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Prediction of the appropriate size of drug molecules that could be released by a pulsatile mechanism from pH/thermoresponsive microspheres obtained from preformed polymers

Gheorghe Fundueanu [a,b,*], Marieta Constantin [a], Ionela Oanea [a], Valeria Harabagiu [a], Paolo Ascenzi [c], Bogdan C. Simionescu [a,d]

[a] Department of Bioactive and Biocompatible Polymers, "Petru Poni" Institute of Macromolecular Chemistry, 700487 Iassy, Romania
[b] Department of Pharmaceutical Sciences, University of Ferrara, I-44100 Ferrara, Italy
[c] Department of Biology and Interdepartmental Laboratory for Electron Microscopy, University Roma Tre, I-00146 Roma, Italy
[d] Department of Natural and Synthetic Polymers, "Gheorghe Asachi" Technical University of Iasi, 700050 Iasi, Romania

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ABSTRACT

Preparation of cross-linked pH/thermoresponsive microspheres from preformed polymers is still lacking in literature since copolymers possessing both temperature- and pH-sensitive units together with a cross-linkable moiety in appropriate ratios are required. Moreover, choosing of the appropriate drugs able to be loaded and then released in a pulsatile manner is randomly performed. Here, we report the synthesis of pH/thermoresponsive cross-linked microspheres based on *N*-isopropylacrylamide and *N*-alloc-ethylenediamine. A chromatographic method was developed to predict the appropriate size of drug molecules that could be loaded and then released in a pulsatile manner. Accordingly, it was established that common drugs (salicylic acid, benzoic acid, nicotinic acid, lidocaine and diclofenac), with molecular weights ranging between 100 and 1000 g mol^{-1}, could be loaded and released in a pulsatile manner. Biologic molecules with higher molecular weights (heparin, lysozyme and bovine serum albumin) are completely excluded from the pores of cross-linked pH/thermoresponsive microspheres both below and above the volume phase transition temperature.

1. Introduction

Stimuli-responsive polymers are a class of materials that undergo a phase transition when small changes in the environmental parameters take place [1–3]. Among stimuli-responsive polymers, pH- and temperature-responsive polymers are the most used for biomedical applications because they use the change in pH and temperature of the human body as triggering agents in drug delivery [4–6]. Poly(*N*-isopropylacrylamide) (poly(NIPAAm)) is the most popular thermoresponsive polymer since it exhibits a sharp phase transition around 32 °C [7,8]. The temperature at which this transition occurs is called the lower critical solution temperature (LCST). Below the LCST, the polymer chain is hydrated and adopts an extended coil conformation, while above it the polymer is dehydrated and adopts a globular conformation. Correspondingly, the cross-linked hydrogels obtained from these polymers swell under the LCST and shrink above it [9,10]. This swelling/shrinking process is usually used to control the delivery of drugs in a pulsatile manner [11,12]. When a pH-sensitive monomer (weak acidic or basic monomer) is copolymerized with *N*-isopropylacrylamide (NIPAAm), a hydrogel with both pH- and thermosensitive properties is obtained [13]. Moreover, if the pH-sensitive monomer is hydrophilic, the LCST of the copolymer could be increased towards the body temperature [14]. However, conventional thermoresponsive hydrogels are limited for practical applications because of their slow swelling and deswelling rates [15,16]. Basically, the rates of swelling and deswelling processes of conventional hydrogels depend on the rate of solvent diffusion and on the gel size and porosity [17,18]. The smaller the size of the hydrogel, the faster the response rate to the input signal. Methods for the preparation of small microspheres with a relative fast swelling/deswelling rate are well known [19,20].

Most microspheres are prepared from monomers by suspension [11] or precipitate polymerization [21]. The main disadvantage of these methods is that it is more difficult for demonomerization to take place in a tridimensional network than in a solution of linear polymer. Moreover, the removal of monomers is often incomplete.

* Corresponding author at: Department of Bioactive and Biocompatible Polymers, "Petru Poni" Institute of Macromolecular Chemistry, 700487 Iassy, Romania. Tel.: +40 232 217454; fax: +40 232 211299.
E-mail address: ghefun@icmpp.ro (G. Fundueanu).

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Thermoresponsive microspheres from preformed polymers can be prepared by dropping a polymer solution into a liquid at a temperature above the LCST [22,23]. However, these microspheres are not stable or easy to handle, and have a reduced number of biomedical applications. Preparation of microspheres by cross-linking the functional amido groups of poly(*N*-isopropylacrylamide-co-acrylamide) was pioneered by our research group. However, due to the low reactivity of the amido groups, the necessary time to produce stable microspheres is very long [24]. The loading of these microspheres with biologically active compounds and the release studies were randomly performed [11]. The size of drug molecules is not correlated with the pore size of thermoresponsive microgels below and above the LCST. Therefore, no a priori information is available about the loading and release mechanism, and attempts must be done before.

In this paper, the preparation of stable pH/thermoresponsive microspheres from well-characterized preformed polymers is reported. Poly(*N*-isopropylacrylamide-co-*N*-alloc-ethylenediamine) (poly(NIPAAm-co-NAEDA)) was prepared as a new pH/thermoresponsive polymer possessing cross-linkable amino groups and having an LCST tailored towards body temperature. This copolymer was transformed into pH/thermoresponsive stable microspheres by an original approach based on the cross-linking of the amino group of *N*-alloc-ethylenediamine (NAEDA) with glutaraldehyde (GA) at a temperature slightly below the LCST. The microspheres were characterized by optical and scanning electron microscopy (SEM) in the dried, swollen and shrunken states. Inverse size exclusion chromatography (ISEC) was used to characterize the permeability of microspheres below and above the LCST, and to predict the appropriate size of drug molecules that can be released by a pulsatile mechanism.

