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Facile and Rapid Access to Glyconanocapsules by CuAAC Interfacial Polyaddition in Miniemulsion Conditions

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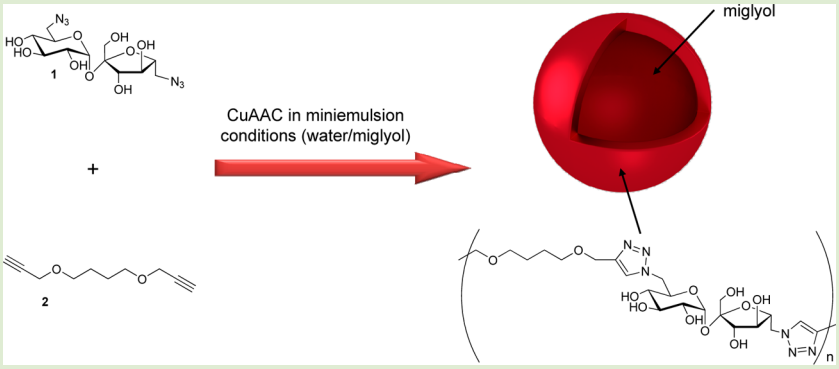
Supporting Information



ABSTRACT: Glyconanocapsules with a biocompatible oily core have been successfully prepared by copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) interfacial step growth polymerization between 6,6′-diazido-6,6′-dideoxysucrose and bis(propargyloxy)butane in oil-in-water miniemulsion conditions. Optimization of the interfacial polymerization process in dispersed medium afforded the rapid and reproducible preparation of stable monodispersed glyconanocapsules having a diameter around 200 nm.

Owing to the crucial role of carbohydrates in a myriad of biological recognition events,[1] synthetic methods affording tailor-made glycomaterials have recently attracted considerable attention. By means of conventional and controlled radical, ionic, ring-opening, or metathesis polymerizations, a large palette of well-defined macromolecular species, that is, linear,[2] star,[3] comb-like,[4] hyperbranched,[5] and dendrigraft glycopolymers,[6] have been described over the last few decades. The preparation of a series of precisely defined glycopolymers has progressively opened the door to the conception of stable nanoscale glyco-objects using self-assembly and micellization concepts.[7] Artificial glyco-based (micro or) nanovehicles for targeted drug delivery, such as micelles or nanocapsules, have been essentially designed by self-organization of amphiphilic block copolymers in water (and subsequent cross-linking within the core, the shell or at the interface of the two blocks)[8] or to a lesser extent by layer-by-layer deposition (and cross-linking) of preformed polymers onto decomposable colloidal templates.[9]

A heterophase polymerization process such as miniemulsion polymerization constitutes a very convenient and popular one-step alternate to generate polymeric nano-objects. Miniemulsions,[10] which are kinetically stable submicronic oil-in-water dispersions with droplet size ranging from 50 to 500 nm generated by high shear devices such as ultrasonicators, have been successfully applied to radical, ionic, or metal-catalyzed polymerizations as well as enzymatic or chemical polycondensation/polyaddition reactions.[11] In miniemulsion processes, polymerization generally occurs in the dispersed phase or at the interface of the droplets. As a consequence, this approach easily lends itself to the preparation of a broad spectrum of polymer colloids, that is, nanoparticles,[12] polymer/polymer hybrid nanoparticles,[13] polymer–inorganic nanocomposites,[14] or nanocapsules of a different chemical nature. The synthesis of nanocapsules by polymerization in miniemulsion conditions has been extensively investigated in the past decade owing to their potential value in pharmaceutical or cosmetic applications. Polyurethane, polyurea, polyamide, poly(vinyl ether), or

