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Entrapment and release of drugs by a strict "on-off" mechanism in pullulan microspheres with pendant thermosensitive groups

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ABSTRACT

Here, we report a new method to predict the appropriate size of drugs which can be entrapped in and released from a hydrogel with pendant thermosensitive units by a strict "on-off" mechanism. Moreover, the valve-type action of the thermosensitive arms has been investigated. Inverse size exclusion chromatography (ISEC) and environmental scanning electron microscopy (ESEM) have been used to characterize the extension and collapse of the pendant thermosensitive units, below and above the lower critical solution temperature (LCST) under physiological conditions, confirming the hypothesis postulated by the "arid" theoretical models.

The functionalized pullulan (Pul) microspheres, here prepared, were coupled with thermoresponsive oligomers by reaction between the $-NH_2$ end-group of oligomers and chlorine present on Pul microspheres. The Pul microspheres with temperature sensitive moieties were packed in a glass column and the elution volume of standard molecule with well-known molecular weights (radius of gyration) was determined below and above the LCST. FITC-Dextran 4000 diffused through the pores of Pul microspheres with short thermosensitive arms (Mw = 1500 g/mol) both below and above the LCST of the thermosensitive units. In contrast, Pul microspheres with long thermosensitive arms (Mw = 3300 g/mol) allowed the diffusion of FITC-Dextran 4000 only above the LCST of the thermosensitive units. Indeed, the long thermosensitive arms are extended below the LCST and FITC-Dextran 4000 is completely excluded from the pores. The loading/release profile of this model molecule follows an "on-off" mechanism, confirming the results obtained by ISEC. ESEM was used as a new technique, taking images of the surface of the thermosensitive pullulan microspheres in their natural swollen state, with no prior specimen preparation, below and above the LCST. The low toxicity of pullulan microspheres observed below and above the LCST of thermosensitive units at high concentrations (10 mg/ml) recommends their potential use for controlled drug delivery applications.

1. Introduction

Thermoresponsive polymers that undergo changes of their physicochemical properties by variation of the environmental temperature have gained attention recently for their relevant technological and biomedical applications [1,2].

Poly(N-isopropylacrylamide) (poly(NIPAAm)) is the most known thermoresponsive polymer because it exhibits a sharp phase transition around 32 °C, which is close to the human body temperature [3,4]. The temperature at which this transition occurs is called the lower critical solution temperature (LCST). Below the LCST the polymer chain is hydrated, adopts an extended coil conformation, and is soluble in water. In contrast, above the LCST the polymer is

Abbreviations: AAm, Acrylamide; AcCl-Pul microspheres, Acethyl chloride pullulan microspheres; AIBN, N,N′-azobisisobutyronitrile; CA, Cysteamine hydrochloride; CAB, Cellulose acetate butyrate; ClAcCl, Chloroacetyl chloride; CP, Cloud point; DMF, Dimethylformamide; ECH, Epichlorohydrin; LCST, Lower critical solution temperature; NIPAAm, N-Isopropylacrylamide; Pul, Pullulan; poly(NIPAAm-co-AAm), poly(N-isopropylacrylamide-co-acrylamide); ST, Semitelechelic NIPAAm/AAm oligomer; TEA, Triethylamine; VPTT, Volume phase transition temperature.

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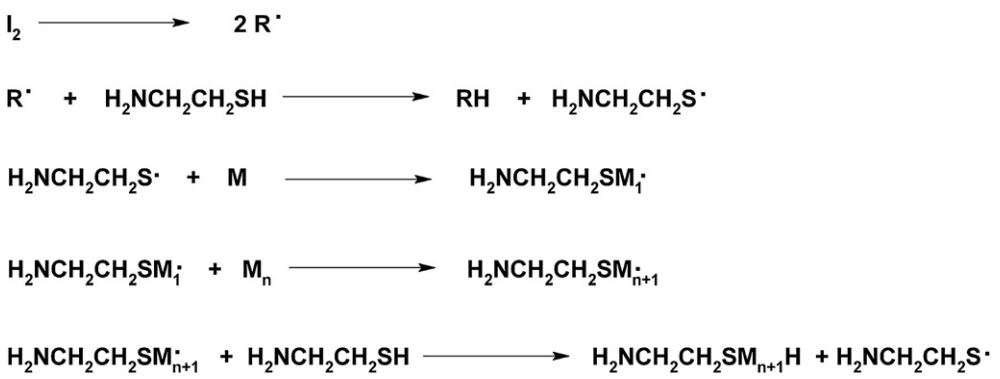

Fig. 1. Radical oligomerization of NIPAAm/AAm.

dehydrated, becomes hydrophobic, adopts a globular conformation and is insoluble. Correspondingly, the cross-linked hydrogels obtained from these polymers swell under the volume phase transition temperature (VPTT; that corresponds to LCST in cross-linking hydrogels) and shrink above it [5,6]. This swelling/shrinking process has been exploited for the development of thermosensitive drug delivery systems. Notably, the drug is usually released when the hydrogel is in the swollen state (i.e., below the VPTT) [7,8]. Above the VPTT, the matrix shrinks impairing the drug release. However, a large amount of drug is released during the swelling/shrinking process and a strict "on-off" release mechanism is not always achieved [7,8]. Moreover, even in the collapsed state, these networks are enough permissible for drugs with small size (low molecular weight) [9]. In order to avoid these drawbacks, hydrogels with pendant thermoresponsive units, that do not change their volume below and above LCST, were synthesized [10,11]. In fact, the release of drugs is controlled by the extension/contraction of the pendant units, acting as a valve, instead of volume changes. Below the LCST, the thermosensitive units are in the extended state and impair the drug release. Above the LCST, the thermosensitive units collapse, allowing the drug release. However, the choice of the appropriate molecule size to be released by a strict "on-off" mechanism is always randomly performed and requires a lot of work [12]. Moreover, the valve-type action of thermoresponsive arms, often depicted by the "arid" theoretical models, is not satisfactory [13]. Most thermosensitive drug delivery systems are used as chemically cross-linked poly(NIPAAm) based hydrogels [14,15]. From viewpoint of some biomedical applications, these hydrogels are not appropriate candidates because they are not biodegradable under physiological conditions.

