Functional Nanostructures

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Simple but Precise Engineering of Functional Nanocapsules through Nanoprecipitation**

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Abstract: *A general, rapid, and undemanding method to generate at will functional oil-filled nanocapsules through nanoprecipitation is reported. On the basis of polymer and hexadecane/water/acetone phase diagrams, the composition can be set so that polymer chains preferentially stick at the interface of the oil droplets to create nanocapsules. The nanocapsules can be decorated with biorelevant molecules (biotin, fluorescent tags, metal nanoparticles) within the shell and loaded with hydrophobic molecules in a simple one-pot procedure.*

The generation of engineered synthetic capsules has attracted considerable attention owing to their promising application in the controlled release of pharmaceuticals, catalysis, imaging, and biochemical reactions.[1] Whereas *micro*capsule generation by chemical-engineering processes has been possible for decades, routes to polymeric *nano*-capsules require soft-matter technologies, such as: 1) well-established self-organization of amphiphilic block copolymers upon solvent displacement into polymersomes or micelles possessing a cross-linkable shell and a core which can be conveniently degraded,[2] 2) core removal of dendrimers,[3] 3) layer-by-layer deposition of polymers on sacrificial template particles,[4] 4) polymerization-induced self-assembly by controlled radical polymerization under dispersion or emulsion polymerization conditions,[5] or 5) interfacial polymerization under miniemulsion conditions.[6]

A simple means of generating colloids relies on the so-called nanoprecipitation technique,[7] the principle of which lies in the supersaturation of whatever hydrophobic solute (oil, polymer solids) primarily dissolved in a hydrophilic solvent (e.g. acetone, THF) when a large excess of the nonsolvent, water, is added. This technique, rediscovered just over a decade ago and coined the "ouzo effect", moved one step forward with the establishment of well-defined phase diagrams.[8] Whereas the generation of plain organic[9] or polymeric[7b] nanoparticles is now well-documented (including encapsulation in a polymeric matrix[10]), little has been reported on the encapsulation of liquids by a polymer by using the ouzo effect. This lack of studies on nanocapsule generation surely lies in the difficulty to reproducibly coprecipitate various substrates (oil, polymer, liposome…) in a one-pot procedure

Herein, we present a general, undemanding method for the construction of multifunctional, oil-filled nanocapsules with controlled morphologies in a simple, straightforward batch experiment. We demonstrate that a deep understanding of phase diagrams allows delimitation of the window in which narrowly dispersed core/shell nano-objects decorated with biologically relevant species can be elaborated (see Figure 1). More specifically, we demonstrate that in a narrow range of polymer/water/acetone/hexadecane (HD) compositions, solvent shifting forces the polymer chains to preferentially stick at the interface of the oil droplets to generate polymeric capsules in the full volume. We also show that the initial incorporation in the organic phase of a diisocyanate[11] with low water sensitivity both enables the structure of the nanocapsules to be locked through cross-linking of the polymer shell (Figure 1 A) and the attachment of molecules of interest primarily introduced in the water phase (Figure 1 C,D). Nanocapsules can also be loaded with hydrophobic molecules (Figure 1 D) or coated with metal nanoparticles (Figure 1 B).

To exemplify this concept, we focus herein on the production of functionalized, surfactant-free glyconanocapsules, which are promising candidates for drug-delivery applications.[12] Although highly attractive, the development of such glyco-nano-objects has been limited so far.[13] Water-soluble glycopolymers of *N*-[7-(α-D-mannopyranosyloxy)-heptyl]methacrylamide (HMM) were first prepared by polymerization mediated by 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (see full details in the Supporting Information). Polymerization reactions proceeded smoothly

