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Synthesis and characterization of new temperature-responsive nanocarriers based on POEOMA-*b*-PNVCL prepared using a combination of ATRP, RAFT and CuAAC

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ABSTRACT

Narrowly distributed diblock copolymers comprising a non-linear poly(ethylene glycol) (PEG) analogue and a biocompatible temperature-responsive block were successfully synthesized for the first time through a "click" coupling approach, using the copper catalyzed azide-alkyne [3+2] dipolar cycloaddition (CuAAC) reaction. The alkyne-terminated poly(*N*-vinyl caprolactam) (PNVCL) was obtained by reversible addition fragmentation chain transfer (RAFT) polymerization and the α-azide terminated poly(oligo(ethylene oxide) methyl ether methacrylate (N_3-POEOMA) was synthesized by supplemental activator and reducing agent (SARA) atom transfer radical polymerization (ATRP). The ensuing temperature-responsive POEOMA-*b*-PNVCL copolymers are hydrophilic but became amphiphilic at temperatures above the low critical solution temperature (LCST) of the PNVCL and self-assemble into organized nanostructures. The thermal induced self-assembly behavior of these block copolymers and its dependence on block composition and solution properties, were accessed by turbidimetry and dynamic light scattering (DLS) analysis. Faster heating rates promote the formation of smaller and more stable particles ($D_h \sim 150–180$) with narrow size particle distributions (PDI < 0.1). TEM results revealed the formation of spherical aggregates at 45 °C. The encapsulation of nile red (NR), a model hydrophobic drug, into the hydrophobic domain of the diblock copolymer nanostructures as well as its temperature-triggered release was demonstrated by fluorescence spectroscopy. A small drop of the solution temperature causes the disruption of the nanostructures and induces the fast release of the hydrophobic content.

1. Introduction

Poly(N-vinyl caprolactam) (PNVCL) is a water soluble, non-ionic, thermo-responsive polymer that undergoes a phase separation in water for temperature above its cloud point (T_{CP}). The T_{CP} of this polymer is dependent on the polymer molecular weight (MW) [1–3] and solution concentration [2]. Due to its biocompatibility and low cytotoxicity, PNVCL stands out as an ideal polymer for biomedical applications [4], especially when compared to poly(*N*-isopropylacrylamide) (PNIPAM) [5]. The combination of such features turns PNVCL a very interesting hydrophobic segment to be used in experiments with solution temperatures above the T_{CP}. The conjugation of PNVCL with permanent hydrophilic segments such as PEG (or derivatives)

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enables the synthesis of temperature-responsive block copolymers. Such block copolymers are amphiphilic for temperatures above the T_{CP} of PNVCL, and therefore can form well-defined nanoaggregates via self-assembly routes. These stimuli-responsive structures have an enormous potential for advanced drug delivery systems (DDS), mostly because the assembly and disassembly of the block copolymers does not require the use of any additive [6,7]. Nevertheless, constrains involving the successful use of reversible deactivation radical polymerization (RDRP) methods for the controlled polymerization of NVCL hampered the wide use of this promising polymer in preparation of nanocarriers. Indeed, the literature reports on RDRP of PNVCL homo and (co)polymers are very scarce and consequentially its full potential has not been exploited yet. NVCL has been synthesized by reversible addition fragmentation chain transfer (RAFT) [1,2,8,9], cobalt-mediated radical polymerization [10–12] and atom transfer radical polymerization (ATRP) [13–15]. The non-activated nature of PNVCL poses several difficulties involving the synthesis of block copolymers with other segments by using a common RDRP method. The use of cobalt as organic metallic complexes allows the synthesis of block copolymers of poly(vinyl acetate)-*b*-PNVCL (PVAc-*b*-PNVCL) [10] as well as poly(vinyl alcohol)-*b*-PNVCL obtained after the hydrolysis of the PVAc segment [7]. Other strategies report the modification of the terminal chain-end groups of a first segment into a macro-RAFT agent for further RAFT polymerization of NVCL [16]. Using this approach, narrowly distributed block copolymers of PEG-*b*-PNVCL were obtained from a poly(ethylene glycol) methyl ether (mPEG)macro-RAFT agent [17] or PNVCL based linear dendritic block copolymers from an dendritic aromatic polyamide macro-RAFT agent [18]. A similar strategy involved the combination of ring opening polymerization (ROP) and RAFT to obtain linear poly(ε-caprolactone)-*b*-PNVCL (PCL-*b*-PNVCL) block copolymers from a macro-PCL RAFT agent [19], from a bi-functional initiator [20] and also using other architectures such as stars with PCL cores [21].