Here, we report a new thermoresponsive drug delivery system based on pullulan (Pul) microspheres coupled with semitelechelic poly(N-isopropylacrylamide-co-acrylamide) oligomers. Firstly, this new drug delivery system is biodegradable because the oligomers (showing a LCST of about 37 °C at pH 7.4, phosphate buffer) are linked to Pul microspheres by an ester group that can be hydrolysed under physiological conditions. Moreover, Pul is a biodegradable polysaccharide. Secondly, the volume of this drug delivery system is unaffected by temperature changes below and above the LCST. Thirdly, the characterization of these microspheres by inverse size exclusion chromatography (ISEC) allowed determination of the length of the thermosensitive units and the exact size of drugs that can be entrapped and released by a strictly "on-off" mechanism. This mechanism, based on the contraction and extension degree of the pendant thermoresponsive oligomers, has been also characterized by environmental scanning electron microscopy (ESEM).

Table 1
Preparation and characterization of semitelechelic NIPAAm/AAm oligomers.

Sample	[T]/[M][a]	Yield (%)	Exchange capacity (meq/g)	M_n (g/mol)
ST_1	0.05	68.4 ± 7.2	0.66 ± 0.02	1500
ST_2	0.025	77.5 ± 8.1	0.3 ± 0.01	3300

Data are the results of three independent experiments.
[a] [T]/[M] represents the molar ratio between the chain transfer agent and the monomers.

2. Materials and methods

2.1. Materials

Pul, Mw = 200,000 g/mol, was purchased from Hayashibara Laboratories LTD (Okayama, Japan). Cellulose acetate butyrate (CAB; Mw = 40,000 g/mol, degree of substitution (%) with acetyl = 0.2, butyryl = 2.4, hydroxyl = 0.4) was obtained from Eastman Inc. (Kingsport, Tennesse, USA). N-isopropylacrylamide (NIPAAm), obtained from Aldrich Chemical Corp. (Milwaukee, WI, USA), was recrystallized with hexane. Acrylamide (AAm), dimethylformamide (DMF), epichlorohydrin (ECH), N,N′-azobisisobutyronitrile (AIBN), chloroacethyl chloride (ClAcCl), triethylamine (TEA), cysteamine hydrochloride (CA), glucose, FITC-Dextran 4000 and standard dextrans with different molecular weights (Mw = 1000, 5000, 12000, 25000, 2000000 g/mol) were supplied from Fluka AG (Buchs, Switzerland). All chemicals were of the highest analytical grade and used without purification unless stated.

2.2. Synthesis of Pul microspheres

Pul microspheres were prepared by suspension cross-linking with ECH of an aqueous solution of the Pul polymer as previously reported [16].

Cross-linked Pul microspheres were obtained using a cylindrical glass reactor provided with an anchor type glass stirrer, and a reflux condenser. The reactor was maintained at a constant temperature with a thermostatic water bath. In detail, 4 g of Pul were dissolved in 35 ml NaOH solution (10%, w/v) under stirring. After a complete removal of the air bubbles under vacuum, the solution was poured in 100 mL of dispersion medium (1,2-dichloroethane) in which 2.4 g of CAB (as dispersion agent) were dissolved. The obtained w/o emulsion was stirred for 1 h, then 4 mL of ECH were added and the cross-linking reaction was carried out for 20 h at 55 °C. The cross-linked microspheres were recovered by filtration through a sintered glass filter, under vacuum. The removal of residuals was performed by washing the microspheres in the following order: 1,2-dichloroethane, acetone, acetic acid/water solution (30%, v/v), distilled water, and methanol. Then, microspheres were completely dried by overnight exposure to 60 °C, under vacuum.

2.3. Synthesis of acethyl chloride Pul (AcCl-Pul) microspheres

Typically, 1 g (6.1 mmol) Pul microspheres were swollen in 25 ml DMF for 24 h. Then, 1.47 mL ClAcCl (18.5 mmol) were added dropwise for 0.5 h, then the reaction continued for 2.5 h at 18 °C. Finally, the microspheres were washed successively with DMF, distilled water, and acetone; lastly, they were dried from diethyl ether.

2.4. Synthesis of semitelechelic NIPAAm/AAm oligomers

Semitelechelic NIPAAm/AAm oligomers were synthesized by free radical copolymerization in DMF using AIBN as the initiator. Typically, 1.13 g NIPAAm (10 mmol), 106.5 mg AAm (1.5 mmol), 65 mg CA (0.57 mmol), and 25 mg AIBN (0.15 mmol) were solubilized in 6 ml DMF. Dried nitrogen was bubbled through the solution for 30 min prior to polymerization. The reaction mixture was allowed to react at 80 °C for 16 h. At the end of the reaction, 0.66 mL triethylamine were added as the hydrochloride captor. The oligomers were precipitated into diethyl ether. The precipitate was