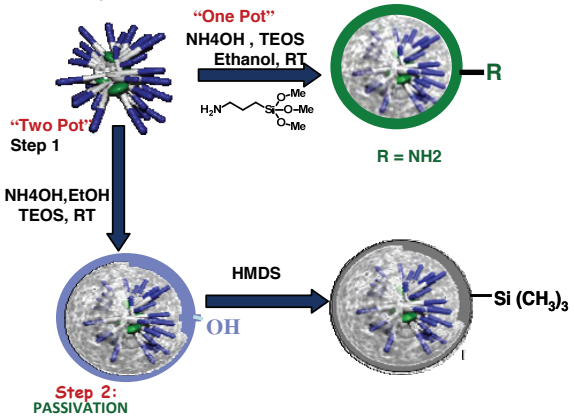


Figure 5: High resolution confocal micrograph of unassisted cellular uptake of nanogel star composed of poly(stryrene-b-DMAEMA and encapsulated porphyrin derivative by primary mouse myoblast stem cells



Scheme II: Formation of core-shell silica coated nanoparticles using a soft colloid template containg an occluded dye



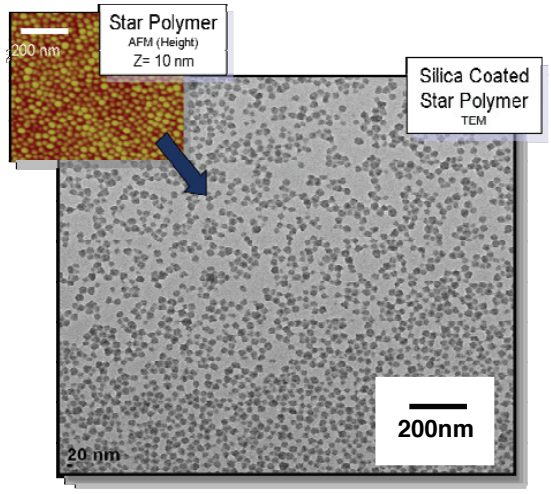


Figure 6: TEM micrograph of silica coated nanogel star polymer. Upper left insert is AFM image of the nanogel star polymer composed of poly(styrene-b-DMAEMA) arms alone deposited from water

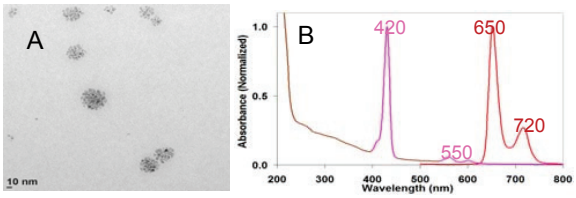


Figure 7: A. TEM micrograph of nanogel star composed of poly(styrene-b-DMAEMA) arms loaded with~ 7nm Fe_3O_4 nanoparticles; B. Absorption-emission spectra of particles in A loaded with porphyrin

Scheme III: Electroless deposition of gold on silicon oxide templated by nanogel star polymers

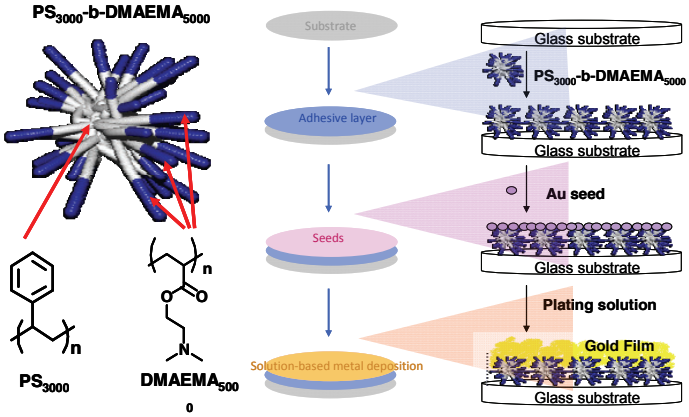


Figure 8: Porphyrin loaded nanogel star polymer coated with gold by electroless deposition in comparision with solid gold particle of the same size. Insert left- solid gold particles; insert right- nanogel star polymer coated with gold