Poly(oligo(ethylene oxide) methyl ether methacrylate (POEOMA) is a brush-type polymer composed by pendent short PEG monomer units that is easily polymerized by RDRP methods [22]. POEOMA based copolymers have been reported for several different applications in the biomedical field, including inorganic nanocarriers [23], biodegradable nanogels [24], bioconjugates [25,26], gene delivery systems [27,28], and stimuli-responsive micelles [29].

In polymer chemistry the "click" inspired type of reactions have been in the spotlight over the last years since these synthetic routes are tolerant to a variety of functional groups, which facilitates the creation of precise molecular architectures [30,31]. The conjugation of different homopolymers through a CuAAC reaction [31–33] has been reported as a convenient and straightforward strategy in macromolecular engineering, for the development of block copolymers otherwise difficult to achieve [34].

In this work, controlled functionalized homopolymers of POEOMA and PNVCL were prepared via ATRP and RAFT, respectivelly. Well-defined block copolymers of POEOMA-*b*-PNVCL were obtained through the CuAAC reaction between azide terminated POEOMA (N_3-POEOMA) and alkyne terminated PNVCL (AT-PNVCL). The block copolymers were characterized and its temperature induced self-assembly was studied. The ability of such block copolymers to encapsulate water-insoluble molecules into its hydrophobic domain was also accessed by fluorescence spectroscopy, using nile red (NR) as a model hydrophobic drug.

2. Experimental

2.1. Materials

N-vinylcaprolactam (NVCL) (98%, Sigma-Aldrich) was purified by passing through a short alumina column and recrystallized in hexanes. Oligo(ethylene oxide) methyl ether methacrylate ($OEOMA_{500}$, with M = 500 g.mol^{-1} and pendent EO units DP ≈ 8) (99%, Aldrich) was purified by passing through a column filled with basic alumina to remove the inhibitor. Sodium dithionite ($Na_2S_2O_4$) (>87%, Merck), copper(II) bromide ($CuBr_2$) (99 + % + extra pure, anhydrous, Acros), sodium ascorbate (NaAsc) (⩾98%, Sigma), copper(II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$) (⩾98.0%, Sigma-Aldrich), tetrabutylammonium fluoride trihydrate ($TBAF{\cdot}3H_2O$) (99%, Acros Organics), 2-(2-chloroethoxy)ethanol (99%, Sigma-Aldrich), sodium azide (NaN_3) (⩾99.5%, Sigma-Aldrich), triethylamine (Et_3N) (⩾99%, Sigma-Aldrich), α-bromoisobutyryl bromide (98%, Sigma-Aldrich), nile red (NR) (TCI Europe), deuterated chloroform ($CDCl_3$) (Euriso-top, Euriso-top, +1% TMS), deuterium oxide (D_2O) (Euriso-top), phosphate buffered saline (PBS) tablets (Sigma), isopropanol (IPA) (Fisher Chemical), ethyl acetate (Fisher Chemical), hexane (⩾98.5%, Fisher Scientific), petroleum ether (Fisher Scientific), and tetrahydrofuran (THF) (Fisher Scientific) were used as received. 2,2′-Azobis(2-methylpropionitrile) (AIBN) (Fluka, 98%) was purified by recrylstallization from methanol before use. The 1,4-dioxane (Acros Organics, 99.8%) was dried over CaH_2 and distilled under reduced pressure prior to use. Purifued water (Milli-Q®, Millipore, resistivity >18 MΩ.cm) was obtained by reverse osmosis. For gel permeation chromatography (GPC), poly(methyl methacrylate) (PMMA) standards (Polymer Laboratories) (Acros, 99%, ~70 mesh) and high performance liquid chromatography (HPLC) dimethylformamide (DMF) (HPLC grade, Panreac) were used as received.

Protected alkyne-terminated RAFT agent ($PAT\text{-}X_1$) [35], tris[2-(dimethyamino)ethyl] amine (Me_6TREN) [36] and 2-(2-azidoethoxy)ethyl bromoisobutyrate (N_3E^iBBr) [37] were prepared following the procedures described in the literature. Briefly, the 2-(2-azidoethoxy)ethanol was synthesized from 2-(2-chloroethoxy)ethanol and sodium azide and then reacted with 2-bromoisobutyryl bromide to give N_3E^iBBr, ^1H NMR (400 MHz, $CDCl_3$, δ (ppm)): 1.95 (s, 6H, $(CH_3)_2C$), 3.39 (t, 2H, CH_2N_3), 3.70 (t, 2H, $N_3CH_2CH_2O$), 3.76 (t, 2H, $COOCH_2CH_2O$), 4.35 (t, 2H, CH_2OCO); ^{13}C NMR (100 MHz, D_2O, δ (ppm)): 50.23 ($N_3—CH_2—$), 60.38 ($—CH_2—OH$), 69.12 ($N_3—CH_2—CH_2—O$), 71.64 ($O—CH_2—CH_2—OH$).