Reactive & Functional Polymers 75 (2014) 31–40

Contents lists available at ScienceDirect

Reactive & Functional Polymers

journal homepage: www.elsevier.com/locate/react

Bisensitive core–shell nanohydrogels by e-Beam irradiation of micelles

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ARTICLE INFO

Article history:
Received 3 July 2013
Received in revised form 3 November 2013
Accepted 30 November 2013
Available online 7 December 2013

Keywords:
Block copolymers
e-Beam irradiation
Nanogels
Micelles
Light scattering

ABSTRACT

Sensitive nanohydrogels based on micellar aggregates from amphiphilic block copolymers were synthesized by means of electron beam irradiation. The crosslinked shell consists of temperature sensitive [*N*-isopropylacrylamide (NIPAAm)] and pH sensitive [5-methacryloyloxy pentatonic acid (5MPA) or 4-methacryloyloxy benzoic acid (4MBA)] units, while the core is a hydrophobic network that may work as a reservoir for drugs. The effect of the irradiation dose and the polymer concentration were tested. Dynamic and static light scattering were combined to investigate the morphology of aggregates prior and after irradiation to obtain nanohydrogels. Hydrodynamic radius of nanogels is in the range from 18 to 44 nm. ρ-Parameter suggests that crosslinked aggregates of flexible chains were obtained. Mixtures of block copolymers with different phase transition behavior in each aggregate lead to the preparation of nanohydrogels with tailored phase transition behavior.

1. Introduction

Nanohydrogels are nanometric sized swollen polymer networks with particular physicochemical properties such as good solubility in aqueous environment, stability in a broad range of pH and temperatures, good degree of flexibility and high surface/volume ratio [1]. In aqueous environment, responsive nanometric crosslinked polymers undergo a reversible phase transition induced, for instance, by temperature or pH [2–5]. These properties of nanohydrogels makes them candidates for applications such as controlled drug delivery, regenerative medicine, bioimaging and sensors [6–10]. Compared with other polymer nanoparticles used for drug delivery, stimulus responsive nanogels are noteworthy for their ability to encapsulate bioactive drugs, their high stability for prolonged circulation in the blood stream, and their controlled release and site-specific targeting of loaded drugs modulated by environment stimuli [8]. For drug delivery applications, the thermal responsiveness of carriers needs to be tuned to human body conditions [11–13]. Copolymerization of NIPAAm with hydrophilic monomers, containing weak carboxylic acids as substituents, can be used to tailor the phase transition temperature of nanogels above human body temperature in the pH range from 5.0 to 7.4 (physiological pH) [4,14]. Nanogels with ideal sensitivity behavior for transport and delivery of drugs could significantly improve the efficacy of therapies [15].

The broad application potential of this biomaterials have increased the interest in developing synthetic strategies that allow the preparation of nanogels with defined morphology, and controlled size and functionality. Various synthetic methods leaded to the preparation of chemically (covalent) crosslinked and physically (non-covalent) crosslinked nanogels [5–7]. The preparation methods are based on (1) polymerization of monomers in a homogeneous phase or in a micro- or nanoscale heterogeneous environment; (2) crosslinking of preformed polymers; (3) template-assisted nanofabrication of nanogel particles; and (4) physical self-assembly of interactive polymers [6]. The synthesis of chemically crosslinked micro- and nanogels from monomers and/or preformed polymers have been carried out by means of conventional and controlled radical crosslinking copolymerization techniques [16,17]. For example, heterogeneous free radical polymerization [18–21], reversible addition–fragmentation chain transfer (RAFT) polymerization [22–25], atom transfer radical polymerization (ATRP) [26–28] and nitroxide-mediated polymerization (NMP) [29–31], have been used. Irradiation methods, such as electron beam [32–34], gamma rays [35,36], and ultraviolet light [37–39] have been also tested. With these synthetic strategies, homogeneous [36,37] and core–shell [5,34,35,39] nanogels have been designed and prepared.

