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Poly(NIPAAm-co-β-cyclodextrin) microgels with drug hosting and temperature-dependent delivery properties ☆

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ABSTRACT

One of the most important drawbacks of the thermosensitive hydrogels based on N-isopropylacrylamide (NIPAAm) is the lack of functional groups able to specifically bind drugs; moreover, these hydrogels are not biodegradable. In order to overcome these inconveniences, poly(NIPAAm-co-β-cyclodextrin) (poly(NIPAAm-co-β-CD)) microgels were obtained by cross-linking polymerization of the corresponding monomers. β-CD was first functionalized with an appropriate amount of vinyl groups, thus acting both as a co-monomer with hosting properties and as a biodegradable cross-linker. The volume phase transition temperature (VPTT) of the microgels was determined under simulated physiological conditions by measuring the swelling degree and by microcalorimetry. The microgels, due to their small size and high porosity, possess a relative rapid swelling/deswelling rate around the human body temperature. The hydrogels were loaded with the model drug diclofenac by inclusion within cyclodextrin cavity and the release studies were performed under simulated physiological conditions, below and the above the VPTT. In the presence of α-amylase (from *Aspergillus Oryzae*), microgels have showed a low degradation rate (15% of initial weight after 7 days), the erosion occurring especially at the surface.

1. Introduction

Controlled drug delivery systems have captured the attention of researchers in the last three decades [1–3]. Although great advances have been made in this area, these delivery systems are not appropriate for any type of disorder. As a result, self-regulated drug delivery systems have been designed and developed [4,5]. These systems have the capacity to perceive themselves small changes in normal physiological parameters and act to redress them.

Among the self-regulated drug delivery systems, thermosensitive hydrogels have a special place because they exploit small changes in the temperature of the human body and use these changes as triggering agents [6,7]. Most of the temperature-sensitive hydrogels are based on poly(*N*-isopropylacrylamide) (poly(NIPAAm)), indeed this polymer, in aqueous solution, possesses a transition temperature (called lower critical solution temperature; LCST), close to that of the human body [8,9]. Below the critical temperature, poly(NIPAAm) is in the hydrated state and is soluble, while above the critical temperature, the polymer is dehydrated and precipitates. Correspondingly, the hydrogel obtained from poly(NIPAAm) swells under the LCST and collapses above the LCST. This swelling/collapsing process is usually exploited for pulsatile release of drugs [10,11].

Three-dimensional hydrogels are usually obtained by dropping a polymer solution in a liquid heated to a temperature higher than the LCST [12]. The main disadvantage of these hydrogels is the lack of chemical and mechanical stability. In fact, hydrogels with good chemical and mechanical stability were synthesized by covalent [5] or radiation cross-linking method [13]. However, most of cross-linked hydrogels are not biodegradable since the water soluble *N*,*N*-methylenebisacrylamide is widely used as cross-linker in aqueous solution [14,15].

Biodegradable hydrogels were synthesized by cross-linking NIPAAm with a biodegradable PEG-co-PCL macromolecular cross-linker under UV irradiation [16]. However, these hydrogels do

Abbreviation: A-CD, acryloylated CD; AC, acryloyl chloride; CD, cyclodextrin; DMF, *N*,*N*-dimethyl formamide; DS, degree of substitution; DSC, differential scanning calorimetry; ESEM, environmental scanning electron microscopy; KPS, potassium persulfate; LCST, lower critical solution temperature; 3-MBA, 3-methylbenzoic acid; NIPAAm, *N*-isopropylacrylamide; PBS, phosphate buffer solution at pH = 7.4; TEA, triethylamine; TEMED, *N*,*N*,*N*′,*N*′-tetramethylethylene-diamine; VPTT, volume phase transition temperature.

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not possess functional groups capable to bind specifically drugs. Copolymerization of NIPAAm with monomers containing functional carboxylic or amino groups seems to be an alternative, however, their presence in copolymers even in small amounts diminishes substantially the thermo-sensitivity [17,18]. Moreover, electrostatic interactions between the functional groups and opposite charged molecules induce a dramatic change of the transition temperature of the complex [19].

Remarkably, copolymerization of NIPAAm with functionalized cyclodextrins (CDs) appears to facilitate drug binding [20]. CDs are well-known as cyclic oligosaccharides capable to specifically bind drugs within the hydrophobic cavity. Moreover, being starch derivatives, CDs may be hydrolysed by starch degrading enzymes. Of note, that α-CD is not hydrolysed by α-amylase while β-CD is degraded to a certain extent [21,22].

In this study, β-CD has been chemically functionalized, thus acting both as a co-monomer with inclusion capacity and as a biodegradable cross-linker. Then, poly(NIPAAm-co-β-CD) cross-linked three-dimensional microgels were synthesized by co-polymerization of the co-monomers in aqueous solution. The microgels display both hosting and thermosensitive properties (relative high swelling/collapsing rates around the human body temperature). The microgels were loaded with the model drug diclofenac by inclusion within cyclodextrin cavity; the release studies were carried out under simulated physiological conditions, below and the above the VPTT.