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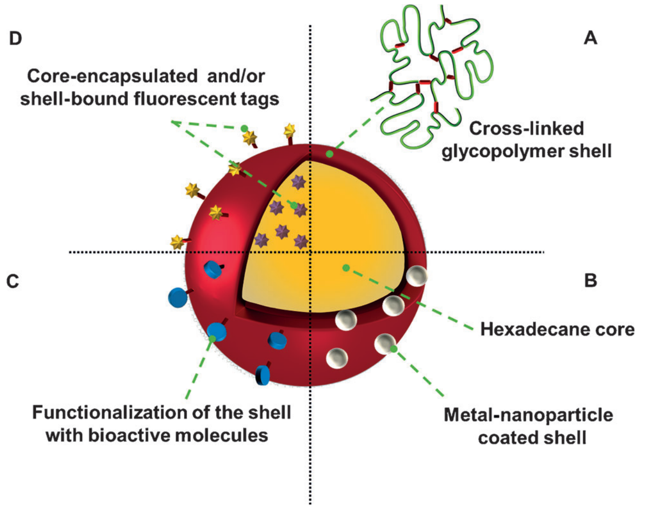


Figure 1. General schematic illustration of functional nanocapsules that can be built by nanoprecipitation: A) cross-linked oil-filled nanocapsules; B) metal-nanoparticle-coated nanocapsules; C) nanocapsules with bioactive molecules within the shell; D) core- or shell-tagged fluorescent nanocapsules.

to yield two polymers (PHMM1 and PHMM2) exhibiting molar masses of 24 and 102 kg mol^{-1} and a $Đ$ value below 1.10 (see Table S1 and Figure S1 in the Supporting Information).

Phase diagrams for the polymers in water (solvent)/acetone (nonsolvent) mixtures and for HD in acetone (solvent)/water (nonsolvent) were then determined (see Figures S2–S4 for the establishment of phase diagrams). The cloud-point boundary of the polymer/water/acetone ternary system was readily determined by the titration of aqueous solutions of different polymer concentrations with the step-by-step addition of acetone until the polymer precipitated. It can basically be described by a straight line at a constant mass fraction of acetone (see Figure 2). The boundary is slightly shifted to a lower mass fraction of the solvent when titrations are performed with the polymer possessing the highest molar mass (see Figure S2). The phase diagram of HD, superimposed on that of the polymerin Figure 2 shows three apparent zones(for the full diagram, see Figure S3). Above the binodal curve, HD is perfectly miscible with the acetone/water mixture (domains c and d). In the ouzo domain (b), metastable emulsions are formed, and for the largest concentrations of HD, that is, beyond the "ouzo limit" (domains a and e), the oil rapidly demixes.[14]

The requirement for nanocapsule formation is that emulsion droplets are generated and adsorption of the polymer at the interface occurs. The domain in which this process occurs (f, colored green in Figure 2) is narrow: Typically, the content of acetone should be between 65 and 70 wt %, whereas the final weight fraction of HD was chosen as 0.2 wt %. Dynamic light scattering (DLS) of the resulting dispersions highlighted the presence of a single population with a z-average diameter (D_z) of 124 nm and a polydispersity index equal to 0.11 (see Figure S4).

To "freeze" the structure of the capsules and simultaneously promote their functionalization, a cross-linker, isophorone diisocyanate (IPDI), was added initially to the organic solution (Scheme 1). Figure 3 shows TEM pictures of a series of nano-objects of increasing complexity. When HD and PHMM were coemulsified without IPDI or with a low content of diisocyanate (0.5 equiv per PHMM chain, 5.7×10^{-4} equiv per OH group), the structures did not survive to TEM analysis (Figure 3 A; see also Figure S5). HD leaked out of the droplets, whereas most of the PHMM chains self-assembled in small, dense dark dots of pure precipitated glycopolymer (see also Figure S2). Upon the addition of 1 equivalent of IPDI per PHMM chain, the D_z value of the capsules increased to 248 nm (PDI = 0.07), and the formation of a thin, fragile, and deformable membrane was observed by TEM at the surface of the droplets (Figure 3 B). HD domains and glycopolymer nanoparticles (from self-assembly of the unreacted free chains during sample casting) were still present. An increase in the IPDI content resulted in progressive shrinkage of the capsule membranes as a result of cross-linking reactions between PHMM chains, and the D_z value of the capsules dropped to 200 and 150 nm upon the addition of 5 and 24 equivalents, respectively, of IPDI (Figure 3 C and Figure S5). The nano-objects also gradually evolved to robust and perfectly spherical glyconanocapsules. Accordingly, leakage of HD was suppressed, thus confirming that the polymer membranes are sufficiently robust not to be broken during the TEM process (casting/analysis). Outside the defined f zone, nanocapsules cannot form (see Figure S6): In domain b, for example, droplets are not covered by the glycopolymer, so that, despite IPDI addition, free emulsion