2. Materials and methods

2.1. Materials

NIPAAm, obtained from Aldrich Chemical Corp. (Milwaukee, WI, USA), was recrystallized with hexane. NAEDA, GA aqueous solution (25% w/v), potassium persulfate and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) were supplied by Fluka AG (Seelze, Germany). Light mineral oil ($d = 0.84\ g\ ml^{-1}$) was supplied by Sigma Chemical Co. (St Louis, MO, USA). Deuterated water (D_2O) (for determination of total volume of the column), blue dextran (BD), $M_w = 2000{,}000\ g\ mol^{-1}$ (for determination of the void volume) and dextrans (DTs) with standard molecular weights (i.e. DT 1000 ($M_w = 1000\ g\ mol^{-1}$), DT 5000 ($M_w = 5000\ g\ mol^{-1}$) and DT 12000 ($M_w = 12{,}000\ g\ mol^{-1}$)) were provided by Sigma–Aldrich GmbH (Buchs, Switzerland). Common drugs (salicylic acid, benzoic acid, nicotinic acid, lidocaine and diclofenac), heparin ($M_w = 12{,}000\ g\ mol^{-1}$), vitamin B_{12} and glucose were supplied by Sigma–Aldrich GmbH (Buchs, Switzerland). Proteins with standard molecular weights (lysozyme, $M_w = 14{,}300\ g\ mol^{-1}$, and bovine serum albumin (BSA), $M_w = 66{,}000\ g\ mol^{-1}$) were provided from Amersham-Pharmacia Biotech Europe GmbH (Freiburg, Germany).

All chemicals were of analytical or reagent grade, and were used without purification unless stated.

2.2. Synthesis of poly(NIPAAm-co-NAEDA)

The synthesis of linear poly(NIPAAm-co-NAEDA) was carried out by free radical copolymerization in aqueous solution. Typically, 1.13 g of NIPAAm (10 mmol) and 0.361 g of NAEDA (2 mmol) were solubilized in 15 ml of 1.0 M KNO_3 aqueous solution. Dried nitrogen was bubbled through the solution for 30 min prior to polymerization. The initiator (0.050 g of $K_2S_2O_8$; 0.074 mmol) and accelerator (20 μl of TEMED) were then added to the solution and copolymerization was achieved within 6 h at 18 °C. Thereafter, the polymer solution was dialyzed for 5 days at 20 °C (molecular weight cut-off 10,000–12,000 $g\ mol^{-1}$; from Medi Cell International, UK) and recovered by freeze-drying.

2.3. Determination of molecular weight

The molecular weight of copolymers was determined by viscosimetric measurements. For this purpose, the copolymers were dissolved in tetrahydrofuran and the viscosity of copolymer solutions (the concentration ranged between 0.25 and 1.0 $g\ dl^{-1}$) was measured at 27 °C. The viscosity-average molecular weight of copolymers (M_v) was calculated according to Eq. (1) [25]:

$$[\eta] = 5.8 \times 10^{-5} M_v^{0.78} \quad (1)$$

where $[\eta]$ is the intrinsic viscosity ($dl\ g^{-1}$), and 10^{-5} and 0.78 are constants (*K* and *a*, respectively) for a given polymer–solvent–temperature system.

2.4. Copolymer composition

The copolymer composition was determined by ^1H nuclear magnetic resonance (NMR) analysis. ^1H NMR spectra of poly(NIPAAm-co-NAEDA) were recorded in D_2O on a Varian Mercury Plus 400/Varian VXR 200 spectrometer operating at 400 MHz. The molar fractions of NIPAAm and NAEDA in copolymer were determined from the area of the peak at 3.90 ppm, due to the methyne group of NIPAAm, and the area of the peak at 3.13 ppm for the methylene protons of NAEDA.

2.5. Determination of the lower critical solution temperature

LCST was determined from the dependence of the absorbance change at 450 nm on temperature. An ultraviolet–visible (UV–Vis) Specord 200 spectrophotometer (Analytic Jena, Jena, Germany), coupled with a temperature controller, was used. The polymer solution (1–10% w/v) was prepared in distilled water, a standard acidic solution (pH 1.2, 64 mM HCl + 50 mM KCl) and a standard phosphate buffer solution (pH 7.4, 50 mM Na_2HPO_4 + NaOH; PB). The heating rate was 2 °C every 10 min and 0.2 °C in the vicinity of the cloud point (CP). CP was defined as the inflexion point in the curve of the absorbance change vs. temperature.

2.6. Microsphere preparation

Typically, 1 g of poly(NIPAAm-co-NAEDA) was solubilized in 12 ml distilled water at 4 °C. Next, 0.4 ml 0.5 M H_2SO_4 and 1 ml of GA solution (25% w/v) were added just before dispersion of the aqueous copolymer solution in 60 ml of the dispersing phase, which consisted of light mineral oil. As a dispersing agent, 0.25 g of soybean lecithin was added. The reactor consists of a cylindrical vessel (*h* = 8 cm, i.d. = 8 cm) with a round bottom. The mixture was stirred at 350 rpm by a three-blade turbine impeller for 12 h at a temperature lower than the LCST (32 °C). Finally, the cross-linked microspheres were washed successively with cyclohexane, methanol, water, 0.1 N HCl, water and acetone, then dried from diethyl ether.

2.7. Morphological and dimensional analysis

The morphology of the microspheres was evaluated by the stereomicroscope AlphaSTO-5 (Elektro-Optika Ltd., Hungary) and by SEM. The mean diameter of the microspheres was determined