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Dual-Reactive Surfactant Used for Synthesis of Functional Nanocapsules in Miniemulsion

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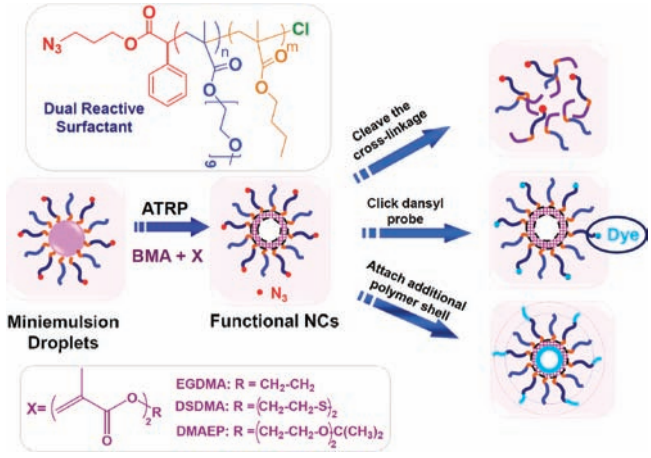
Herein, a new concept for the facile preparation of stable multifunctional polymeric nanostructures in miniemulsion polymerizations is introduced.[1] The concept involves the use of a dual-functional reactive surfactant (DRS). The DRS is an amphiphilic block copolymer that is designed to carry one halogen end group capable of initiating atom-transfer radical polymerization (ATRP)[2] and a second orthogonal functionality (e.g., azide) to be used later for further modifications of the formed particles by click chemistry.[3] The amphiphilic nature of DRS should locate the surfactant at the oil−water interface in miniemulsion, providing inward growth of polymer chains in the organic phase[4] while the second functionality is situated in the aqueous phase. More stable polymeric nanoparticles (nanogels or nanocapsules) with reactive functional groups on the surface can be obtained through copolymerization of hydrophobic monomers with divinyl cross-linkers in a "one-pot" reaction. The concentrations of reactive functional groups in the two phases can be controlled by diluting the DRS with different proportions of a monofunctional reactive surfactant. Functional polymeric nanostructures can also be prepared through the self-assembly of well-defined block copolymers with preserved functionalities in selective solvents.[5] In this case, however, subsequent stabilization of these assembled structures via cross-linking is often required to afford a more stable nanostructure.

In this communication, we mainly focus on the preparation of polymeric nanocapsules, which consist of a polymer shell and an "empty" interior. This encapsulated "space", which is isolated from the external environment by the polymer barrier, can be utilized as a reservoir for active substances to protect them against a potentially harsh environment. Such polymeric nanoparticles have found use in catalysis, cosmetics, food additives, and pharmaceutics.[6] To date, however, the facile preparation of multifunctional polymeric nanocapsules has remained challenging.[7] Miniemulsion polymerization allows a single-step synthesis of nanocapsules with high encapsulation efficiency.[1b,8] We previously reported the successful preparation of stable polymeric nanocapsules via an interfacially confined ATRP in miniemulsion using an amphiphilic block copolymer as a stabilizer and macroinitiator.[4c]

We now introduce a DRS as a new reagent for the preparation of functional polymeric nanocapsules. An amphiphilic block copolymer with heterotelechelic structure was synthesized as a DRS: α-azido-ω-2-chloroisobutyrate-poly(oligo(ethylene oxide) monomethyl ether methacrylate)-*b*-poly(*n*-butyl methacrylate) (N_3−POEOMA−PBMA−Cl). This was used together with a monofunctional reactive surfactant, poly(ethylene oxide)-*b*-poly(*n*-butyl methacrylate) (PEO−PBMA−Cl), to copolymerize *n*-butyl methacrylate (BMA) with various cross-linkers in miniemulsion, forming nanocapsules with cross-linked shells. The latent azide functionality was then used for covalent attachment of small molecules (e.g., fluorescent dyes) or converted to ATRP initiators to grow a second layer. Cleavable cross-linking units can be degraded to decompose nanocapsules and deliver cargo and to help verify the attachment of new polymer chains in the form of block copolymers (Scheme 1).

Scheme 1. Preparation of Polymeric Nanocapsules in Miniemulsion Using a Mixture of Dual and Monofunctional Reactive Surfactants



Three different dimethacrylate cross-linkers, ethylene glycol dimethacrylate (EGDMA), bis(2-methacryloyloxyethyl)disulfide (DSDMA), and di(methacryloyloxy-1-ethoxy)isopropane (DMAEP), were copolymerized with BMA to prepare nanocapsules containing either permanent or degradable cross-linking units in the shells. Typical results are summarized in Table 1. The initial monomer droplets and resulting polymer latexes displayed good colloidal stability with well-maintained particle size, suggesting that the majority of the amphiphilic copolymers were located at the particle surface. Thus, polymerization should start and be constrained to the interfaces, with the polymer chains growing inward in a controlled manner by activators generated by electron transfer (AGET)[9] ATRP.