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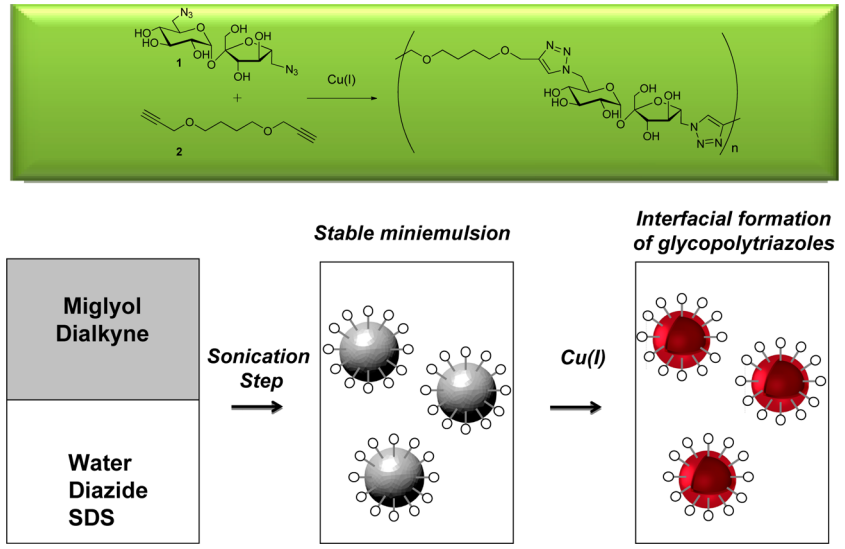
Scheme 1. CuAAC Polyaddition of 1 and 2 (top) and General Approach Towards Glyconanocapsules from Interfacial CuAAC Polyaddition (bottom)



poly(butyl cyanoacrylate) based nanocapsules have for instance recently been described.[15]

Herein we explore the preparation of monodisperse glyconanocapsules from copper(I)-catalyzed azide−alkyne cycloaddition (CuAAC) interfacial step growth polymerization in oil-in-water miniemulsion conditions using miglyol, a biocompatible neutral oil as dispersed phase (see Scheme 1). Despite its high efficiency, its well-established orthogonality to most of other chemistries employed in macromolecular science, and its compatibility with aqueous media,[16] the scope of CuAAC as a tool to grow polymeric shells in interfacial step growth polymerizations remains unexplored so far.[17]

The couple of monomers, a carbohydrate-based diazide, 6,6′-diazido-6,6′-dideoxysucrose (**1**), and an aliphatic dialkyne, bis(propargyloxy)butane (**2**), was selected in regard to the solution behavior of the catalyst system, that is, sodium ascorbate/$CuSO_4$, which is expected to be located in the aqueous phase in the course of the miniemulsion polymerization. The construction of the polymeric membrane by interfacial polyaddition requires the diffusion of one of the monomers at least toward the adverse phase. In this context, a short miglyol-soluble dialkyne ether, dialkyne (bis-(propargyloxy)butane), exhibiting a value of the partitioning coefficient toward the continuous phase (water), ca. ∼ 3.1, was prepared in high yield (16 g, 86% yield) from the alkylation of 1,4-butanediol with propargyl bromide. The complementary water-soluble carbohydrate-based diazide monomer, 6,6′-diazido-6,6′-dideoxysucrose, was selected for the high availability and water solubility of its parent structure, sucrose. A multigram scale synthesis using a single step procedure allowed the direct regioselective diazidation of sucrose under Mitsunobu conditions (18 g, 70% yield).[18] Importantly, this sugar monomer is not soluble in the lipophilic dispersed phase, and the construction of the glycomembrane should thus be governed by the diffusion of the lipophilic monomer toward the aqueous continuous phase.

For a full understanding of the polymerization process, the CuAAC step growth polyaddition of diazide **1** and dialkyne **2** was primarily investigated in homogeneous conditions in DMSO-d_6 (20 wt %) at 60 °C. Polyaddition was proven to proceed very fast (75% of conversion in less than 30 min). The resulting glycomaterial was not soluble in water or miglyol but readily dissolved at room temperature in DMF or DMSO. On the basis of this solution behavior, we anticipated that the interfacial polyaddition in miniemulsion conditions should lead to the generation of the desired glyconanocapsules.

The preparation of the glycopolytriazoles was clearly demonstrated by ^{1}H, ^{13}C NMR, and 2D NMR (in DMSO-d_6) with the appearance of peaks between 7.65 and 8.00 ppm (two main peaks at 7.85 and 7.98 ppm together with other minor peaks) and between 124 and 145 ppm (two groups of peaks at 124−125 and 144−145 ppm) corresponding, as confirmed by HSQC and HMBC experiments, to the resonances of the two (nonequivalent) formed 1,4-disubstituted 1,2,3-triazole rings. The observation of such complex patterns may reflect the concomitant formation of cyclic and linear polytriazoles and/or the preparation of oligomers. The reaction was also followed by IR spectroscopy by monitoring the dramatic intensity decrease of the typical azide stretching band at 2130 cm^{-1}. In the course of the polyaddition process, SEC analysis of the dry samples (in DMF) confirmed the formation of glycopolytriazoles with apparent molar mass of ca. 1400 g/mol and a molar mass distribution equal to 2.3. The preparation of polytriazoles was further corroborated by matrix-assisted laser desorption ioniazation time-of-flight (MALDI-TOF) experiments that brought to light the presence of species corresponding to populations with a degree of polymerization ranging between 2 and 7 (See Table 1). The formation of low molar mass polytriazoles is somewhat counterintuitive in view of the quantitative conversion of the monomers and formation of triazole linkages. However, this discrepancy between theoretical masses (determined from Carothers equation) and experimental molar masses together with the NMR data suggests the formation of a nonnegligible amount of low molar mass cyclic species in the course of the interfacial polymerization.