2. Experimental

2.1. Materials

N-isopropylacrylamide (NIPAAm), obtained from Aldrich Chemical Co. (Milwaukee, WI, USA), was recrystallized with hexane. β-CD was provided from Roquette Frères (Lestrem, France). 3-methylbenzoic acid (3-MBA), acryloyl chloride (AC), *N*,*N*-dimethyl formamide (DMF), potassium persulfate (KPS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), triethylamine (TEA) were supplied from Fluka AG (Buchs, Switzerland). α-amylase from *Aspergillus Oryzae* (35.4 U/mg) was provided from Sigma–Aldrich (St. Louis, USA). Phosphate buffer solution at pH = 7.4 (PBS) (50 mM NaH_2PO_4 + NaOH) were prepared in our laboratory. All chemicals were of analytical or reagent grade and were used without purification unless stated.

2.2. Synthesis of acryloyl-β-cyclodextrin

Acryloyl β-CD (A-β-CD) was synthesized as follows. 11.35 g of β-CD (10 mmol) were solubilized in 50 mL of DMF at 50 °C, under stirring. The resulting clear solution was cooled down to room temperature and 8.36 mL of TEA (60 mmol) were added as HCl neutralizer. Thereafter, 4.87 mL of AC (60 mmol) in 50 mL DMF were added drop-wise (during one hour) to the mixture cooled down to −5 °C. The reaction continued for one hour at −5 °C, and for 10 h at 22 °C; then, the TEA hydrochloride was separated by filtration, and the filtered solution was precipitated in acetone. The precipitate was washed several times with acetone and chloroform and dried under vacuum. The resulting white powder was analysed by ^{1}H NMR.

2.3. Double bonds estimation

The number of acryloyl groups introduced per unit of β-CD was determined by ^{1}H NMR analysis. ^{1}H NMR spectra were recorded in deuterated water on a Varian Mercury Plus 400/Varian VXR 200 spectrometer operating at 400 MHz.

2.4. Synthesis of poly(NIPAAm-co-β-CD) microparticles

The synthesis of poly(NIPAAm-co-β-CD) microparticles was carried out by free radical co-polymerization in aqueous solution. 1.13 g of NIPAAm (10 mmol) and 0.5 g of A-β-CD_3 (0.41 mmol) were solubilized in 20 mL distilled water. Dried nitrogen was bubbled through the solution for 30 min prior to polymerization. Then, the initiator (0.025 g of KPS; 0.037 mmol) and the accelerator (30 μL of TEMED) were added to the solution and co-polymerization was achieved within 8 h at 22 ± 2 °C. Thereafter, the copolymer gel was extensively washed with distilled water and recovered by drying at 40 °C, under vacuum. Finally, the cross-linked co-polymer was ground with a grinder. Microparticle separation was performed by using a system of sieves with the exclusion diameter of 60, 125, and 220 μm. The fraction between 60–220 μm was used for subsequent experiments.

2.5. Morphological and dimensional analysis

The size and morphology of the microparticles were evaluated by optical and environmental scanning electron microscopy (ESEM).

2.6. FT-IR characterization of microparticles

The microparticles were dried under vacuum at 50 °C for 24 h, then they were prepared accordingly to the KBr technique and analysed by FT-IR (Vertex 70, Bruker, Austria) spectroscopy.

2.7. Determination of β-CD in microparticles

β-CD determination in microparticles was carried out by the absorption of typical organic compound (3-methylbenzoic acid (3-MBA)) that forms strong 1:1 inclusion complexes with β-CD [23,24]. 100 mg of poly(NIPAAm-co-β-CD) microparticles were dispersed in 10 mL of a solution of 3-MBA (0.5 mg/mL) in PBS, and kept 48 h under gentle stirring. The total amount of 3-MBA included in the β-CD cavity was determined by UV–Vis spectrophotometrical analysis, accounting for the difference between the initial concentration of 3-MBA and the amount of 3-MBA in the supernatant after 48 h. The amount of effective CD in microspheres was determined:

$$CD\% = Q \times q \quad (1)$$

where *Q* is the percentage of 3-MBA retained by poly(NIPAAm-co-β-CD) microparticles, and *q* is the ratio between the molecular weight of A-β-CD and 3-MBA.

2.8. PBS retention

The amount of PBS retained in the pores of microparticles, at room temperature (22 ± 2 °C), was determined by immersing 100 mg of dried microparticles in 10 mL of PBS containing 3-MBA (0.5 g/mL) (see previous section). After 48 h, the microspheres were filtered under vacuum, and weighed. The amount of retained PBS was determined by subtracting the weight of dried microparticles from that of swollen microparticles.

2.9. Swelling factor

The volume expansion of microparticles at different temperatures was determined at equilibrium, by placing microparticles in PBS in a graduated cylinder (i.d. = 12 mm). The swelling factor (*s*) was calculated:

$$s = \frac{V_s}{V_d} \quad (2)$$