Table 1. Nanocapsules with Different Cross-Linkages in the Shell

entry[a]	reactive surfactant	X[a]	D_n (nm)[b]	CV[b]
S1	N_3−$POEOMA_{11}$−$PBMA_{22}$−Cl + PEO_{120}−$PBMA_{30}$−Cl	EGDMA	180	0.11
S2	N_3−$POEOMA_{11}$−$PBMA_{22}$−Cl + PEO_{120}−$PBMA_{30}$−Cl	DSDMA	195	0.13
S3	N_3−$POEOMA_{14}$−$PBMA_{25}$−Cl + PEO_{120}−$PBMA_{20}$−Cl	DSDMA	150	0.20
S4	N_3−$POEOMA_{14}$−$PBMA_{25}$−Cl + PEO_{120}−$PBMA_{20}$−Cl	DMAEP	140	0.20

[a] Polymerization conditions: [BMA]/[X]/[N_3−POEOMA−PBMA−Cl]/[PEO−PBMA−Cl]/[$CuBr_2$/bis(2-pyridylmethyl)octadecylamine]/[ascorbic acid] = 220:20:0.5:0.5:0.5:0.15; 25 wt % hexadecane compared with BMA and anisole; 30 vol % anisole relative to BMA; 3 wt % BMA relative to water; 65 °C; reaction times of 2.6 h for S1 and S2, 3.6 h for S3, and 2.0 h for S4. [b] The average diameter of polymer nanocapsules (D_n) and coefficient of variation (CV) in water were determined by dynamic light scattering.

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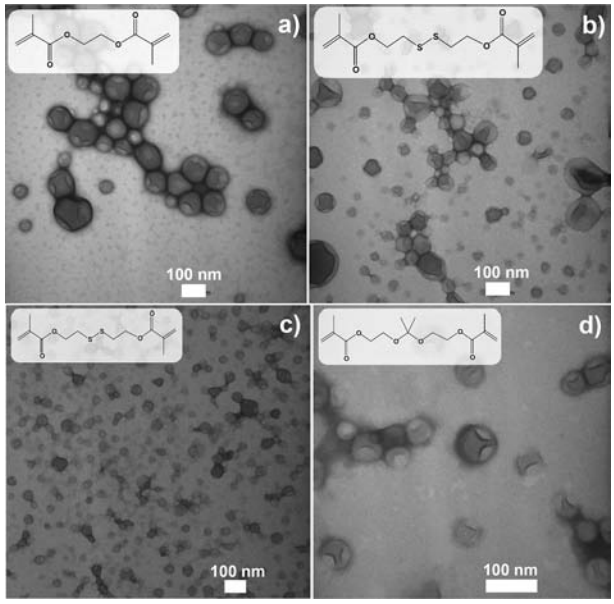

Figure 1. TEM images of polymeric nanocapsules formed via miniemulsion ATRP of BMA and various cross-linkers (the chemical structures of the cross-linkers are shown in the insets) using N_3−POEOMA−PBMA−Cl and PEO−PBMA−Cl as the reactive stabilizer: (a) S1; (b) S2; (c) S3; (d) S4.

Particles with deformed and indented structures were observed in the transmission electron microscopy (TEM) images (Figure 1), suggesting the original formation of polymer particles with liquid cores (solvent, hexadecane, and unreacted monomer). The interior space allowed the structure to collapse when samples were dried on the TEM grids. The smaller particle size shown in the TEM images, in comparison with the values determined by dynamic light scattering (DLS), could be due to the significant shrinkage. The morphology of the formed polymeric nanocapsules was also analyzed by tapping-mode atomic force microscopy (AFM). Sample S1 showed a collapsed structure after drying (Figure 2a), similar to what was observed in the TEM images. Moreover, sample S2 could be squeezed by harder tapping conditions, as confirmed by the significant deformation when the tapping force was gradually increased (Figure 2b−d). The deformed nanocapsule reformed the original spherical structure when the tapping force was reduced (Figure 2e). These observations support the presence of a squeezable core inside the nanocapsules.