CuAAC interfacial polyaddition of **1** and **2** was subsequently investigated in miniemulsion conditions. As no 1,3-dipolar Huisgen cycloaddition was observed at room temperature in the absence of copper catalyst, a pre-emulsion containing a stoichiometric ratio of the two monomers respectively dissolved in the aqueous and organic phases (water/miglyol 3:1 v/v), and

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Table 1. Populations Detected under Homogeneous and Miniemulsion Conditions (Na^+ Cationization)

population[a]	DP	calcd mass (g/mol)	expt mass under homogeneous conditions[b]	expt mass under miniemulsion conditions[c]
AB	2	581.2	581.2	581.3
ABA	3	747.3	747.3	747.3
BAB	3	973.3	not detected	974.3
BABA	4	1139.4	1139.4	1139.4
BABAB	5	1531.6	1531	not detected
ABABA	5	1305.5	1305.4	1303.2
BABABA	6	1697.6	1698.0	1698.6
BABABAB	7	2089.8	not detected	not detected
ABABABA	7	1863.8	1864.4	not detected
ABABABAB	8	2255.9	2258.3	not detected

[a]A: motif based on dialkyne, bis(propargyloxy)butane, B: motif based on 6,6′-diazido-6,6′-dideoxysucrose. [b]Determined by MALDI-TOF. [c]Determined by ESI MS.

the surfactant (SDS, 3 wt % relative to the organic phase) was first generated. It is worth mentioning that miglyol as the dispersed phase simultaneously plays the role of costabilizer, classically added to the dispersed phase to prevent Ostwald ripening.[19] Ultrasonication of the resulting emulsion for 6 min at 25 °C afforded a stable monodisperse miniemulsion presenting a *z*-average diameter of around 200 nm and a polydispersity index of 0.107. Importantly, no methodology based on the incorporation of the catalytic system during the pre-emulsion step (catalyst in water + alkyne in miglyol or catalyst and azide in water + miglyol) allowed for preparing stable submicrometer oil-in-water droplets. The miniemulsion polyaddition was finally triggered upon addition of the catalytic system, and the glass tube was heated at 60 °C. The kinetics of polymerization were conveniently monitored by ^{1}H NMR. In analogy with the polymerization carried out under homogeneous conditions, the peaks corresponding to the triazoles moieties between 7.65 and 8.00 ppm progressively grew in the course of the polymerization (see Figure 1A).

Conversion was determined from relative integration of these rising peaks and of the unchanged peak corresponding to the H1 proton of the sugar monomer at 5.18 ppm (see Figure 1A). Investigation by ^{1}H NMR of the CuAAC interfacial polymerization kinetics showed that the polyaddition in miniemulsion proceeds smoothly and full conversion was finally attained after 4 h of reaction.

Relying on previous works,[20] it was hypothesized that performing the CuAAC polyaddition under microwave irradiation could improve the kinetics of the reaction in dispersed medium. The innocuousness of microwave irradiation toward the miniemulsion in terms of colloidal properties and stability was primarily confirmed on copper-free systems (200 nm vs 190 nm after irradiation), and microwave-assisted CuAAC interfacial polyaddition of **1** and **2** in miniemulsion was then investigated at 60 °C. As illustrated in Figure 1C, similar ^{1}H NMR profiles were observed in the course of the microwave-assisted polyaddition. Importantly, the kinetics of

