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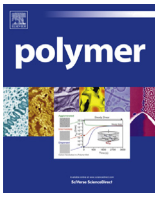

From well-defined poly(*N*-acryloylmorpholine)-stabilized nanospheres to uniform mannuronan- and guluronan-decorated nanoparticles by RAFT polymerization-induced self-assembly

Isabelle Chaduc [a], Eric Reynaud [b], Lionel Dumas [b], Luca Albertin [b, 1], Franck D'Agosto [a, *], Muriel Lansalot [a, **]

[a] *Université de Lyon, Univ. Lyon 1, CPE Lyon, CNRS UMR 5265, Laboratoire de Chimie, Catalyse, Polymères et Procédés (C2P2), LCPP Team, Bat 308F, 43 Bd du 11 Novembre 1918, F-69616 Villeurbanne, France*

[b] *Centre de Recherches sur les Macromolécules Végétales (CERMAV-CNRS), Grenoble, France*

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Non-ionic poly(*N*-acryloylmorpholine) (PNAM)-decorated polystyrene (PS) particles were synthesized by polymerization-induced self-assembly (PISA) in emulsion, mediated by the reversible addition-fragmentation chain transfer (RAFT) technique, in a one-pot/two-step process. PNAM was first prepared by RAFT polymerization in water using 4-cyano-4-thiothiopropylsulfanyl pentanoic acid (CTPPA) as chain transfer agent. Chain extension of PNAM by a PS block was then accomplished by the polymerization of styrene in water. Spherical nanoparticles (number-average diameter < 60 nm) exclusively composed of well-defined PNAM-*b*-PS amphiphilic block copolymers ($1.1 < Đ < 1.4$) were successfully obtained under a broad range of conditions (PNAM number-average molar mass of 2000, 4000 and 8000 g mol^{-1}, and average polymerization degree of the PS block from 150 up to 1600). Mannuronan ($ManA_{17}$)- and guluronan ($GulA_{20}$)-decorated nanoparticles were further synthesized according to a similar PISA process. Glycuronan macromonomers carrying a methacrylate polymerizable group ($ManA_{17}MA$ or $GulA_{20}MA$) were first copolymerized with *N*-acryloylmorpholine (NAM) under successful RAFT control using CTPPA. The resulting hydrophilic P(NAM-*co*-$ManA_{17}MA$) and P(NAM-*co*-$GulA_{20}MA$) macroRAFT agents were then used to polymerize styrene in water. Spherical glycuronan-decorated nanoparticles composed exclusively of amphiphilic block copolymers were successfully obtained for both glycuronan-based macroRAFT agents.

1. Introduction

The self-assembly of amphiphilic block copolymers in water is a very active field of research pioneered and popularized by Eisenberg, Discher and Zhang [1–5]. It enables the preparation of aggregates with morphologies ranging from spherical, to rods, lamellae, vesicles and large compound micelles [6] that find very promising application in nanoscience [7,8]. In a typical procedure (the co-solvent method) [2,6] the block copolymer is dissolved in a water-miscible common solvent (*i.e.* a good solvent for all blocks) and water is slowly added to desolvate the hydrophobic blocks and induce their aggregation into the hydrophobic part of the aggregates. Water addition is continued until well after the critical water content at which aggregation starts, and the aggregates are quenched in an excess of water to freeze the kinetic processes and morphologies. The common solvent is finally eliminated by dialysis of the resulting dispersion against water. The final morphology will be dictated by various parameters such as the nature and molar mass of the hydrophilic and hydrophobic blocks, the composition (*i.e.* hydrophile/hydrophobe ratio) and concentration of the block copolymer, the nature of the common solvent, the water content before quenching, the pH and/or the temperature. Although widespread, this process is unsuitable for routine and industrial applications since (i) it requires large amounts of water and long equilibration times and (ii) it invariably leads to dilute dispersions

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* Corresponding author.