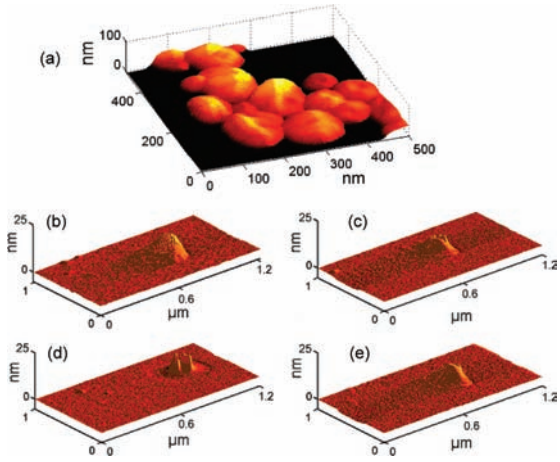

Figure 2. AFM images of polymeric nanocapsules: (a) S1 after drying under ambient conditions. (b−e) S2 at different set-point values A/A_0 (see the Supporting Information): (b) 0.8; (c) 0.6; (d) 0.4; (e) returned to 0.8. S2 observations were done within 2 h after drop-casting of a THF dispersion.

Cross-linkers with cleavable disulfide (DSDMA) and acetal (DMAEP) linkers provided functional polymeric nanocapsules that could be degraded under specific conditions. These degradable nanoparticles can potentially be used for delivery applications, such as drug delivery, especially since the primary chain size controls the size of degraded fragments and is easily tuned by the targeted degree of polymerization. Nanocapsules containing either redox-cleavable disulfide bond or acid-degradable acetal-based cross-linking units in the shells were synthesized (Table 1, S2−S4) under similar polymerization conditions. The size of the segments of the amphiphilic DRS should affect the emulsification ability, and consequently, nanocapsules with smaller sizes were obtained with S3 and S4. However, even the small particles, with diameters of less than 80 nm after drying on the TEM grids, still showed the collapsed shell morphology, suggesting the formation of nanocapsules originally containing a liquid core.

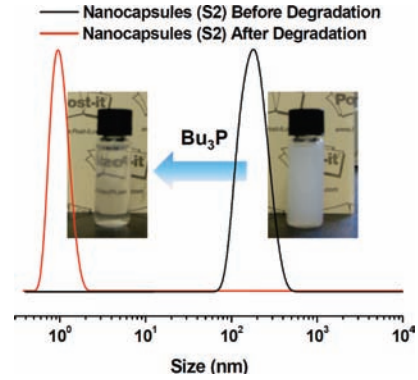

Figure 3. DLS results and images of disulfide-containing polymeric nanocapsules (S2) before and after degradation in THF.

The polymeric nanocapsules with the disulfide cross-linkage (S2) were cleaved into soluble polymers in the presence of tri(*n*-butyl)phosphine (Bu_3P) as a reducing agent in THF.[10] The significant decrease in the nanocapsule size, from hundreds to a few nanometers after degradation, confirmed the successful cleavage of the disulfide linkage (Figure 3). The transparent THF solution of formed free polymers after reduction further confirmed the complete cleavage process. The number-average molecular weight and the molecular weight distribution of the formed polymers were $M_n = 36\,000$ and $M_w/M_n = 1.84$, respectively, as determined by gel-permeation chromatography (GPC) with a refractive-index detector and linear poly(methyl methacrylate) as a standard. On the basis of the molecular weight of the degraded polymers, the monomer conversion was ~68% when the polymerization was stopped after 2.6 h. On the other hand, the cross-linked nanocapsules were obtained with essentially 100% efficiency, since no free polymer chains could be extracted from the final products. The introduction of acid-sensitive cross-linking units into the polymer shell allowed the formation of nanocapsules that should be hydrolyzed under acidic conditions (S4). The successful degradation process was also confirmed by DLS and GPC results. The particle size decreased to several nanometers after acid hydrolysis. GPC results showed the formation of soluble polymers after the cleavage of acetal-based cross-linkage in the presence of aqueous HCl. The number-average molecular weight of the cleaved polymers was $M_n = 34\,000$ with $M_w/M_n = 1.77$.

The azido groups from the DRS (N_3−POEOMA−PBMA−Cl), which were preserved in the nanocapsules, were utilized for the further modification of the formed nanocapsules. A dansyl probe containing an alkyne group (see the Supporting Information) was

attached to the nanocapsules (S2) by click reaction. After the reaction, the formed product was dialyzed against THF for 3 days (with the solvent changed on a daily basis) to remove any unreacted dansyl probe. The modified nanocapsules displayed very strong fluorescence under UV irradiation, indicating the successful incorporation of the dansyl probe into the nanocapsules (Figure 4). They showed an emission peak at 506 nm when excited with 339 nm light, similar to the pure dansyl probe. These results confirmed that the azido groups were well-preserved and still accessible for subsequent modification after formation of the nanocapsules. The modified nanocapsules were then cleaved with tri(*n*-butyl)phosphine, and the degraded polymers were purified again by dialysis to remove any dye that could be trapped inside the original nanocapsule cores. The fluorescence spectra of the samples before and after degradation essentially overlapped, indicating similar concentrations (Figure SI 1 in the Supporting Information).