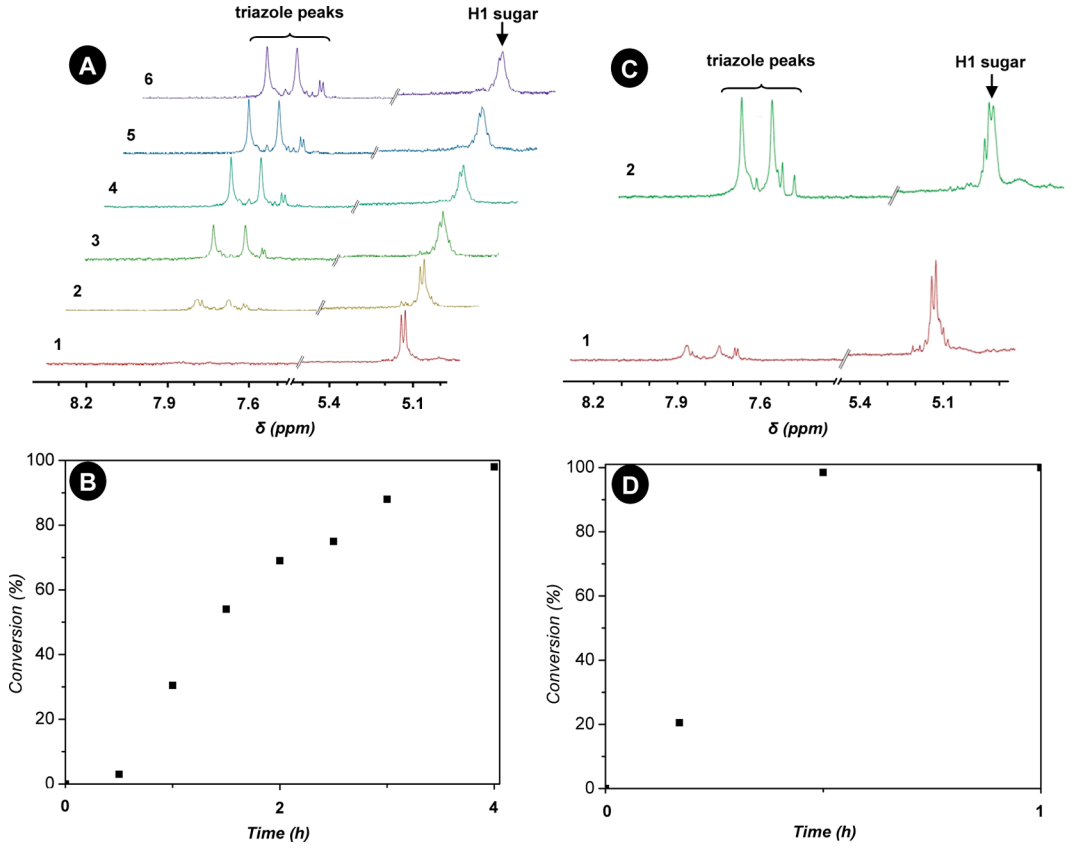

Figure 1. (A) ^{1}H NMR monitoring (DMSO-d_6) of CuAAC interfacial polyaddition of **1** and **2** in miniemulsion. From 1 to 6: 0.5, 1, 1.5, 2, 2.5, and 3 h of reaction (B) kinetics of CuAAC polymerization of **1** and **2** at 60 °C. (C) ^{1}H NMR monitoring (DMSO-d_6) of microwave-assisted CuAAC interfacial polyaddition of **1** and **2** in miniemulsion. From 1 to 2: 0.2 and 0.5 h (D) kinetics of microwave-assisted CuAAC polymerization of **1** and **2** at 60 °C.

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polymerization were considerably impacted by microwave irradiation. After 30 min of reaction, the microwave-assisted polyaddition in miniemulsion was indeed nearly complete (98%, see Figure 1D), whereas only 3% of conversion is achieved under conventional miniemulsion conditions after the same lapse of time.

The chemical microstructure of the glycopolytriazoles (that constitute the nanocapsule membranes) was further investigated by mass spectrometry analysis. The polymerization solutions were analyzed without any purification. Surprisingly, MALDI-TOF experiments performed in analogous conditions as those used for the polytriazoles synthesized under homogeneous conditions were unproductive. The polymers were consequently analyzed by ESI MS (see Table 1 and Supporting Information). The quality of the spectra was relatively poor, and many peaks could not be assigned due to the complex composition of the dispersion. However, collision-assisted dissociation (CAD) experiments clearly underlined the presence of species perfectly matching with the populations expected for the CuAAC polyaddition between **1** and **2** (Table 1), confirming the formation of oligotriazoles with DP ranging between 2 and 5.