Electron beam (e-Beam) irradiation is a standard tool for the construction of nanogels. The procedure applied is usually based on the irradiation of dilute polymer solutions with a sufficient dose to obtain nanogels [32,33]. When dilute aqueous polymer solutions are subjected to ionizing radiation, polymer radicals are formed, which may decay by disproportion and recombination (crosslinking) [40]. Depending on the polymer concentration and on the dose, two

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http://dx.doi.org/10.1016/j.reactfunctpolym.2013.11.010

different crosslinking reactions can take place: inter and intramolecular crosslinking. If the irradiation dose and the distance between single polymer chains are high, the recombination of the formed radicals results mainly in an intramolecular crosslinking [32,33]. The e-Beam irradiation methodology has been used to prepare a variety of nanogels starting either with single polymer chains or aggregates of amphiphilic copolymers. For example Schmidt et al. [33] reported the synthesis of homogeneous nanogels by means of pulsed electron beam irradiation of dilute aqueous poly(vinyl methyl ether) solutions. Henke et al. [41] carried out the preparation of nanogels based on crosslinked interpolymer complexes by using high-molecular-weight polyvinylpyrrolidone and oligomeric poly(acrylic acid) as substrates. That report showed that radiation technique may be a useful tool to obtain multicomponent nanogels based on polymer complexes of hydrogen-bonding macromolecules. Zschoche et al. [42] prepared T/pH-responsive core–shell nanogels from poly(NIPAAm)-*graft*-poly(2-carboxyethyl-2-oxazoline)s. For that, micelle-like aggregates based on graft polymers were preformed and then irradiated at a dose of 20 kGy.

In this work, bisensitive nanohydrogels with well-defined core–shell morphology based on amphiphilic block copolymers crosslinked in the micellar state were prepared by means of e-Beam irradiation. This synthetic strategy may allow the preparation of nanohydrogels with controlled size, molecular weight and responsive behavior. In these materials, the shell is a T/pH-sensitive network that stabilizes the nanohydrogels in aqueous solution; while the core is a hydrophobic network that could be used as a reservoir for drugs and other hydrophobic molecules. The goal nanohydrogels have a great potential for application as tailored biomaterials for controlled drug delivery.

2. Experimental

2.1. Materials

The monomers, 4-methacryloyloxybenzoic acid (4MBA) and 5-methacryloyloxypentanoic acid (5MPA) were synthesized according to literature reports [43,44]. The RAFT agent (CTA), 2-hydroxyethyl 2-phenylacetate dithiobenzoate (HPDB) was synthesized following a previously reported procedure [45]. 4,4′-azobis(4-cyanopentanol) (ACP) was also prepared as described in literature [46]. All other chemicals were purchased. *N*-Isopropylacrylamide (NIPAAm, 97%, Aldrich) was purified by recrystallization from *n*-hexane. Hexyl acrylate (HA, 98.0%, Aldrich) and styrene, (St., 99.0%, Spectrum) were purified by passing through an inhibitor remover column (Aldrich). *p*-dioxane (ACS grade, Fermont), diethyl ether (ACS grade, Fermont), *n*-hexane (ACS grade, Fermont) and tetrahydrofuran (HPLC grade, Aldrich) were used as received.