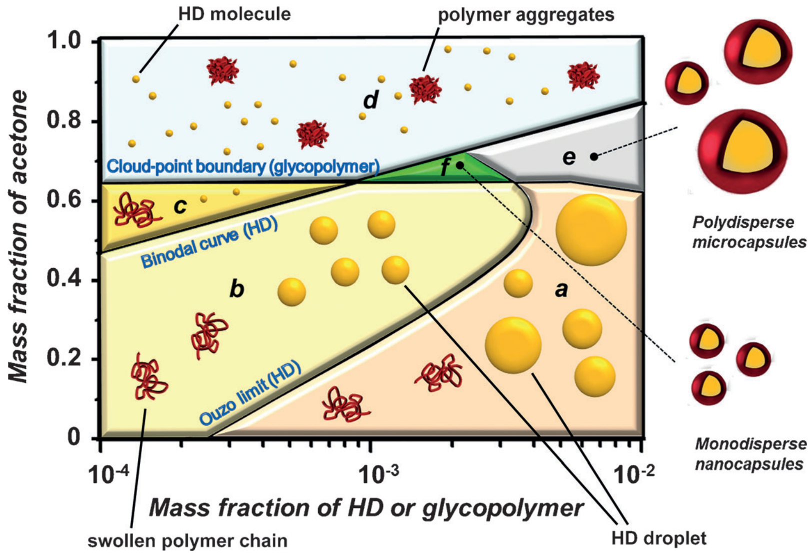
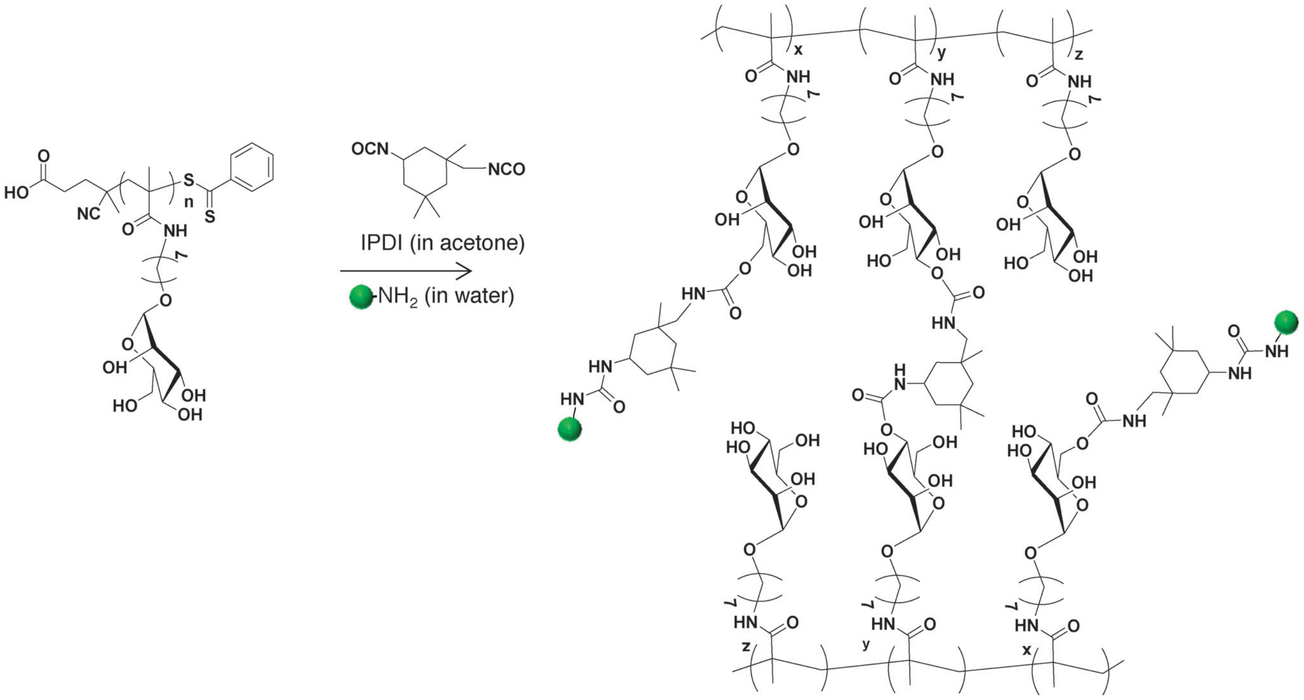