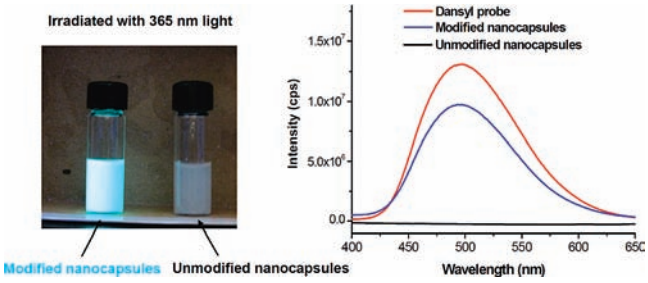

Figure 4. (left) Images during UV irradiation and (right) fluorescence spectra of polymeric nanocapsules with and without dansyl probe modification in THF. Concentrations for fluorescence analysis: dansyl probe, 9.1×10^{-4} mg/mL; modified and unmodified nanocapsules, 0.1 mg/mL.

Comparison of the fluorescence intensities of the pure dansyl probe and the modified nanocapsules provided the amount of accessible azido groups in the product as 0.0143 mmol/g. On the basis of the molecular weight of degraded polymers ($M_n = 36\,000$), the theoretical amount of azide groups in the product should be ~0.0139 mmol/g, which is in good agreement with the measured value. These results confirm that the dansyl dye was quantitatively covalently attached to the azide functionalities. The amount of azide groups per nanocapsule could be easily programmed by changing the ratio of the DRS (N_3−POEOMA−PBMA−Cl) to the monofunctional reactive stabilizer (PEO−PBMA−Cl) as well as the reaction time and monomer conversion.

A click reaction was used to attach an ATRP initiator with an alkyne group (prop-2-ynyl 2-bromoisobutyrate) to the periphery of the nanocapsules in order to build an additional polymer shell. Chain extension with di(ethylene glycol) monomethyl ether methacrylate (MEO_2MA) was conducted from the ATRP initiator sites (surface and also interior). The disulfide-containing nanocapsules (S3) were used to verify the growth of the $PMEO_2MA$ shell from the nanocapsules. The molecular weight of the polymers formed after degradation increased from $M_n = 43\,000$ to $M_n = 61\,000$, confirming the chain extension of MEO_2MA from the nanocapsules. The molecular weight of the $PMEO_2MA$ block was $M_n = 18\,000$ (Figure 5). With the ATRP initiator sites both in the core and on the periphery, it was possible to grow various polymer shells simultaneously from the nanoparticle surface and inside the core of the hollow structure, resulting in a stable vesicle-like structure. As shown in Figure SI 2b, the functionalized nanocapsules retained the spherical shape and core/shell structure, meaning that the particle morphology was preserved after the introduction of the new tethered polymer shell.

Ethyl 2-bromoisobutyrate (EBiB) was also used as a co-initiator to generate untethered chains; in this case, the growth of the polymer shell could be followed without destroying the nanocapsules. The molecular weight of the free polymer chains gradually increased as the polymerization continued, and the value of $M_w/M_n = 1.3$ was relatively low, suggesting a well-controlled chain extension (Figure SI 2a).

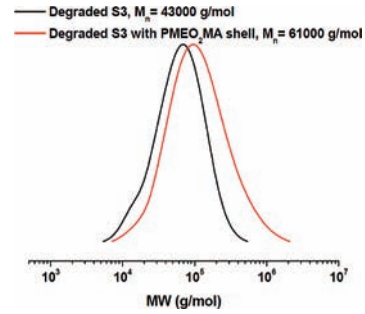

Figure 5. GPC traces of degraded nanocapsules (S3) before and after chain extension with MEO_2MA.

Functional polymeric nanocapsules were successfully prepared via miniemulsion copolymerization of BMA and various cross-linkers by AGET ATRP using a mixture of a dual-reactive surfactant, N_3−POEOMA−PBMA−Cl, and monoreactive PEO−PBMA−Cl. The use of cleavable cross-linkers resulted in the formation of degradable nanocapsules. The α-azido functionality on the DRS was preserved in the nanocapsule structure and remained accessible for further modification. Both a fluorescent dansyl probe and a tethered linear polymer shell were successfully introduced onto the shell of the nanocapsules, resulting in multifunctionalized polymeric nanoparticles.

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Supporting Information Available: Procedures for the preparation of polymeric nanocapsules in miniemulsion and the subsequent modification by click reaction. This material is available free of charge via the Internet at http://pubs.acs.org.

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