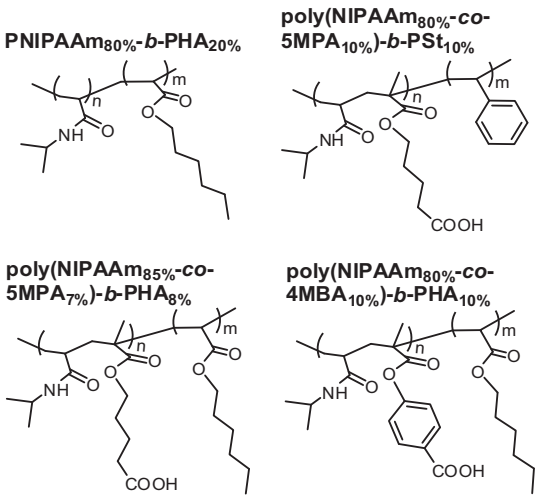
2.2. Preparation of block copolymers

Block copolymers were prepared following a two-step synthetic strategy described previously [14]. In the first step, macro-CTAs were synthesized with prescribed molecular weights via reversible addition–fragmentation chain transfer (RAFT) polymerization. Poly(NIPAAm)-S(C=S)Ph and poly(NIPAAm-*co*-4MBA$_{5\%}$)-S(C=S)Ph were prepared using molar ratios of monomer/CTA/initiator of 180:1.0:0.2. Poly(NIPAAm-*co*-5MPA$_{5\%}$)-S(C=S)Ph was synthesized using a molar ratio of 180:1.0:0.4. In each case the total monomer concentration used was 3.0 mol L^{-1}. As an example, the preparation of poly(NIPAAm-*co*-5MPA$_{5\%}$)-S(C=S)Ph using a molar ratio of 180:1.0:0.4, is described in detail: *N*-Isopropylacrylamide (3.010 g, 26.600 mmol), 5-methacryloyloxypentanoic acid (0.260 g, 1.400 mmol), 2-hydroxyethyl 2-phenylacetate dithiobenzoate (0.052 g, 0.156 mmol) and 4,4′-azobis(4-cyanopentanol) (15.743 mg, 0.062 mmol) were dissolved in *p*-dioxane (9.33 mL). The solution was added to an ampoule and degassed by three freeze–evacuate–thaw cycles; afterwards the ampoule was sealed with flame under vacuum. The reaction was carried out with magnetic stirring at 70 °C for 16 h. The reaction was stopped by freezing and the ampoule opened. *p*-Dioxane was removed by evaporation and the residual polymer was redissolved in acetone. Polymer solution was precipitated using an excess of diethyl ether for removing monomers and other non-polymeric residues. The polymer product was redissolved in acetone and precipitated again using an excess of diethyl ether. This procedure was repeated three times. The polymer was collected and dried under vacuum at room temperature overnight.

In the second step, block copolymers were prepared using styrene and hexyl acrylate for the second block (see Scheme 1). Macro-CTAs from PNIPAAm-S(C=S)Ph, poly(NIPAAm-*co*-5MPA$_{5\%}$)-S(C=S)Ph and poly(NIPAAm-*co*-4MBA$_{5\%}$)-S(C=S)Ph with molecular weight between 1.7×10^4 and 2.2×10^4 g mol^{-1} were used in molar ratios monomer:Macro-CTA:initiator of 50:1.0:0.2 for HA and of 100:1.0:0.2 for St. In each case the macro-CTA was dissolved in *p*-dioxane under mechanical stirring for 4 h to achieve a concentration of 7 mmol L^{-1}. Afterwards, the monomer and initiator were dissolved in the macro-CTA solution. Solutions were added to an ampoule, degassed by three freeze–evacuate–thaw cycles, sealed under vacuum and placed in a thermostated oil bath (70 °C). Copolymerization was continued for 24 h under magnetic stirring. Polymer solutions were precipitated into an excess of cold diethyl ether without removing the *p*-dioxane. In this way, residual monomers and also homopolymers were removed. The residual block copolymer was re-dissolved in acetone and precipitated again in an excess of cold diethyl ether. This procedure was repeated three times. The block copolymer products were finally dried under vacuum at room temperature overnight.

2.3. Preparation of nanogels by electron beam irradiation

Nanogels based on amphiphilic block copolymers crosslinked in the micellar state were prepared by means electron beam irradiation (e-Beam, see Scheme 2). In the first step aggregates were prepared either via direct dispersion in water (deionized water or buffer at pH 7.4) or by emulsion using dichloromethane (DCM) as oily phase. Concentrations of 0.25, 0.5, 0.75 and 1 mg mL^{-1} of amphiphilic block copolymers were tested. PNIPAAm$_{85\%}$-*b*-PSt$_{15\%}$ of 2.21×10^4 g mol^{-1}, PNIPAAm$_{80\%}$-*b*-PHA$_{20\%}$ of 2.34×10^4 g mol^{-1}, poly(NIPAAm$_{80\%}$-*co*-5MPA$_{10\%}$)-*b*-PSt$_{10\%}$ of 2.4×10^4 g mol^{-1}, poly(NIPAAm$_{85\%}$-*co*-5MPA$_{7\%}$)-*b*-PHA$_{8\%}$ of 2.66×10^4 g mol^{-1}, and



Scheme 1. Amphiphilic block copolymers prepared by RAFT.