Figure 2. Overlapped phase diagrams of PHMM and HD in acetone/water mixtures. PHMM is soluble in zones a, b, and c, and micellizes in d, e, and f; HD is fully dissolved in zones c and d, generates metastable emulsions in b and f, and demixes in a and e. The domain f in which well-defined nanocapsules are built through the conjoint precipitation of HD and glycopolymer is shown in green (see also Figure S6). The cloud-point boundary in this diagram was established with PHMM2.



Scheme 1. One-pot cross-linking and functionalization of the polymer shell.

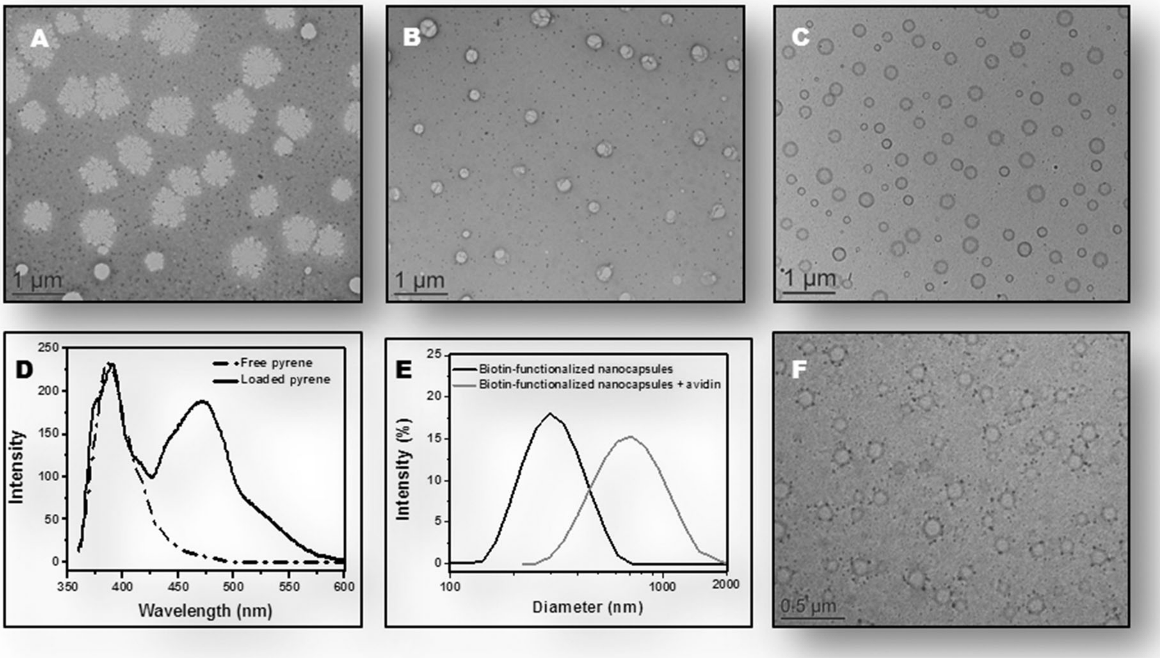


Figure 3. A, B, C) TEM images of nanocapsules obtained by adding a solution of HD (2 mg) in acetone (0.65 g) to an aqueous polymer solution (0.35 g of water and 1 mg of PHMM2) and cross-linking with 0.5 (A), 1 (B), and 24 equivalents (C) of IPDI with respect to the glycopolymer chains. D) Fluorescence emission spectra of an initial solution of pyrene in acetone and of pyrene-loaded capsules. E) Aggregation of biotin-tagged nanocapsules in the presence of avidin, as observed by DLS. F) TEM image of AuNP-functionalized nanocapsules.

droplets destabilize with time; on the other hand, in domain e, polydisperse (and quite robust) microcapsules have been spotted.

The versatility of the ouzo procedure also enables the generation of more complex nanocapsule-like structures, including the sequestration of hydrophobic molecules in the

HD core and the covalent attachment of hydrophilic molecules to the nanocapsule shell[15] (Scheme 1; see also Figures S7–S10). Dye-loaded nanocapsules were readily prepared through the direct addition of hydrophobic probes (e.g. pyrene) to the organic solution prior to the nanoprecipitation procedure. The encapsulation of pyrene was assessed by the fluorescence emission spectrum of the resulting dispersion. The spectrum showed an ensemble of monomeric peaks (between 375 and 410 nm) and an additional broad unstructured band (from 425 to 550 nm) due to excited-state-dimer (excimer) emission, which reflects the spatial proximity of the fluorophores in the nanocapsule cavity (Figure 3D; see Figure S10).