Colloidal properties of the glyconanocapsules were further investigated. To evaluate the robustness of our strategy, CuAAC polyadditions were then repeatedly performed under conventional and microwave-assisted miniemulsion conditions. In all cases, dynamic light scattering (DLS) analysis of the final dispersed systems, diluted in a SDS solution, revealed the presence of nanocapsules with a polydispersity index below 0.20 and a *z*-average diameter of around 200 nm confirming that the process is reproducible and that the identity of the droplets generated during the emulsification step is maintained during the interfacial CuAAC polyaddition (see Figure 2). Importantly, all of the dispersions exhibited a perfect stability over months.

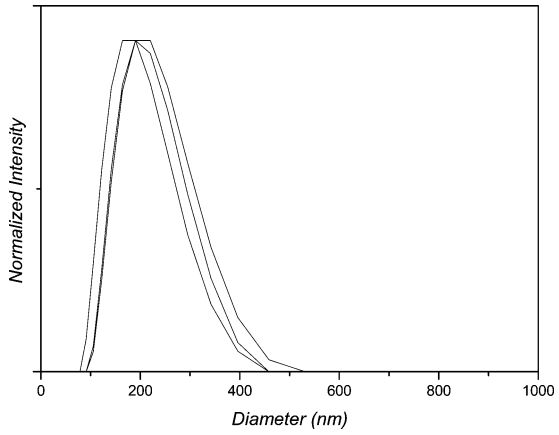

Figure 2. DLS of glycopolytriazole nanocapsule dispersions obtained under similar thermal CuAAC conditions (*z*-average diameters = 197, 218, and 219 nm).

Electron microscopy observation of dry preparations of the glycopolytriazole dispersion attested the preparation of robust spherical submicrometric particules. Although slightly smaller owing to drying treatment, the size of the nano-objects determined by scanning electron microscopy (SEM) was in good agreement with the DLS values observed in aqueous solutions (see Figure 3, top pictures). The formation of glyconanocapsules was undoubtedly assessed by transmission

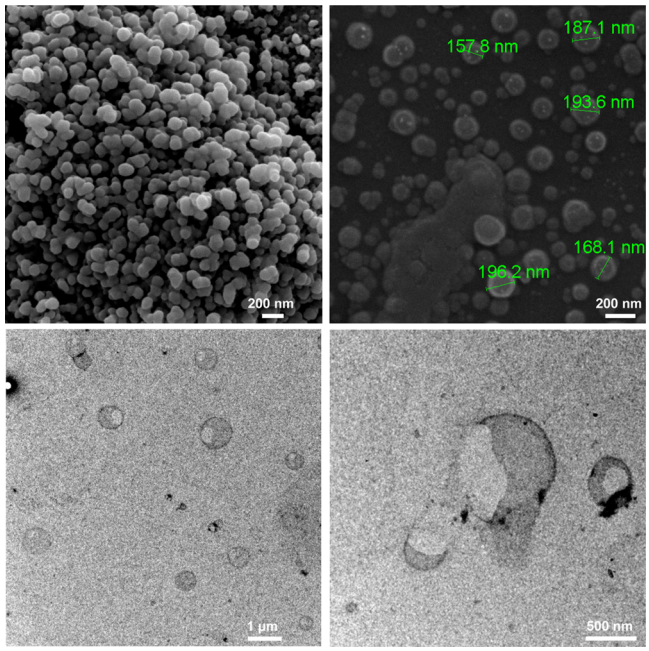

Figure 3. SEM (top) and TEM (bottom) images of the glyconanocapsules generated by interfacial CuAAC polyaddition.

electron microscopy images (TEM) that highlighted a difference of contrast between the center and the edge of the nanocapsules supporting the formation of a core−shell morphology with a glycopolymer shell of around 20 ± 6 nm (Figure 3, bottom pictures). The hollow structure of the nano-objects was further corroborated by the observation of several broken nanocapsules together with undamaged ones.

In summary, we have demonstrated that CuAAC interfacial polyaddition in oil-in-water miniemulsion conditions constitutes an original, robust, and reproducible method to straightforwardly generate stable glyconanocapsules with an average diameter of 200 nm. Employing microwave irradiation was shown to significantly enhance the kinetics of the CuAAC interfacial polyaddition of 6,6′-diazido-6,6′-dideoxysucrose and dialkyne affording the rapid preparation of glyconanocapsules (98% of conversion after 30 min of reaction) without disturbing the colloidal stability of the suspension. The formation of glyconanocapsules was finally confirmed by SEM and TEM.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental methods and ESI MS of the glyconocapsule membranes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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