** Corresponding author.

E-mail addresses: franck.dagosto@univ-lyon1.fr (F. D'Agosto), muriel.lansalot@univ-lyon1.fr (M. Lansalot).

[1] Present address: Laboratoire de Chimie et Biologie des Métaux, UMR 5249 - Université Grenoble Alpes, CEA, CNRS, 17 rue des Martyrs, 38054 Grenoble, France.

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of low solids content (typically < 2 wt%).

The large scale development of high performance materials based on block copolymer nanoparticles will depend on the availability of simple procedures to self-assemble and isolate large amounts of the targeted structures. In the case of water-borne nanoparticles, the so-called Polymerization-Induced Self-Assembly (PISA) process has considerably eased their preparation by combining the synthesis and self-assembly of amphiphilic block copolymers in a single operation [9–14]. It relies on the chain extension of living hydrophilic polymer chains with a hydrophobic monomer via controlled radical polymerization directly in water. Either performed under dispersion or emulsion polymerization, PISA allows to build phase diagrams [15,16] similar to those previously depicted for the self–assembly of preformed amphiphilic block copolymers [6] but obtained here under much more favorable conditions, *i.e.* at high solids content and short reaction times. Simply varying the relative fraction of each block in the copolymer usually dictates the final morphology of the aggregates, although kinetically trapped morphologies are quite common. Accordingly, most of the studies dedicated to PISA and targeting the formation of spheres, fibers or vesicles depict the effect of the increase of the molar mass of the hydrophobic block on the morphology of the final nano-objects, which most of the time follows the spheres - to worms - to vesicles transition [9–13,17,18] previously described by studies with the co-solvent method [6].

In our laboratory we are investigating Polymerization-Induced Self-Assembly in emulsion for the facile synthesis of polymer nano-objects with original and well-defined surface chemistry [19–22]. In this context, an original two-step one-pot RAFT-mediated PISA process was developed [23] that was successfully applied to the synthesis of polystyrene particles stabilized by various hydrophilic and pH-sensitive polymers. Thus, poly(acrylic acid) [24], poly(methacrylic acid) [25] or poly(methacrylic acid-*co*-poly(ethylene oxide) methyl ether methacrylate) [26] macroRAFT agents were first formed in water, and then directly used to mediate the emulsion polymerization of styrene in the same reaction vessel. It is worth noting that this strategy requires a very good control over the polymerization of the hydrophilic monomer(s) up to complete conversion [23].

Alginate is a family of binary heteropolysaccharides produced by brown algae [27] and by some soil bacteria [28]. They are linear unbranched copolymers of (1 → 4)-linked β-D-mannuronic acid (ManA) and α-L-guluronic acid (GulA) residues distributed in long homopolymeric blocks, *i.e.* (1 → 4)-β-D-mannuronan (ManA$_X$) and (1 → 4)-α-L-guluronan (GulA$_X$, where X is the degree of polymerization), and in shorter, mostly alternating, copolymer blocks [27,29]. Since they are entirely composed of glycuronic acid residues, alginates are glycuronans. A striking feature of alginates is their ability to undergo ionotropic gelation, *i.e.* to form transparent hydrogels upon addition of some divalent cations in aqueous solution (e.g. Ca^{2+}, Ba^{2+}, Sr^{2+} and Zn^{2+}). This is due to the intermolecular complexation of said ions by (1 → 4)-α-L-guluronan and alternating sequences [30–32] and to the formation of junction zones. Said complexation is the basis of the high affinity and selectivity of binding of alginates for some divalent metal ions [30,33–35] which is exploited for the sequestration of heavy metals [36–39] and radioactive isotopes in solution [40–42]. Another attractive characteristic of alginates is the possibility to extract their constituting blocks by controlled hydrolysis and selective precipitation [43,44]. This way homopolymeric guluronan and mannuronan samples with number-average degree of polymerization $X_n = 30 \pm 10$ and low molar mass dispersity ($Đ \leq 1.2$) can be obtained in good yield on a multi-gram scale. These oligosaccharides are stiff, negatively charged at pH ≥ 4, and retain the ion-binding properties of the parent homopolysaccharides. Concerning the biological activity of (1 → 4)-β-D-mannuronan and (1 → 4)-α-L-guluronan, it is demonstrated that they are potent immune-modulators [45,46].