[16] Fluorophores or biotin were attached to the polymer shell by incorporating an amino-functionalized fluorescein (Fc) tag or amino-functionalized biotin in the initial aqueous solution (see Figures S8 and S9). The addition of these coreactants has no impact on the nanoprecipitation and cross-linking processes. Fc-labeled and biotinylated nanocapsules with a z-average diameter of 210 and 245 nm, respectively, were generated. The covalent attachment of Fc tags to the polymer shell during the nanoprecipitation process was confirmed by a characteristic fluorescence emission at 518 nm (see Figure S9). Consistent with a successful functionalization, biotinylated nanocapsules were proven to disrupt 4′-hydroxyazobenzene-2-carboxylic acid (HABA)-avidin complexes owing to the favored binding of avidin to biotin ($K_d = 10^{-15}$ M versus 5.8×10^{-6} M for HABA-avidin; see also Figure S8). Accordingly, DLS confirmed the formation of large aggregates (967 nm) upon the addition of tetravalent avidin to biotinylated nanocapsules (Figure 3E). One-pot procedures with concomitant shell functionalization and core loading were equally effective (see Figure S11). Importantly, it was also shown that the initial incorporation of dodecanethiol-functionalized gold nanoparticles (AuNPs) or iron oxide nanoparticles within the organic phase leads to the preparation of NP-coated nanocapsules (Figure 3F; see also Figures S12 and S13).

To emphasize the general applicability of the method, we finally explored the preparation of nanocapsules from a different oil, miglyol 812, and a new glycopolymer, poly(N-[2-(α-D-mannopyranosyloxy)ethyl]methacrylamide). In analogy with the PHMM/water/acetone/HD system, well-defined miglyol-filled glyconanocapsules were generated, but with a smaller diameter and lower polydispersity index ($D_z = 130$ nm, PDI = 0.04; see Figure S14).

In conclusion, we have demonstrated that the exploitation of phase diagrams of different solutes solvent-shifted by the ouzo effect enables the generation of well-defined nanocapsules by an extremely fast and straightforward method. The nanocapsules can be shell-cross-linked, shell-functionalized, and loaded with active components at the same time in a rapid one-pot procedure. This simplified route to functional nanocapsules is general and can be readily transposed to other systems to design new materials of practical applicability in different fields in which colloids are used.

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