To the best of our knowledge, only four previous papers describe the synthesis of carbohydrate-decorated nanoparticles by RAFT-mediated PISA in dispersed aqueous media, and then only neutral monosaccharides were considered. Bernard et al. [47] reported the use of a xanthate end-functionalized dextran (dextran-CTA, functionalization rate ca. 30%) to mediate vinyl acetate (VAc) emulsion polymerization. The *in situ* formation of dextran-*b*-PVAc block copolymers afforded particle stabilization and monodisperse PVAc latex particles were obtained with fast kinetics for low amounts of dextran-CTA (2–4 wt%). Ting et al. carried out the emulsion polymerization of styrene in the presence of hydrophilic poly((2-methacrylamido)-glucopyranose) prepared by RAFT [48] leading to the formation of spherical glyco-particles bearing glucose units. Very recently, Hatton et al. [49] used hydrophilic dithioester-functionalized xyloglucan (XG) chains for the synthesis of poly(methyl methacrylate) (PMMA) particles by RAFT emulsion polymerization. The nanoparticles were subsequently adsorbed onto cellulose through non-covalent interactions. Ladmiral et al. used hydrophilic polymer chains containing galactose units for the RAFT dispersion PISA of 2-hydroxypropyl methacrylate (HPMA) [50]. By varying the molar mass of the HPMA block and the solids content of the dispersion, glycopolymer-decorated nanospheres, worm-like micelles and vesicles were obtained. Concerning glycuronan-stabilized polymer particles, those described so far [51,52] are micrometric in size and stabilized by the physical adsorption of high molar mass alginates. Very recently, Kapishon and coworkers reported the synthesis of alginate-based micelles by a single electron transfer living radical polymerization (SET-LRP) PISA process [53]. Non-uniform alginate fragments of ca. 20 000 g mol^{-1} were obtained by the random depolymerization of native alginate and partially esterified with bromoisobutyryl initiator groups. Graft chains of poly(methyl methacrylate) were then grown by SET-LRP in homogeneous water/MeOH mixtures, leading to the *in situ* formation of alginate-g-PMMA copolymers. The latter self-assembled into micelles with sizes ranging from 50 nm to 300 nm and *Poly* values comprised between 0.06 and 0.20 (after dialysis), depending on the initial conditions of the polymerization.

In this paper, we first report the synthesis of polystyrene particles by the one-pot PISA process utilizing for the first time a non-ionic hydrophilic monomer (*N*-acryloylmorpholine, NAM). The RAFT homopolymerization of NAM in water and the chain extension of the resulting macromolecules with styrene in emulsion polymerization are first investigated in details under a broad range of conditions. We then describe the preparation of (1 → 4)-β-D-mannuronan- and (1 → 4)-α-L-guluronan-decorated nanospheres by PISA. Uniform guluronan and mannuronan samples ($Đ \leq 1.2$) were functionalized with a polymerizable group at their reducing end and the resulting macromonomers were copolymerized with NAM by aqueous RAFT. The resulting hydrophilic macroRAFT agents were then used to mediate the emulsion polymerization of styrene in the same reactor (Scheme 1).

2. Experimental

2.1. Materials

4,4'-azobis(4-cyanopentanoic acid) (ACPA, Fluka, >98%), sodium hydrogen carbonate ($NaHCO_3$, Aldrich, >99.7%) and *N*-acryloylmorpholine (NAM, Aldrich 97%) were used as received. Styrene (S, Aldrich, 99%) was purified by removing the inhibitor by filtration with aluminium oxide. 4-Cyano-4-thiothiopropylsulfanyl pentanoic acid (CTPPA) was obtained by reaction of ACPA with

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